Oxybenzone HEL Monograph - 1 of 7

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Joe DiNardo <jmjdinardo@aol.com>

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To:IEM Committee <IEM.Committee@mauicounty.us>; County Clerk <County.Clerk@mauicounty.us>;

Cc:cadowns@haereticus-lab.org <cadowns@haereticus-lab.org>;

4 attachments (4 MB)

Oxybenzone HEL Monograph.pdf; 1 Tsui Occurence Distribution and Ecological Risk Assessment 2.pdf; 2 Balmer Occurrence of Some Organic UV Filters in Wastewater.docx; 3 Tashiro Concentrations of organic sun-blocking.docx;

Please note - I the Oxybenzone HEL Monograph is 7 Emails total ... I accidentally number Email number 6 ... 6 of 9 ... there are only 7 Emails ... Sorry! Joe

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,
Craig Downs – Executive Director – Haereticus Environmental Laboratory
Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:
- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.
- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.
- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:
1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone
2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

Oxybenzone

Chemical Identity

Chemical Abstract Service (CAS) Registry Number: 131-57-7

Molecular Weight (MW) – 228.25

United Nations Global Harmonized System (GHS) – Hazard Statements: H413 – May cause long lasting harmful effects to aquatic life [Hazardous to the aquatic environment, long-term hazard]

IUPAC Name: (2-hydroxy-4-methoxyphenyl)-phenylmethanone

Trade Names/Supplier: AEC Benzophenone-3 (A & E Connock (Perfumery & Cosmetics) Ltd.); CSS-II Sun Screening Agent (Spec-Chem Industry Inc.); Custom B-3 (Custom Ingredients, Inc.); Escalol 567 (Ashland Inc.); Eusolex 4360 (Merck KGaA /EMD Chemicals); Eusolex 4360 (EMD Performance Materials Corp.); EUSORB 228 (Aceto Corporation); Jeescreen Benzophenone 3 (Jeen International Corporation; Neo Heliopan BB (Symrise); OriStar BP3 (Orient Stars LLC); Protaphenone-3 (Protameen Chemicals); Uvasorb MET/C (3V Sigma USA Inc.); Uvinul M 40 (BASF Corporation); UVSOB 350 (LC United Chemical Corp.).

FDA Voluntart Cosmetic Registration Program (VCRP): Use as of 01/2015 = 912

Use Level: Up to 6.0% in Sunscreens in the United States; Up to 10% in other countries.

Reported Product Categories: Aftershave Lotions; Baby Shampoos; Basecoats and Undercoats; Bath Capsules; Bath Oils, Tablets, and Salts; Bath Preparations, Misc.; Bath Soaps and Detergents; Blushers (All types); Body and Hand Preparations (Excluding Shaving Preparations); Bubble Baths; Cleansing Products (Cold Creams, Cleansing Lotions, Liquids and Pads); Colognes and Toilet Waters; Eye Lotions; Eye Makeup

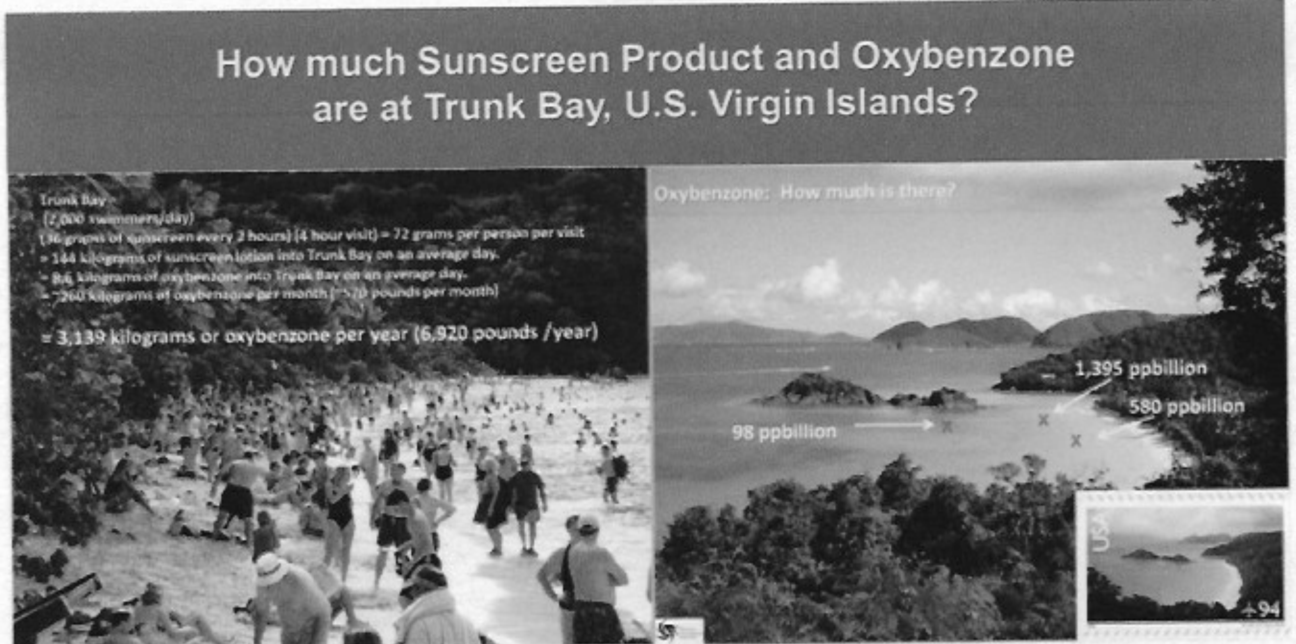
Preparations, Misc.; Eye Shadows; Eyebrow Pencils; Eyeliners; Face Powders; Face and Neck Preparations (Excluding Shaving Preparations); Feminine Hygiene Deodorants; Foot Powders and Sprays; Foundations; Fragrance Preparations, Misc.; Hair Coloring Preparations, Misc.; Hair Conditioners; Hair Dyes and Colors (All Types Requiring Caution Statements and Patch Tests); Hair Preparations (Non-coloring), Misc.; Hair Shampoos (Coloring); Hair Sprays (Aerosol Fixatives); Hair Wave Sets; Indoor Tanning Preparations; Lipsticks; Makeup Bases; Makeup Preparations (Not eye), Misc.; Manicuring Preparations, Misc.; Moisturizing Preparations; Nail Creams and Lotions; Nail Polish and Enamel Removers; Nail Polish and Enamels; Night Skin Care Preparations; Perfumes; Personal Cleanliness Products, Misc.; Shampoos (Non-coloring); Shaving Preparations, Misc.; Skin Care Preparations, Misc.; Skin Fresheners; Suntan Gels, Creams, and Liquids; Suntan Preparations, Misc.; Tonics, Dressings, and Other Hair Grooming Aids

Oxybenzone Contamination in the Environment and the Public

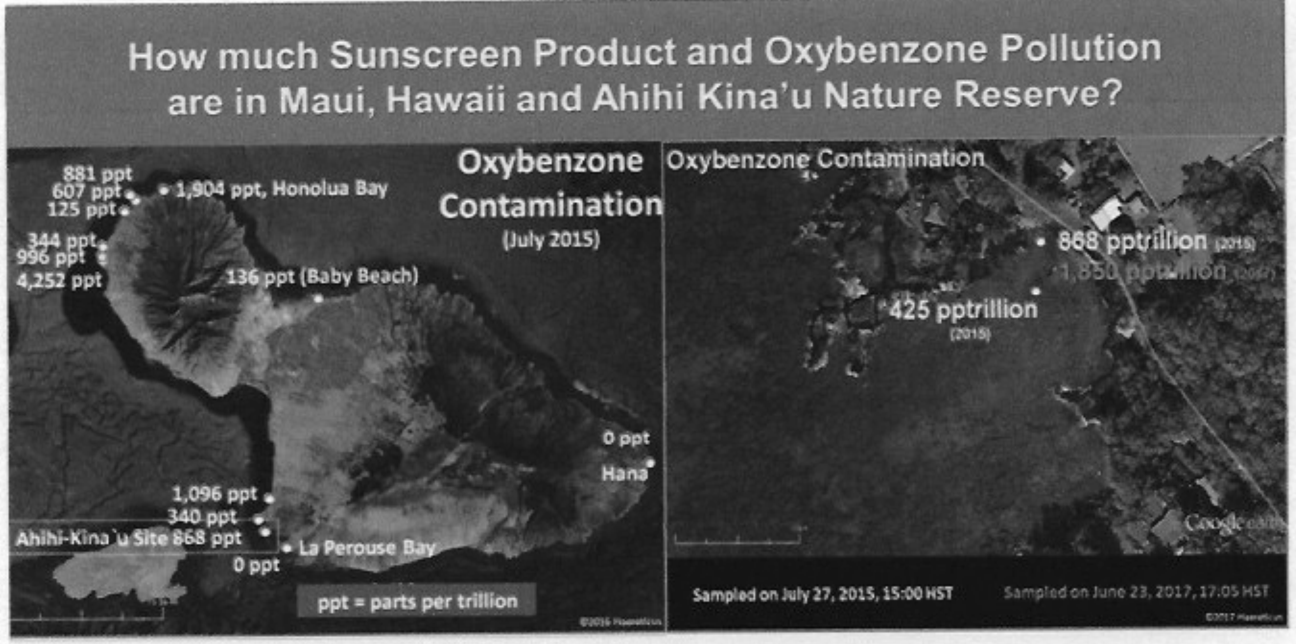
Oxybenzone is a ubiquitous environmental contaminant – it is found in streams, rivers, lakes, and in marine environments from the Arctic Circle (Barrow, Alaska) to the beaches and coral reefs along the equator [1,2,3,4]. It is considered an environmental hazard in many locations [5]. It is found in very high concentrations in swimming pools and hot tubs [6] and even in our drinking water (municipal treated and desalinated sources) [7,8]. Swimmers directly contaminate water sources, but point and non-point sewage and treated waste-water effluent discharges are the largest source of contamination.

Oxybenzone can be absorbed directly through the skin, either from application of sunscreen product onto the skin, or by absorption from swimming in either swimming pools or along beaches [9,10,11]. Oxybenzone in a commercial sunscreen formulation can transfer from the lotion or spray into the body, and be detected in urine within 30 minutes to several hours of application [12,13]. Oxybenzone body-contamination is widely prevalent in the general human population, with some nationalities having higher levels than others [14]. One study found that 96.8% of participants' urine was contaminated with oxybenzone, indicating the almost-universal prevalence of exposure in the human population [15,16]. Oxybenzone can contaminate semen [17], placenta and breast milk of marine mammals and humans [18,19,20,21]. Oxybenzone can both bioaccumulate, and can be biomagnified [22]. Oxybenzone has been found in bird eggs, fish, coral, humans, and other marine mammals [23,24,25].

Oxybenone is found in many aquatic environments. It is found in parts per trillion concentrations off the coast of Barrow, Alaska, U.S.A, to parts per billion on coral reefs in the Caribbean, Pacific, and Red Sea1 [-4]. One of the highest concentrations measured in the marine environment was in Trunk Bay in the U.S. Virgin Islands National Park, in St. John Island, U.S. Virgin Islands. This beach can get between 2,000 to 5,000 people in a day.



In Hawaii, on the island of Maui, the oxybenzone contamination of the west coast is extensive. Honolua Bay on the northern end of Maui saw 1.9 parts per billion oxybenzone. In the Ahihi Kina'u Nature Reserve, oxybenzone levels are increasing from 2015-2017. The near-shore reef and corals in the Fish Cove area is highly degraded.



Oxybenzone Ecotoxicology

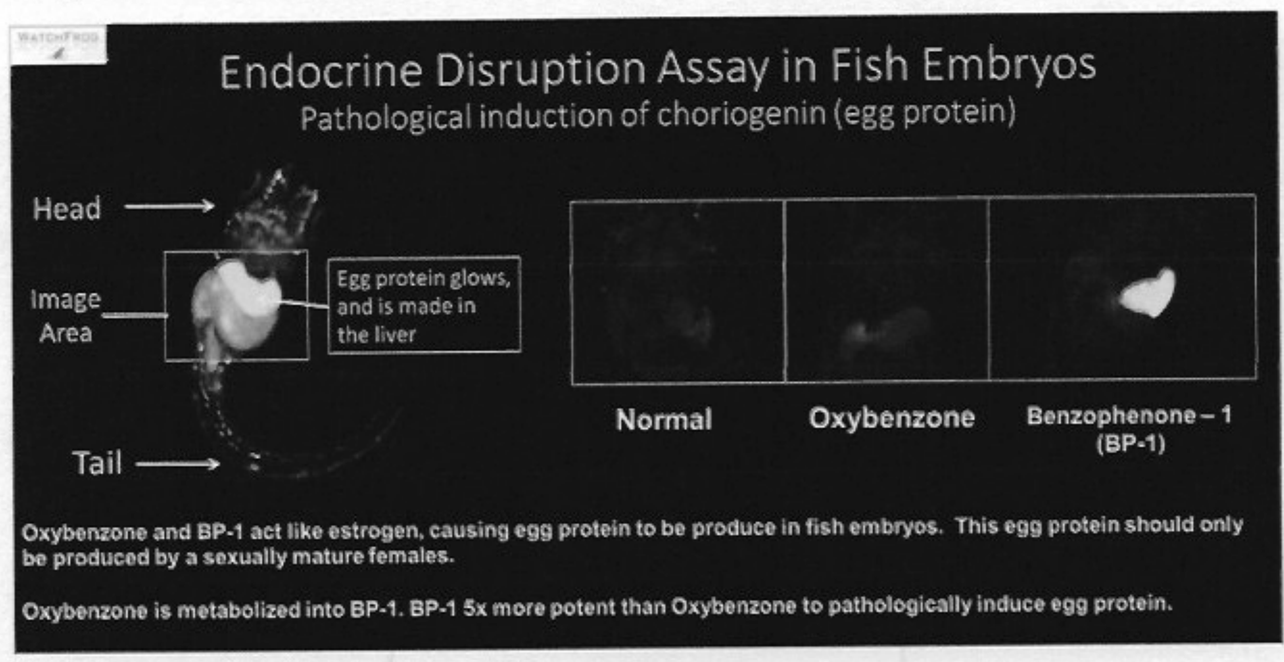
Oxybenzone and many of its metabolites are documented mutagens, especially when exposed to sunlight [26,27,28,29,30]. They can cause genotoxicity either from induction of photo-oxidative stress or adduction to DNA directly through bio-activation by cytochrome P450 enzymes [31,32,33]. Oxybenzone and other benzophenones can induce pro-carcinogenic activities by inducing cell proliferation in cancer cells lines that are receptive to estrogenic compounds [34,35,36]. Recent studies have also documented that oxybenzone increases metastasis potential (cellular proliferation and migration) via a non-estrogenic

mechanism [37,38].

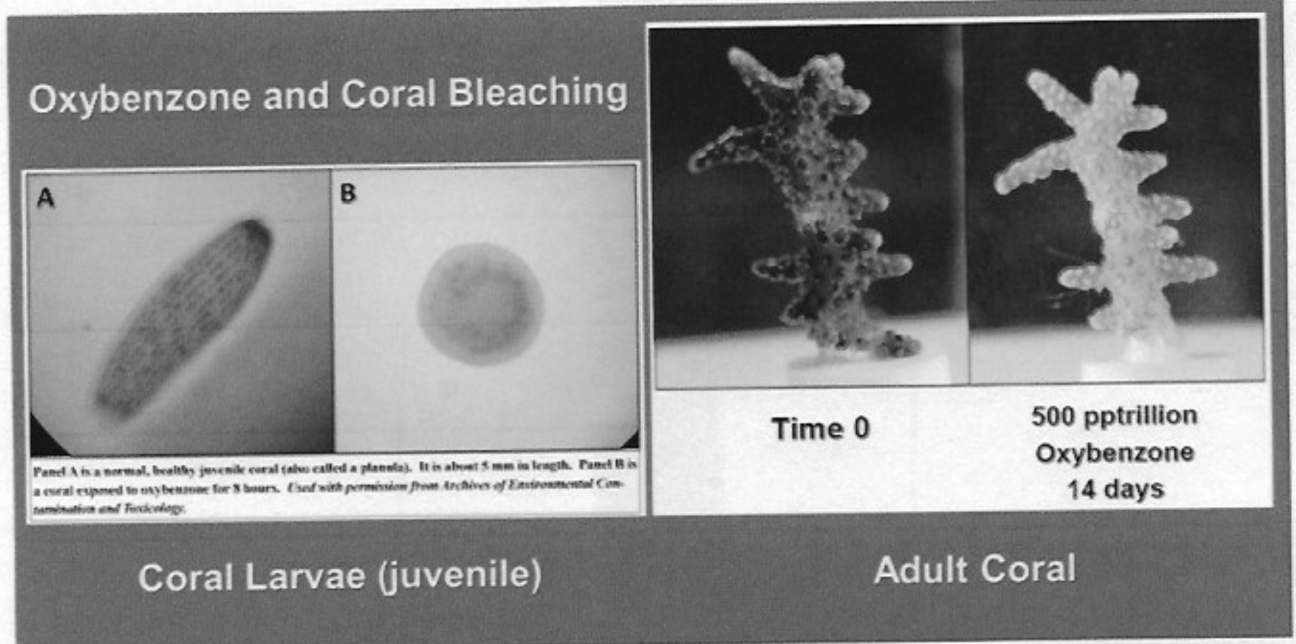
Oxybenzone is a photo-toxicant, especially in the presence of ultraviolent light. This means that the greater the light intensity, especially in the UV and near-UV spectrum, new forms of toxicity manifest, and usually in a dose-dependent manner of both oxybenzone and light.

In mammals, especially humans, oxybenzone has been shown to induce photo-allergic contact dermatitis in 16%-25% of the population [39,40,41]. Oxybenzone causes toxicity to sperm development and sperm viability, reduced prostate weight in mature males, and reduced uterine weight in juvenile females [42,43]. In rodents, it reduced fecundity and induced idiopathic sudden death in lactating mothers [44]. Several recent studies have shown a strong association between urinary and seminal oxybenzone concentrations and increased reproductive diseases and reduced fecundity [45,46]. There is a building body of evidence of the estrogenic and anti-androgenic endocrine disrupting mechanisms of oxybenzone in mammals [47,48]. One study indicated an increased occurrence of endometriosis in women exposed to concentrations of oxybenzone, while another study showed a positive association with uterine leiomyoma [49,50]. Oxybenzone has also been associated with altered timing of breast development in girls [51].

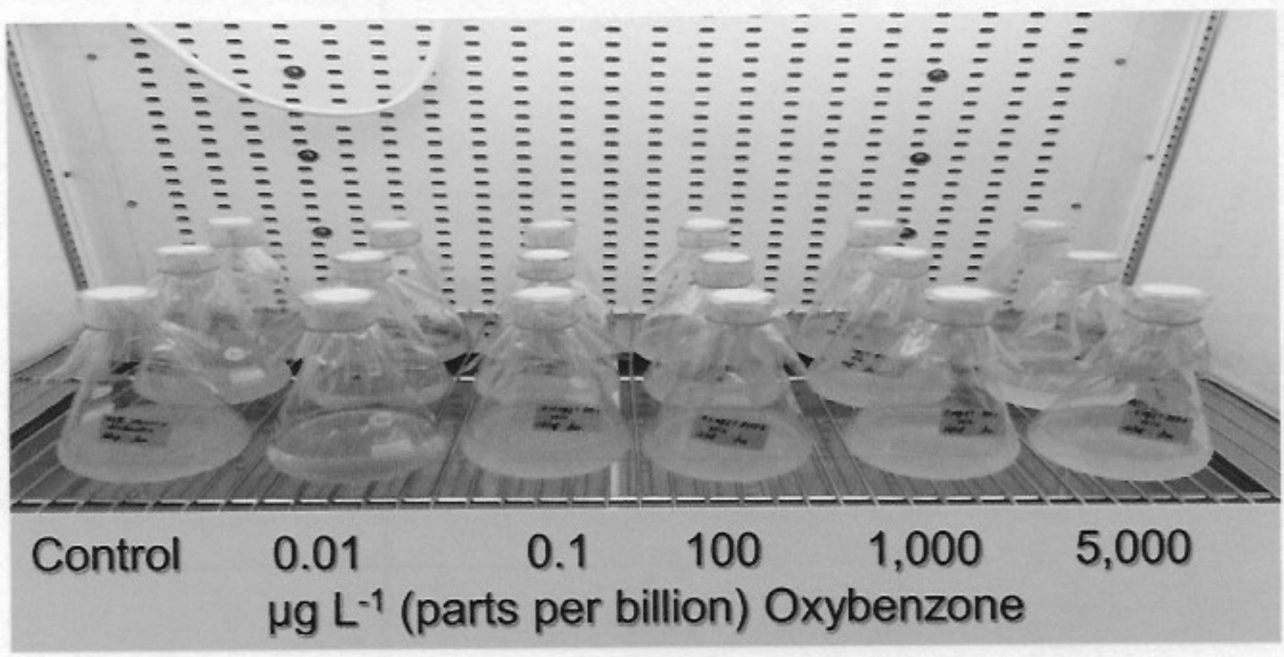
Oxybenzone is a notorious estrogenic endocrine disruptor, causing male fish to become feminized and inducing egg protein production in males and juveniles [52,53,54]. Oxybenzone causes a reduction in the number of eggs a female fish will produce [55,56,57]. In fish, oxybenzone is metabolized into benzophenone-1, a much more potent estrogenic disruptor [58]. Oxybenzone will also cause radical behavioral changes in fish, causing them to lose "territorial" behavior [59].



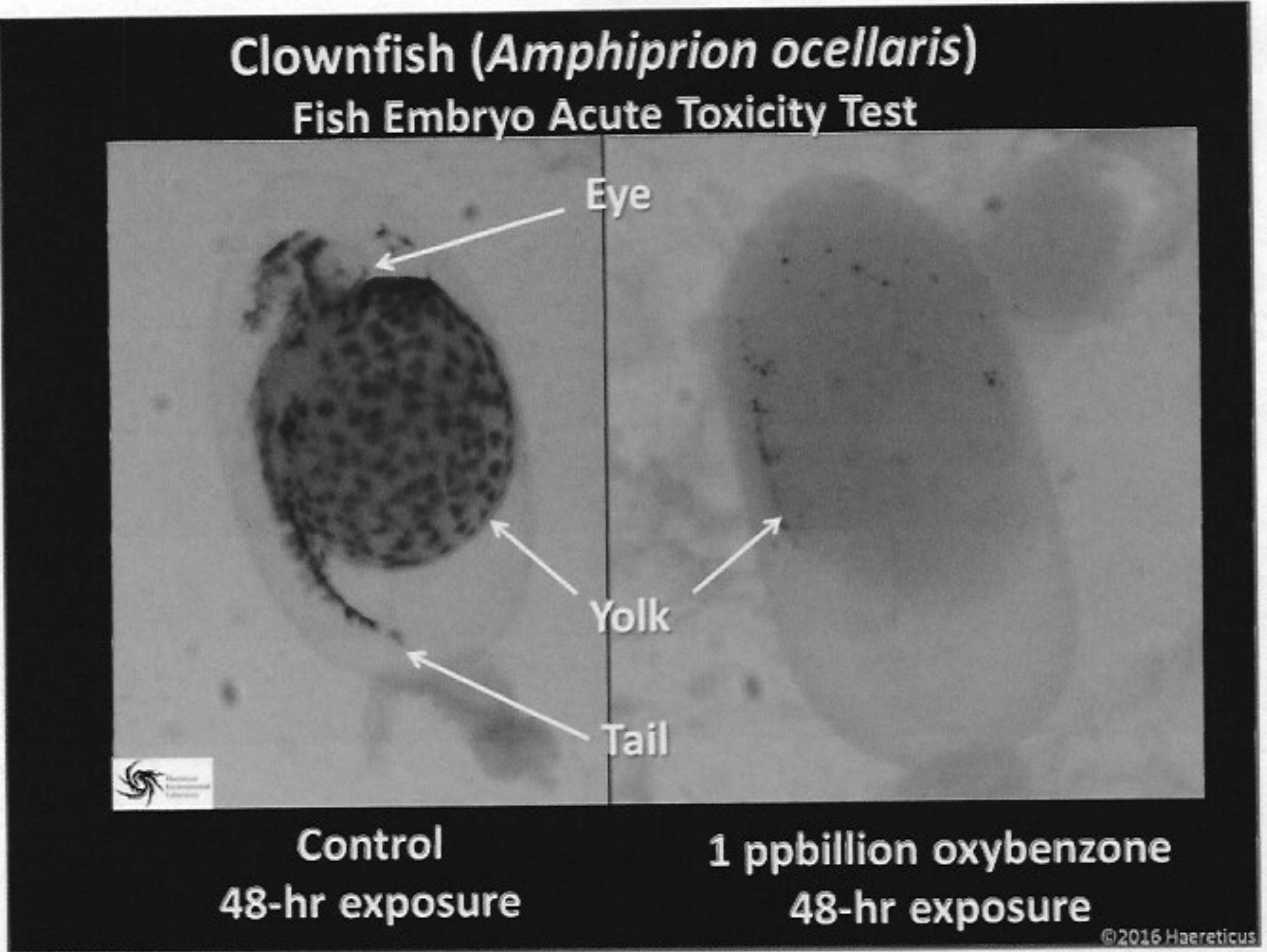
Oxybenzone can have devastating effects on invertebrates, especially on juvenile developmental stages[4]. In coral, it can cause coral bleaching, DNA damage, planula deformity, mortality, and skeletal endocrine disruption[4]. For coral planula, gross toxicological effects were seen as low as 6.5 ppbillion in a 24-hour period, and cellular effects were seen as low as 72 pptrillion in a 4-hour period. In bivalves, growth inhibition occurred around 2-3 ppmillion[1]. In shrimp larvae, growth inhibition was seen around 421 ppbillion[49].



Oxybenzone is even toxic to microalgae, such as *Isochrysis galbana*, at levels comparable to coral, such as 4 ppbillion[49]. A study in 2017 examined the toxicity on mortality, cell growth, and photosynthetic pigments of two important algal species, *Chlamydomonas reinhardtii* and *Microcystis aeruginosa* [1]. Changes in the amount of chlorophyll in these algae occurred in response to oxybenzone concentrations as low as 10 parts per trillion. This toxic response to oxybenzone suggests that the autrophic level of the food web in marine and aquatic systems could be severely challenged.



Oxybenzone can cause developmental deformities and diseases. In coral, it causes severe deformities in larvae, expanding the mouth (oral pore) more than 10x its normal size, exposing the yolk. In fish embryo, depending on the concentration, it can cause deformities in the eye, heart, and spine, and even severely lethal changes where no development occurs at all.



Humans can also exhibit developmental pathologies, especially fetal-development diseases associated with prenatal exposure to oxybenzone [1,2]. Hirschsprung's disease, a development abnormality thought to afflict every 1 in 3,000 births, has been linked to maternal exposure to oxybenzone, by interfering with the migration neural crest cells during embryonic development [3]. Environmentally relevant concentrations of oxybenzone induced a number of cellular pathologies to brain cells, especially neurons in the developing fetus and infant [4,5].

Emerging Science regarding Oxybenzone: a human-health warning.

Oxybenzone can contaminate hot-tubs and swimming pools with concentrations in the parts per billion[5]. If these pools use chlorine or bromine as a disinfectant, the oxybenzone undergoes a chemical reaction and can become "chlorinated" or "brominated – meaning a chlorine or bromine is conjugated to the oxybenzone, changing its chemical structure and chemistry[1,2]. Very recent studies show that the chlorinated forms of oxybenzone are *significantly* more toxic than normal oxybenzone, acting as significant DNA damage agents[57,58,3]. A by-product of this oxybenzone chlorination is chloroform [4].

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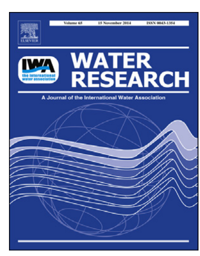

Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries

Mirabelle M.P. Tsui [a,b], *H.W. Leung* [a,b,c], *Tak-Cheung Wai* [b], *Nobuyoshi Yamashita* [d], *Sachi Taniyasu* [d], *Wenhua Liu* [e], *Paul K.S. Lam* [a,b], *Margaret B. Murphy* [a,b,*]

[a] *Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China*
[b] *State Key Laboratory in Marine Pollution, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China*
[c] *Department of Applied Science, Hong Kong Institute of Vocational Education, Chai Wan, Hong Kong, China*
[d] *National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan*
[e] *Marine Biology Institute, Science Center, Shantou University, 243 Daxue Road, Shantou, Guangdong, 515063, PR China*

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ABSTRACT

Organic UV filters are common ingredients of personal care products (PCPs), but little is known about their distribution in and potential impacts to the marine environment. This study reports the occurrence and risk assessment of twelve widely used organic UV filters in surface water collected in eight cities in four countries (China, the United States, Japan, and Thailand) and the North American Arctic. The number of compounds detected, Hong Kong (12), Tokyo (9), Bangkok (9), New York (8), Los Angeles (8), Arctic (6), Shantou (5) and Chaozhou (5), generally increased with population density. Median concentrations of all detectable UV filters were <250 ng/L. The presence of these compounds in the Arctic is likely due to a combination of inadequate wastewater treatment and long-range oceanic transport. Principal component analysis (PCA) and two-way analysis of variance (ANOVA) were conducted to explore spatiotemporal patterns and difference in organic UV filter levels in Hong Kong. In general, spatial patterns varied with sampling month and all compounds showed higher concentrations in the wet season except benzophenone-4 (BP-4). Probabilistic risk assessment showed that 4-methylbenzylidene camphor (4-MBC) posed greater risk to algae, while benzophenone-3 (BP-3) and ethylhexyl methoxycinnamate (EHMC) were more likely to pose a risk to fishes and also posed high risk of bleaching in hard corals in aquatic recreational areas in Hong Kong. This study is the first to report the occurrence of organic UV filters in the Arctic and provides a wider assessment of their potential negative impacts in the marine environment.

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* *Corresponding author*. Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China. Tel.: +852 3442 6858; fax: +852 3442 0522.
E-mail address: mbmurphy@cityu.edu.hk (M.B. Murphy).

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1. Introduction

Organic ultraviolet (UV) filters are widely used as UV radiation-absorbing substances in personal care products (PCPs) to protect human skin from the negative effects of sunlight as well as in materials and paints to prevent product photodegradation. Authorized contents of organic UV filters in PCPs vary according to regulations in the countries/regions of their manufacture, where they may comprise up to 20% of product mass (Chisvert and Salvador, 2007). Owing to their large annual production quantities and widespread usage, particularly because of greater awareness of skin cancer risks in recent decades, organic UV filters can enter the aquatic environment (i) indirectly from wastewater treatment plants (WWTPs) after entering sewage systems following bathing or from industrial discharge due to incomplete removal as well as surface runoff and (ii) directly from recreational activities (e.g. swimming) (Giokas et al., 2007).

As a result of their extensive application and continuous release into aquatic systems, organic UV filters are regarded as pseudo-persistent environmental contaminants, and their ubiquity has raised concerns about their potential environmental impacts (Giokas et al., 2007). They have been found in various environmental samples including surface water, wastewater and sediment (e.g. Tsui et al., 2014; Kameda et al., 2011) generally at ng/L to sub-ug/L levels for aqueous matrices and sub-ng/g levels for solid matrices. However, only a few studies have reported the occurrence of UV filters in the marine environment, and only a limited number of globally authorized compounds have been investigated; for example, benzophenone-3 and -4 (BP-3 and BP-4), ethylhexyl methoxycinnamate (EHMC) and octocrylene (OC) were detected in surface waters in some European countries and Japan (Tashiro and Kameda, 2013; Tovar-Sánchez et al., 2013; Rodil et al., 2008).

Many organic UV filters have high lipophilicity, with octanol-water partition coefficients (log K_{ow}) values generally greater than 3. They have been detected in various aquatic organisms such as brown trout (*Salmo trutta fario*) up to 1800 ng/g (4-methylbenzylidene camphor, 4-MBC) and 2400 ng/g (OC) lipid weight (lw) in Swiss rivers (Buser et al., 2006) and in marine mussels (*Mytilus edulis*) up to 256 ng/g (EHMC) and 7112 ng/g (OC) dry weight (dw) along the French Atlantic and Mediterranean coasts (Bachelot et al., 2012). Moreover, Fent et al. (2010b) suggested food chain accumulation of EHMC, reporting its concentrations in fish and cormorants (*Phalacrocorax* sp.) from six Swiss rivers up to 337 and 701 ng/g lw, respectively. Accumulation of these compounds in organisms is a concern because organic UV filters and their metabolites have been shown to interfere with endocrine function by acting as environmental estrogens both *in vitro* and *in vivo* (Schlumpf et al., 2001; Kunz and Fent, 2006). Moreover, they have been shown to induce bleaching in corals by promoting viral infections (Danovaro et al., 2008).

Data on the occurrence of organic UV filters in fresh surface waters are available for several developed countries (e.g. Kameda et al., 2011; Fent et al., 2010b), but relevant information is lacking for the marine environment in countries outside of Europe or Japan for certain uniformly approved and widely consumed UV filters (e.g. butyl methoxydibenzoylmethane (BMDM) and homosalate (HMS)). Moreover, previous studies have reported the occurrence of UV filters at beaches, but little information is known about coastal waters. In contrast to other organic contaminants (e.g. perfluoroalkyl substances (PFAS) and pharmaceuticals) which have been studied in detail (Richardson and Ternes, 2014), information on the occurrence, distribution, transport pathways and risks of organic UV filters in the aquatic environment is lacking. Therefore, it is of crucial importance to study the environmental distribution and concentrations of these emerging contaminants in order to evaluate their ecological risks.

In light of these considerations, the objectives of this study were to (i) determine the concentrations and spatial occurrence of twelve commonly consumed UV filters, including benzophenone-1, -3, -4 and -8 (BP-1, -3, -4 and -8), ethylhexyl salicylate (EHS), isoamyl p-methoxycinnamate (IAMC), octyl dimethyl-p-aminobenzoic acid (ODPABA), BMDM, EHMC, HMS, 4-MBC and OC in surface water samples collected from different countries including China (Hong Kong, Shantou and Chaozhou), the United States (New York City and Los Angeles), Japan (Tokyo Bay), Thailand (Bangkok) and the Arctic region, as well as their seasonal variation in Hong Kong over the course of one year; and (ii) conduct an ecological risk assessment by using the measured environmental concentrations and available toxicity data.

2. Materials and methods

2.1. Chemicals and materials

Information on chemical standards and preparation of standard solutions can be found in the Supplementary material. Standard purities were all ≥97%. Detailed information on the targeted UV filters is shown in Table A1.

2.2. Sampling

Surface water samples were collected from eight locations (Hong Kong, $n = 60$; Tokyo, $n = 8$; New York, $n = 6$; Los Angeles, $n = 4$; Shantou, $n = 4$; Chaozhou, $n = 3$; Bangkok, $n = 2$) and the Arctic ($n = 14$) from 2012 to 2013 using plastic or stainless steel buckets or glass bottles which were pre-cleaned by rinsing (in sequence) with methanol, Milli-Q water, and water from the specific location. All samples were marine surface water samples except those collected from Bangkok which were freshwater samples. Most of the selected cities are metropolitan areas featuring both commercial and industrial development. Temporal and spatial samples were collected in Hong Kong in both the wet and dry seasons; spatial samples were collected from Tokyo Bay, Los Angeles, New York City and the Arctic, while only a single location was sampled in Bangkok. Detailed information on the sampling locations is shown in Supplementary material Table A2 and Figs A1–5.

Surface water samples were collected from 20 points in Hong Kong in August 2012, February and June 2013; June and August samples represented the wet season, while the February samples represented the dry season. The sampled

points were expected to reflect WWTPs, beaches and aquatic recreational activities (further details are given in the Supplementary material). The Tokyo samples were collected in Tokyo Bay, while the New York samples were collected in Jamaica Bay, Upper New York Bay and the East River near WWTP discharge points. In Los Angeles, Shantou and Chaozhou, surface water samples were collected at beaches and near WWTP discharge points while only river water receiving municipal wastewater was collected in Bangkok. Arctic samples were collected in the Arctic Ocean and Chukchi Sea between 65 and 75 °N.

Water samples were stored in glass bottles pre-rinsed with Milli-Q water and methanol. All glass bottles were wrapped with aluminum foil to avoid contamination and photodegradation of the target compounds. Samples were stored in the dark at 4 °C prior to analysis.

2.3. Analytical procedures

Chemical analysis of the 12 target compounds was modified from a previously reported method (Tsui et al., 2014). Briefly, the analytical procedures consisted of addition of 5% (w/v) Na_2EDTA to each sample, solid phase extraction (SPE) with Bond Elut C18 cartridges, elution by 3 × 4 mL of 50:50 v/v methanol: ethyl acetate (MeOH: EA), concentration under nitrogen flow to less than 0.5 mL, reconstitution to 0.5 mL by MeOH and analysis by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). Detailed analytical procedures and optimal parameters of HPLC-MS/MS for quantification are shown in the Supplementary material.

2.4. Method validation

Details on method validation are given in Table A4 in the Supplementary material. Recoveries ranged from 63% to 106%, while the relative standard deviations (RSD) of target compounds ranged from 1.5% to 7.9%. The results presented in this study were not corrected by recoveries. The method limit of detection (MLOD) was defined as three times the standard deviation of procedural blank peak areas plus their mean value and then corrected by a matrix-induced interference factor which was the slope difference (ratio) of two calibration curves separately constructed in methanol and in water sample extracts (Leung et al., 2012). MLODs ranged from 0.03 to 1.38 ng/L. Field and procedural blanks were analyzed for each sampling trip and for each batch of samples in the laboratory by using Milli-Q water. All of the target compounds were below MLODs in both field and procedural blanks.

2.5. Statistical analyses

Normality tests (Kolmogorov–Smirnov) were performed before statistical analyses. Parametric Pearson correlation analysis was used for the examination of significant correlations among concentrations of different UV filters in surface water from different sampling cities/regions. Log_{10}-transformed values were used to perform the Pearson correlations in all locations except Hong Kong, for which principal component analysis (PCA) and permutational analysis of variance (PERMANOVA) were conducted to explore spatiotemporal patterns in organic UV filter levels because of the larger sample size for this city. Two-way analysis of variance (ANOVA) and *post hoc* Student-Newman-Keuls (SNK) tests were carried out to test spatiotemporal differences in compound concentrations in Hong Kong samples. Samples with concentrations < LOD were treated as zero in the analysis. The significance level was set at $\alpha = 0.05$. Univariate statistical analyses were carried out using SigmaStat 3.5 (Systat Software Inc, Chicago, USA) or SPSS 17 (SPSS Inc.). Multivariate analyses were carried out using PRIMER 6 & PERMANOVA+ (PRIMER-E Ltd, Plymouth, UK).

2.6. Environmental risk assessment (ERA)

Hazard quotients (HQs) for individual UV filters were obtained by dividing measured environmental concentrations (MECs) obtained in this study by predicted-no-effect concentrations (PNECs) calculated by dividing the effect concentrations (ECs) by a standard assessment factor, 1000, to account for intra- (factor = 10) and inter-species variability (10) and chronic exposure conditions (10) (European Commission, 2003). Toxicity data were obtained from the literature focusing on aquatic organisms at different trophic levels including protozoa, algae, crustaceans, invertebrates and fishes. Because of the lack of toxicological literature on many organic UV filters, the risk assessment was only conducted for six compounds: BP-1, BP-3, BP-4, EHMC, 4-MBC and ODPABA.

Preliminary screening of the potential ecological risks of organic UV filters was carried out using the worst-case scenario, HQ_{worst}, in which the maximum MECs of each compound and minimum PNECs were applied in the hazard assessment (Table 1 and A5). The risk classification was based on risk ranking criteria in which HQ < 0.01: “Unlikely to pose risk”; 0.01 ≤ HQ < 0.1: “Low risk”; 0.1 ≤ HQ < 1: “Medium risk” and HQ ≥ 1: “High risk” (Hernando et al., 2006). Probabilistic risk assessment was conducted if the HQ_{worst} of UV filters exceeded 1 by plotting cumulative probability on a log scale. Risk probabilities (*p*) were calculated by substituting the log PNECs of each species in the linear equations for each sampled city, in which (100-*p*)% would be the percentage of samples containing concentrations of that compound exceeding the PNEC of a particular species and thus posing risk based on the assessed endpoint.

3. Results and discussion

3.1. Occurrence and composition of UV filters in surface waters

A total of 101 surface water samples collected from August 2012 to October 2013 were analyzed, and median concentrations in the samples ranged from <LOD to 230 ng/L (Table 1). The number of compounds detected was Hong Kong (12), Tokyo (9), Bangkok (9), New York, Los Angeles (8), Arctic (6), Shantou (5) and Chaozhou (5).

BP-3, EHMC and OC were detected in all cities and in the Arctic with detection frequencies ≥30% in each location (calculated by dividing the number of positive detections by

Table 1 – Concentrations (ng/L) and detection frequencies of 12 UV filters in surface water samples from different cities. Conc.: (median-maximum); DF: detection frequencies (calculated by dividing the number of positive detections by the total number of samples from each location); LOD: limit of detection.

Compounds	Hong Kong ($n = 60$)		Tokyo ($n = 8$)		New York ($n = 6$)		Los Angeles ($n = 4$)		Shantou ($n = 4$)		Chaozhou ($n = 3$)		Bangkok ($n = 2$)		Arctic ($n = 14$)	
	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF	Conc.	DF
ODPABA	95–182	17	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
4-MBC	173–379	12	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
BMDM	24–721	97	78–104	100	70–87	100	67–109	100	53–100	75	<LOD	0	36–38	100	18–70	57
EHMC	89–4043	93	46–95	63	89–150	83	91–138	75	52–78	75	<LOD-79	33	88–95	100	25–66	71
IAMC	63–173	27	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0	<LOD	0
OC	103–6812	100	87–108	75	117–128	83	145–377	100	75–107	75	36–102	67	153–205	100	26–31	43
BP-3	39–5429	95	24–86	100	23–178	100	227–601	100	55–188	100	37–49	100	86–116	100	17–33	71
EHS	61–1030	59	71–95	88	<LOD	0	53–120	50	<LOD	0	121–128	67	28–56	50	<LOD	0
BP-4	54–389	49	71–136	100	89–574	100	<LOD	0	<LOD	0	<LOD-49	33	80–95	100	<LOD	0
HMS	66–2812	76	65–110	100	91–114	67	142–270	100	<LOD	0	<LOD	0	29–59	50	<LOD	0
BP-1	82–135	76	52–95	63	<LOD-74	33	100–117	75	22–58	50	<LOD	0	127–166	100	2.5–5	36
BP-8	64–117	88	76–96	100	72–92	100	29–96	50	<LOD	0	<LOD	0	63–71	100	2–3.3	50

the total number of samples in each place), showing their widespread distribution in the marine environment. Among these three frequently detected UV filters, the highest median concentrations were found in Los Angeles (227 ng/L; BP-3), New York (100 ng/L; EHMC) and Bangkok (153 ng/L; OC), while the lowest median concentrations were found in the Arctic (16.6, 25.4 and 25.8 ng/L for BP-3, EHMC and OC, respectively). The concentrations of five compounds including BP-3, EHMC, EHS, HMS and OC exceeded 1000 ng/L in surface water samples collected on hot summer days with strong UV radiation in June and August 2013 at a popular beach in Hong Kong. Apart from recreational activities and surface runoff, the incomplete removal of organic UV filters in WWTPs is a major contributor to their ubiquitous occurrence in the environment; in Hong Kong, their environmental loading can reach 200 g/day (Tsui et al., 2014). BP-4, with median concentrations lower than 100 ng/L at all locations, was only detected in indirect sources (i.e. through WWTP discharge) because it is used primarily in PCPs such as hand washes/soaps, shower gels and shampoo rather than sunscreen products and it is poorly removed in WWTPs (Tsui et al., 2014). In contrast, 4-MBC (173–378 ng/L), IAMC (62.7–173 ng/L) and ODPABA (95.1–182 ng/L) were only detected at snorkeling hot spots and other recreational beaches in Hong Kong, indicating that recreational activities would be the main sources of these three compounds instead of wastewater effluent discharge. The low detection frequencies of these compounds in WWTP-influenced samples are likely due to their relatively lower use (used in less than 2% of commercially available PCPs in Swiss and British markets; Manová et al., 2013; Kerr, 2011) together with the stronger dilution effects of ocean currents in Victoria Harbour.

Generally, the occurrence of individual UV filters at each location (calculated by dividing the total concentration of each UV filter by the total concentration of UV filters at that location) was $<30\%$, except for EHS in Chaozhou which was $>40\%$ (Fig. A6). The composition profiles of UV filters in surface waters from Hong Kong, Tokyo, New York, Los Angeles and Bangkok were similar while those in samples from Shantou, Chaozhou and the Arctic showed higher percentages of each detectable compound due to the relatively lower number of positive detections. BP-3, EHMC and OC were the dominant compounds detected in all samples. Hong Kong imports a wide variety of PCPs from several countries/regions and has no local regulations for organic UV filter content in products, and therefore more compounds from several chemical classes were detected in these samples. The number of compounds detected in Shantou and Chaozhou were the lowest among all sampled cities, likely because of their lower population densities (2655/km^2 and 849/km^2, respectively; Shantou and Chaozhou Government, 2010) and development level. Liao and Kannan (2014) reported overall geometric mean levels of BP-3 in PCPs purchased in China and the United States, which were 20.1 and 1200 ng/g, respectively. Moreover, the maximum authorized concentrations of some of the targeted compounds in China are lower than those in other countries (e.g. up to 20% of product mass can consist of EHMC in Japan, but only 10% of product mass is permitted in China) (MoH, 2007), indicating comparatively lower application of these UV filters in PCPs in China. Sediments with high organic

carbon content are an environmental sink for contaminants with high log K_{ow} and UV filters can also be detected in sediment (e.g. Kameda et al., 2011).

3.2. Distribution and source determination of UV filters in surface water

Correlation analyses were performed among individual UV filters from Tokyo, the Arctic and the United States for source determination (Table A6). Significant positive correlations ($p < 0.05$) were observed between BP-3 and BP-8 ($r = 0.879$) and BP-3 and EHMC ($r = 0.774$) in the Arctic samples. Higher detection frequencies of UV filters were found in samples collected near Alaska (<72°N) than in those from the open ocean (>72°N). The overall detection frequencies of BMDM, BP-3 and EHMC were >50%, while concentrations of all detectable compounds were <70 ng/L (Table 1). This is the first report of the occurrence and distribution of organic UV filters in the Arctic, for which there are two possible pathways: (i) oceanic transport via ocean currents or (ii) atmospheric transport; these pathways may be either long-range or short-range. Inadequate wastewater treatment facilities could result in the direct release of untreated or undertreated wastewater to the marine environment via oceanic currents (Gunnarsdóttir et al., 2013) and thus wastewater runoff could be one of the local contamination sources of UV filters as some of the sampling points are located offshore of Point Hope and Point Barrow in Alaska (population: 674 and 4212, respectively; United States Census, 2010), both of which employ sewage lagoons as the major wastewater treatment method (BUECI and URS Corporation, 2005). Some compounds such as BP-3 and OC have been reported to be highly photostable towards UV irradiation (half-lives >72 h; Rodil et al., 2009), and they may undergo long-range or local transport via oceanic currents. However, information about the environmental half-lives of organic UV filters is limited. Though some of these compounds have similar Henry's Law constants (ranging from 10^{-5} to 10^{-15} atm·m^3/mol, Table A1) as other organic contaminants known to undergo long-range atmospheric transport such as PFAS and endosulfan (Butt et al., 2010; Weber et al., 2010), there is currently not enough evidence to conclude that they partition into the gas phase as no studies have reported the occurrence of UV filters in air samples or wet or dry deposition, and their atmospheric half-lives are also unknown. More work should be conducted to investigate the fate on these compounds in order to understand their occurrence in aquatic environments.

In Tokyo Bay, significant positive correlations ($p < 0.05$) were observed between EHMC and OC ($r = 0.957$); EHS and HMS ($r = 0.795$) and BP-1 and BP-8 ($r = 0.885$), suggesting that these compounds likely share contamination sources such as wastewater effluents from urban and industrial areas in Tokyo. Moreover, Tokyo Bay receives fresh water from the Tama, Arakawa and Edo Rivers, which flow through densely populated areas with WWTPs at different treatment levels. All wastewater collected from public sewers in Japan is treated with secondary treatment, while 15% is further treated with tertiary methods, including sewage discharged to Tokyo Bay (Ueda and Benouahi, 2009). Chemicals used in PCPs (e.g. UV filters and synthetic musks) were detected in these rivers in previous studies (Kameda et al., 2011; Yamagishi et al., 1983). Kameda (2007) reported the occurrence of EHMC in sediment core samples collected in Tokyo Bay (1977–1997) showing the long history of the occurrence of this compound in the Bay, and the present study showed its wide distribution in surface water in Tokyo Bay as well.

Significant positive correlations were observed between BMDM and OC ($r = 0.971$), BP-3 and BP-4 ($r = 0.903$) as well as HMS and BP-8 ($r = 0.985$) in the samples from New York City, suggesting that they shared similar contamination sources (WWTP effluents). There are 14 WWTPs in New York City serving 7.8 million people (New York City Department of Environmental Protection), and wastewater is treated by secondary treatment and chlorination disinfection, neither of which completely remove organic UV filters from effluents (Tsui et al., 2014). In Los Angeles, the highest concentrations of UV filters were detected at a popular beach, Huntington Beach.

No significant correlations were observed among UV filters in surface water from the two Chinese cities, suggesting that UV filters in the marine environment in these locations have distinct contamination sources such as both municipal and industrial wastewater discharge.

3.3. Seasonal variation of UV filter concentrations in surface waters in Hong Kong

The occurrence of organic UV filters in the Hong Kong samples was investigated in greater depth using PCA. Significant seasonal and zone differences (all $p < 0.0001$, PERMANOVA) were observed. The sample patterns were considered in monthly chronological order without considering the sampling year, and monthly data are presented individually to illustrate spatial patterns (Fig. 1a–c). In all sampling months, the concentrations measured at the sampled points largely conformed to expectations about the major sources of organic UV filters in each zone. February is the end of the dry season, when water temperatures are colder (Table A7) and there is little marine or coastal recreational activity, and thus it was assumed that there would be no or small inputs of organic UV filters at sites representing direct sources and at beaches; these sites cluster together in the PCA (Fig. 1a, I and III) and apart from sites representing indirect sources (WWTPs; Fig. 1a, IV). In contrast, samples collected at the beginning of the wet summer season in June from indirect and direct sources showed less separation, likely due to marine recreational activities increasing with warmer temperatures (Fig. 1b, V and VII), while beach samples (Fig. 1b, VI) were dissimilar to both of these groups. The collected samples were most similar to one another in August, when temperatures were highest (Fig. 1c), and increased usage of organic UV filters at the sampled beaches (Fig. 1c, IX) was evident. Surface water samples collected from Point-16 and -17 were dissimilar to the other beach sampling locations, grouping apart from all other points in February (Fig. 1a, II) and grouping with samples representing direct sources in June and August (Fig. 1b, V and 1c, VIII, respectively). These two points were located on a small beach adjacent to a village community where snorkeling and diving are the major recreational activities, and where there may also be some local wastewater release by

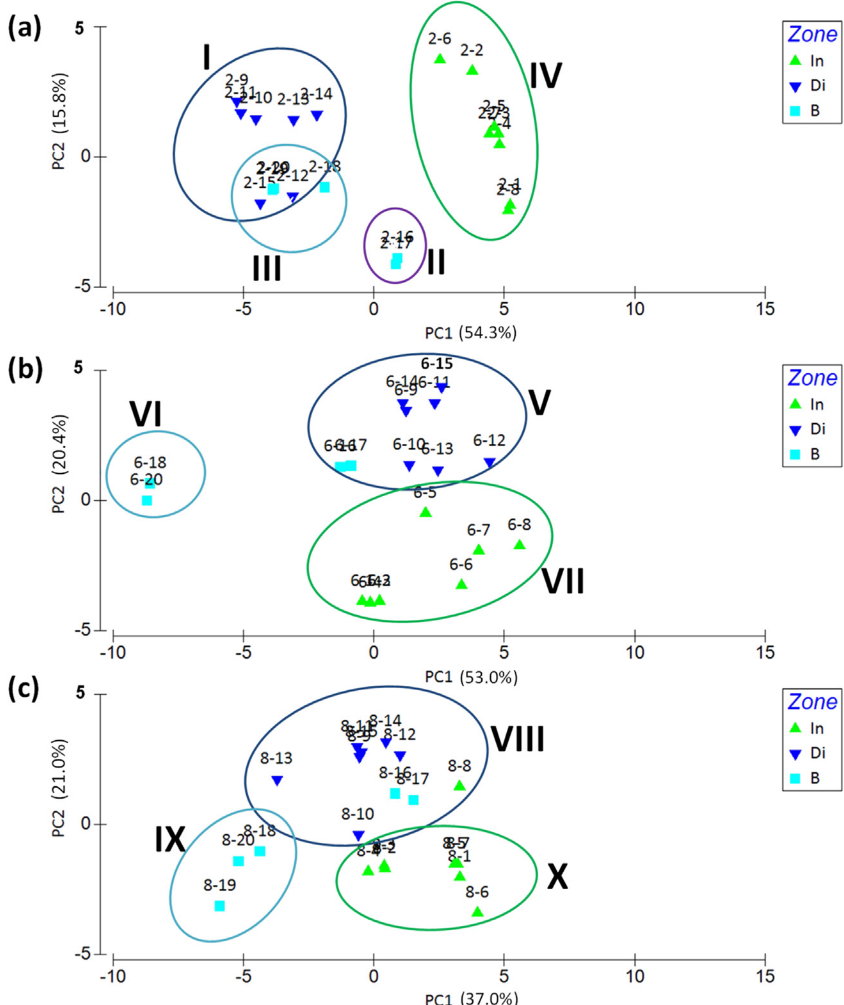

Fig. 1 – Principal Component Analysis (PCA) plots for surface water samples collected in (a) February 2013, (b) June 2013, and (c) August 2012 from Hong Kong (In: Indirect sources (WWTPs), Di: Direct sources (water sports & marine recreational activities), B: Beaches).

residents or tourists. These points therefore showed a distinct pattern compared to the other locations.

Concentrations of all the tested compounds varied according to season and/or sampling zone (ANOVA, Tables A8a & b). The results of *post hoc* SNK tests illustrated that all compounds showed significantly higher concentrations in the wet season in at least one sampling zone with the exception of BP-4, generally reflecting greater usage of PCPs containing organic UV filters during the sunnier and hotter wet season (Tables A7 and A8). BP-4 concentrations were greater in the dry season perhaps because it is increasingly used as a photodegradation retardant and shelf life extension ingredient in many types of PCPs (Hughes and Stone, 2007) that are not used seasonally. Moreover, it is the most hydrophilic of the target compounds (log K_{ow}: 0.89), and therefore this disparity may also be due to differences in precipitation in the dry and wet seasons. IAMC, 4-MBC and ODPABA were mainly detected in the wet season and at locations reflecting direct sources and beaches. This finding is consistent with our previous study of wastewater in Hong Kong which reported low detection frequencies of these compounds in effluent (Tsui et al., 2014).

The detected levels of EHMC showed no significant spatial differences in both seasons and its high detection frequency indicated continuous release of this compound to the marine environment throughout the year. On the other hand, the significantly higher concentration of BP-4 found in locations representing indirect sources in both seasons confirmed that WWTP effluent is a major source of this compound in the aquatic environment. However, the lack of information on product composition/formulations and usage by consumers in Hong Kong makes it difficult to understand how the occurrence patterns of organic UV filters in the environment are related to their use and release.

3.4. *Global comparison of UV filters in surface water*

3.4.1. *Marine environment*

To date, few studies have reported the occurrence of UV filters in the marine environment, and published reports have focused primarily on European countries and reported levels of a small number of compounds. One recent study reported that the maximum concentration of UV filters

detected at coral reef sites in Okinawa Island, Japan was 8.1 ng/L (OC) (Tashiro and Kameda, 2013), which was 28 times less than that detected in coral reef areas in Hong Kong (OC, 231 ng/L). A global comparison of median-to-maximum levels of the target compounds in the present study is shown in Fig. 2. This study is the first report of BMDM, BP-8 and IAMC in the marine environment. Maximum concentrations of BMDM were <200 ng/L in all sampled locations except Hong Kong (BMDM: 721 ng/L), while detection frequencies for BMDM and BP-8 were >60% and IAMC was only detected in Hong Kong. The maximum concentrations of OC, BP-3 and EHMC found in this study were 6810, 5420 and 4040 ng/L, respectively, which were comparable, 1.5 and 10 times higher than those detected in Norway and Spain (7300, 3300 and 390 ng/L, respectively; Langford and Thomas, 2008; Tarazona et al., 2010). The maximum concentration of BP-4 (574 ng/L) was found in a sampling point near the effluent discharge of a WWTP in Jamaica Bay in New York, but it was <LOD in all samples from beaches in this study; in contrast, BP-4 was detected at a Spanish beach at a maximum concentration of 138 ng/L (Rodil et al., 2008). 4-MBC is not permitted for use as a cosmetic ingredient in Japan and the United States, and thus it was not detected in seawater there, but it was detected in Europe and Hong Kong at maximum concentrations of 799 (Langford and Thomas, 2008) and 379 ng/L (present study), respectively.

3.4.2. *Freshwater environment*

The organic UV filter concentrations reported for river surface water samples from Bangkok in the present study are the first reported in Southeast Asia. The maximum concentration of BP-4 detected in Bangkok was 95 ng/L, which was 1.5 and 9 times lower than that in rivers in the United Kingdom and Spain (Kasprzyk-Hordern et al., 2008; Rodil et al., 2008), suggesting lower consumption of BP-4 in Bangkok. The median concentration of EHMC (88 ng/L) in Bangkok falls between those measured in heavily and moderately polluted rivers in Japan (266 and 26 ng/L, respectively) receiving industrial and domestic wastewater discharge (Kameda et al., 2011). Only 23% of wastewater in Bangkok is treated in WWTPs using conventional secondary treatment with activated sludge, and thus a high percentage of untreated wastewater is discharged directly to rivers (Tsuzuki et al., 2009), which likely explains the high number of UV filters detected in these samples. Moreover, incomplete removal of UV filters by secondary treatment with activated sludge in WWTPs may also contribute to their high detection (Tsui et al., 2014).

3.5. *Ecological risk assessment*

A preliminary screening of the worst-case scenario was conducted to assess the potential hazards of UV filters to the aquatic environment (Table A9). In the worst case scenario, BP-1 and BP-4 posed low to medium risk to crustaceans based

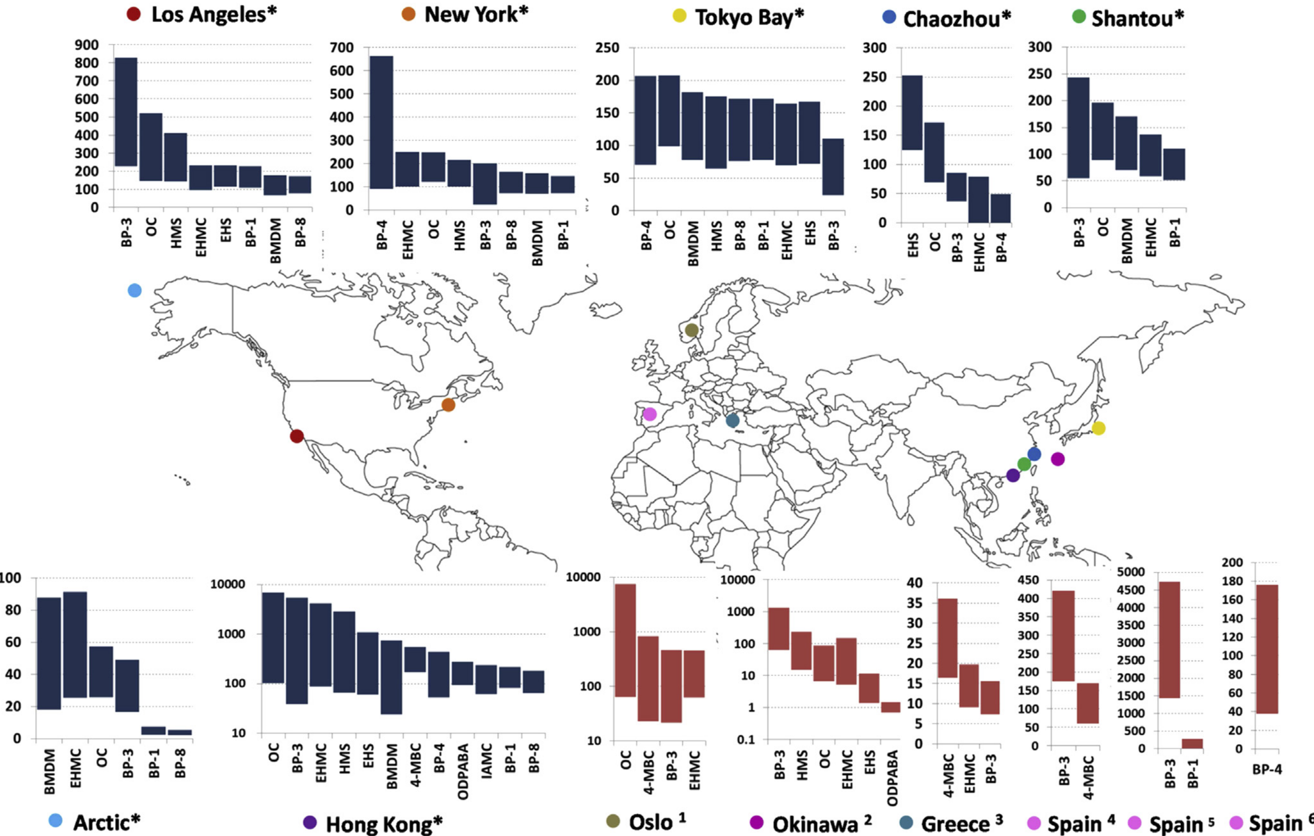

Fig. 2 – Global comparison of UV filters concentration (median-maximum, ng/L) in marine surface waters (*: this study; 1: Langford and Thomas, 2008; 2: Tashiro and Kameda, 2013; 3: Giokas et al., 2005; 4: Tovar-Sánchez et al., 2013; 5: Tarazona et al., 2010; 6: Rodil et al., 2008).

on the endpoints of immobilization and lethality in *Daphnia magna*, as well as in fish based on changes in the expression of endocrine genes in fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) with HQ_{worst} ranging from 0.001 to 0.06 for BP-1 and 0.001 to 0.19 for BP-4 (Sieratowicz et al., 2011; Fent et al., 2010a; Zucchi et al., 2011). The medium risk posed by BP-4 for zebrafish occurred in Hong Kong and New York, with HQ_{worst} at 0.13 and 0.19, respectively. ODPABA posed medium risk (HQ_{worst}: 0.18) to invertebrates based on changes in endocrine-related genes in *Chironomus riparius* as the endpoint (Ozáez et al., 2013).

As some of the HQ_{worst} values for BP-3, EHMC and 4-MBC exceeded 1, probabilistic plots were constructed for their concentrations in surface water samples (Fig. 3a and b, A7). Because of the small number of sampling points in Bangkok, Shantou and Chaozhou, probabilistic risk assessment was not performed for these cities. As a distinct pattern was observed for the concentrations of UV filters in the samples collected at beaches in Hong Kong, these data were considered separately. Detailed information on regression coefficients is shown in Table A10. Multiple threshold values were available for freshwater fish, and all of these were used for the risk assessment to include a range of sensitivities among species (shown as thresholds F_1–F_4 on Fig. 3a and b). The probabilities of 4-MBC causing growth inhibition in algae (based on the inhibitory concentration-10% (IC_{10}) for *Desmodesmus*

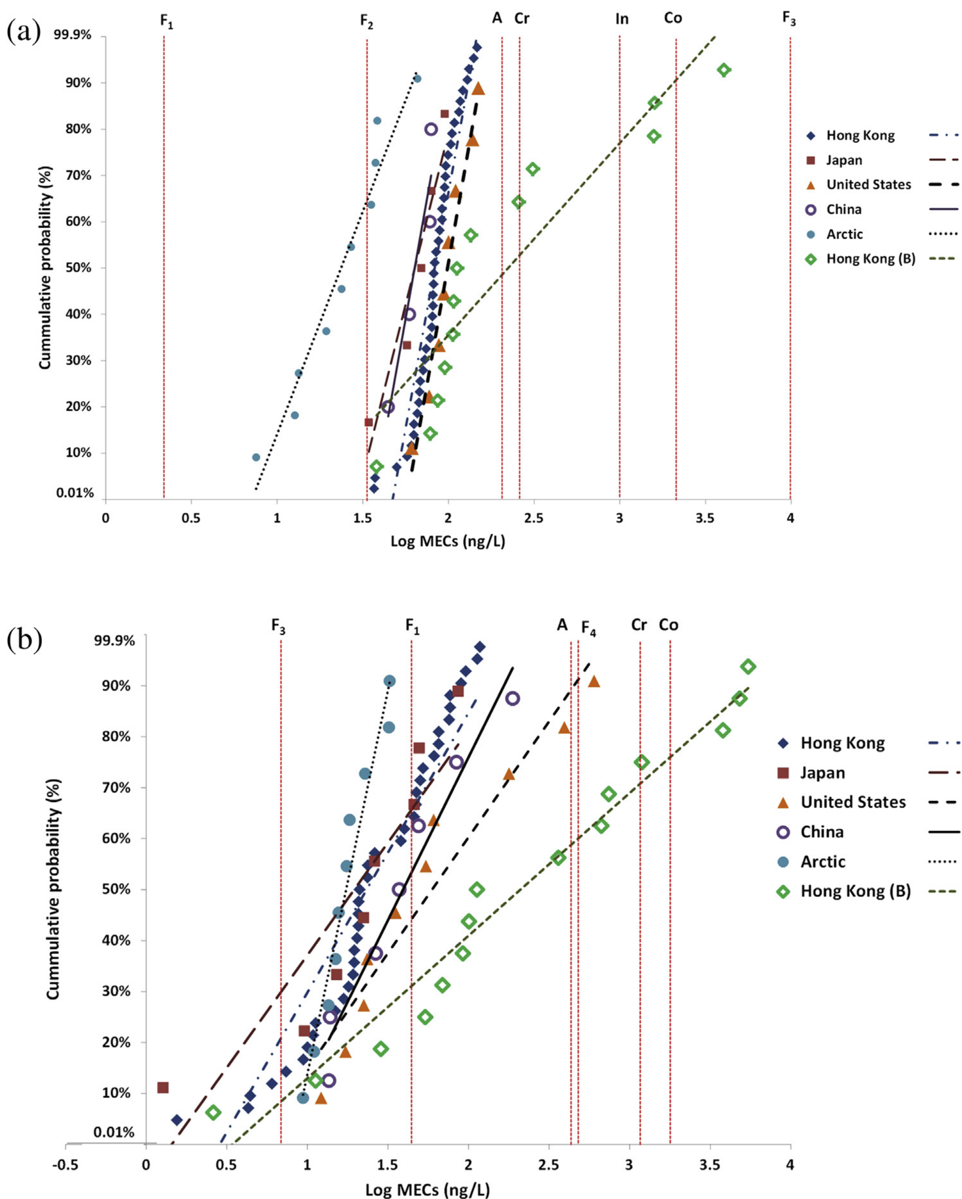

Fig. 3 – a. Probabilistic risk assessment of EHMC in marine surface waters from different locations. "F" thresholds are those derived from toxicity data for different fish species (F_1: Zebrafish; F_2: Fathead minnow; A: Algae; Cr: Crustacean; In: Insect; Co: Coral; F_3: Japanese medaka). Toxicity thresholds and endpoints are given in Table A5. 3b. Probabilistic risk assessment of BP-3 in marine surface water from different locations. "F" thresholds are those derived from toxicity data for different fish species (F_3: Japanese medaka; F_1: Zebrafish; A: Algae; F_4: Rainbow trout; Cr: Crustacean; Co: Coral). Toxicity thresholds and endpoints are given in Table A5.

subspicatus), altering endocrine genes in mosquito larvae (*C. riparius*) and inducing oxidative stress in a protozoan (*Tetrahymena thermophila*) were 34%, 68% and 99.9%, respectively (Gao et al., 2013; Ozáez et al., 2013; Sieratowicz et al., 2011). This result showed that organisms at lower trophic levels were more susceptible to 4-MBC. The probability of risk to fish based on transcriptional changes of endocrine genes in zebrafish was 99.9% for EHMC for all samples with positive detections in all locations, while that based on toxicity in fathead minnow was over 75% in all places except the Arctic, for which the risk was 31%. Moreover, EHMC posed high risk to both cladocerans and algae based on immobilization and growth inhibition as the endpoints (24 and 29%, respectively). For BP-3, the probability of effects on induction of vitellogenin in fish based on data from rainbow trout (*Oncorhynchus mykiss*), egg development in fish based on data from Japanese medaka (*Oryzias latipes*) and induction of oxidative stress in protozoans were over 34%, 50% and 99.9% in all locations, respectively (Coronado et al., 2008; Gao et al., 2013).

It should be noted that the majority of the toxicity values used for the assessment were derived from tests using freshwater organisms, as little information is available in the literature for marine species. The sensitivities of freshwater and saltwater species to different organic contaminants (e.g. pesticides, trace elements) are known to vary, and species sensitivity distributions have been used to understand whether freshwater datasets are protective enough; in some cases, saltwater species have been found to be less sensitive to contaminant effects than freshwater species, though the information available, both in terms of number of species and number of chemicals, is far from comprehensive (Wheeler et al., 2002). Because of the scarcity of information for marine species for UV filters, an inter-species safety factor of 10 was used in this study. Both BP-3 and EHMC posed 21% and 11% risk, respectively, of causing bleaching of hard corals (*Acropora* sp. and *A. pulchra*) at some beaches in Hong Kong located near snorkeling hotspots. It should be noted that these two compounds were detected widely and frequently at high concentrations at the majority of the sampled locations, and therefore their ecological risks and negative impacts should be investigated further.

4. Conclusions

Data on the international distribution and possible negative impacts of organic UV filters in the aquatic environment and the first report of their occurrence in the Arctic have been presented in this study. BP-3 and EHMC showed high detection frequencies at all sampled locations as well as high concentrations in recreational areas; probabilistic risk assessment indicated that these compounds posed various ecological risks to marine ecosystems, including causing coral bleaching and affecting reproduction in fish, though toxicity data for several compounds were not available. The pathways by which organic UV filters are transported to remote Arctic areas remain to be elucidated. These findings indicate that there is a need for greater understanding of the toxicities of these chemicals, both singly and in mixtures, and to consider the current extent of their use, particularly in potentially sensitive ecosystems such as coral reefs.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.09.013.

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2) Occurrence of Some Organic UV Filters in Wastewater, in Surface Waters, and in Fish from Swiss Lakes

Marianne E. Balmer ,* Hans-Rudolf Buser , Markus D. Müller , and Thomas Poiger
Agroscope FAW, Swiss Federal Research Station, CH-8820 Wädenswil, Switzerland
Environ. Sci. Technol., 2005, 39 (4), pp 953–962

Abstract
Organic UV filters are used in personal care products such as sunscreen products, and in cosmetics, beauty creams, skin lotions, lipsticks, hair sprays, hair dyes, shampoos, and so forth. The compounds enter the aquatic environment from showering, wash-off, washing (laundering), and so forth via wastewater treatment plants (WWTPs) ("indirect inputs") and from recreational activities such as swimming and bathing in lakes and rivers ("direct inputs"). In this study, we investigated the occurrence of four important organic UV filter compounds (benzophenone-3, BP-3; 4-methylbenzylidene camphor, 4-MBC; ethylhexyl methoxy cinnamate, EHMC; octocrylene, OC) in wastewater, and in water and fish from various Swiss lakes, using gas chromatographic/mass spectrometric analyses. All four UV filters were present in untreated wastewater (WWTP influent) with a maximum concentration of 19 μg L-1 for EHMC. The data indicate a seasonal variation with influent loads higher in the warmer season (June 2002) than in the colder one (April 2002). The influent loads were in the order EHMC > 4-MBC □ BP-3 > OC. The concentrations in treated wastewater (WWTP effluent) were considerably lower, indicating substantial elimination in the plants. 4-MBC was usually the most prevalent compound (maximum concentration, 2.7 μg L-1), followed by BP-3, EHMC, and OC. UV filters were also detected in Swiss midland lakes and a river (Limmat) receiving inputs from WWTPs and recreational activities. However, all concentrations were low (<2−35 ng L-1); no UV filters (<2 ng L-1) were detected in a remote mountain lake. Data from passive sampling using semipermeable membrane devices (SPMDs) supported the presence of these UV filters in the lakes and the river and suggested some potential for accumulation of these compounds in biota. SPMD-derived water concentra tions increased in the order Greifensee < Zürichsee < Hüttnersee. This order is reversed from that observed for methyl triclosan, used as a chemical marker for WWTP-derived lipophilic contaminants in the lakes. This indicated inputs of UV filters from sources other than WWTPs to the lakes during summer, for example, inputs from recreational activities. Fish (white fish, Coregonus sp.; roach, Rutilus rutilus; perch, Perca fluviatilis) from these lakes contained low but detectable concentrations of UV filters, in particular, 4-MBC (up to 166 ng g-1 on a lipid basis). 4-MBC concentrations relative to methyl triclosan were lower in fish than in SPMDs exposed in the same lakes, suggesting that 4-MBC is less bioaccumulated than expected or metabolized in fish. The lipid-based bioconcentration factor (BCFL) estimated from the fish (roach) data and SPMD-derived water concentrations was about 1−2.3 × 104 and thus approximately 1 order of magnitude lower than expected from its Kow value.

3) Mar Pollut Bull. 2013 Dec 15;77(1-2):333-40. doi: 10.1016/j.marpolbul.2013.09.013.

Concentration of organic sun-blocking agents in seawater of beaches and coral reefs of Okinawa Island, Japan.

Tashiro Y[1], Kameda Y.
Author information
1 School of International Studies, Meio University, 1220-1, Bimata, Nago, Okinawa 905-8585, Japan. Electronic address: tashiro@meio-u.ac.jp.

Abstract
The concentration of UV filters (UVFs) and UV light stabilizers (UVLSs) were measured in seawater and river water collected from sites at four beaches, two reefs, and one river on Okinawa Island, Japan. UVFs and/or UVLSs of 8-10 types were detected in beaches samples and 6-9 types were detected in reef samples. The total UVF concentrations at the beach sites were highest either in July or August with a maximum of 1.4 μg L(-1). The concentrations at the reef sites did not show peaks in summer and the maximum values were close to 10 ng L(-1). The detected UVF profiles reflected the ingredients of sunscreens used in each region. The highest UVLS concentrations at the reefs were observed not only in summer but also in June and September. The UVLS concentrations at the reefs were similar to or even higher than that at the beaches or in the river.

Oxybenzone HEL Monograph - 2 of 7

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4 attachments (7 MB)

4 Downs_et_al 2015 Arch_Env_Contam_Tox__SS.pdf; 5 Sang Environmental occurrence and ecological risk.pdf; 6 Ekowati Occurrence of pharmaceuticals and UV filters in swimming pools and spas.docx; 7 Diaz Cruz Analytical and Bioanalytical Chemistry.docx;

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,
Craig Downs – Executive Director – Haereticus Environmental Laboratory
Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:
- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.
- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.
- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:
1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone
2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

Arch Environ Contam Toxicol
DOI 10.1007/s00244-015-0227-7

Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands

C. A. Downs[1] · Esti Kramarsky-Winter[2,3] · Roee Segal[2] · John Fauth[4] · Sean Knutson[5] · Omri Bronstein[2] · Frederic R. Ciner[1] · Rina Jeger[3] · Yona Lichtenfeld[6] · Cheryl M. Woodley[7,8] · Paul Pennington[8] · Kelli Cadenas[9] · Ariel Kushmaro[3] · Yossi Loya[2]

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Abstract Benzophenone-3 (BP-3; oxybenzone) is an ingredient in sunscreen lotions and personal-care products that protects against the damaging effects of ultraviolet light. Oxybenzone is an emerging contaminant of concern in marine environments—produced by swimmers and municipal, residential, and boat/ship wastewater discharges. We examined the effects of oxybenzone on the larval form (planula) of the coral *Stylophora pistillata,* as well as its toxicity in vitro to coral cells from this and six other coral species. Oxybenzone is a photo-toxicant; adverse effects are exacerbated in the light. Whether in darkness or light, oxybenzone transformed planulae from a motile state to a deformed, sessile condition. Planulae exhibited an increasing rate of coral bleaching in response to increasing concentrations of oxybenzone. Oxybenzone is a genotoxicant to corals, exhibiting a positive relationship between DNA-AP lesions and increasing oxybenzone concentrations. Oxybenzone is a skeletal endocrine disruptor; it induced ossification of the planula, encasing the entire planula in its own skeleton. The LC_{50} of planulae exposed to oxybenzone in the light for an 8- and 24-h exposure was 3.1 mg/L and 139 µg/L, respectively. The LC_{50}s for oxybenzone in darkness for the same time points were 16.8 mg/L and 779 µg/L. Deformity EC_{20} levels (24 h) of planulae exposed to oxybenzone were 6.5 µg/L in the light and 10 µg/L in darkness. Coral cell LC_{50}s (4 h, in the light) for 7 different coral species ranges from 8 to 340 µg/L, whereas LC_{20}s (4 h, in the light) for the same species ranges from 0.062 to 8 µg/L. Coral reef

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✉ C. A. Downs
cadowns@haereticus-lab.org

[1] Haereticus Environmental Laboratory, P.O. Box 92, Clifford, VA 24533, USA

[2] Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

[3] Avram and Stella Goldstein-Goren Department of Biotechnology Engineering and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, 84105 Beer Sheva, Israel

[4] Department of Biology, University of Central Florida, 4000 Central Florida Boulevard, Orlando, FL 32816-2368, USA

[5] Pacific Biosciences Research Center, University of Hawaii, Honolulu, HI 96822, USA

[6] Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel

[7] Hollings Marine Laboratory, U.S. National Oceanic & Atmospheric Administration, 331 Ft. Johnson Rd., Charleston, SC 29412, USA

[8] Center for Coastal Environmental Health and Biomolecular Research, U.S. National Oceanic & Atmospheric Administration, 219 Ft. Johnson Rd., Charleston, SC 29412, USA

[9] National Aquarium, 501 East Pratt Street, Baltimore, MD 21202, USA

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contamination of oxybenzone in the U.S. Virgin Islands ranged from 75 μg/L to 1.4 mg/L, whereas Hawaiian sites were contaminated between 0.8 and 19.2 μg/L. Oxybenzone poses a hazard to coral reef conservation and threatens the resiliency of coral reefs to climate change.

Oxybenzone (BP-3; benzophenone-3; 2-hydroxy-4-methoxphenyl phenylmethanone; CAS No. 131-57-7) often is used as an active ingredient in sunscreen lotions and personal-care products, such as body fragrances, hair-styling products, shampoos and conditioners, anti-aging creams, lip balms, mascaras, insect repellants, as well as dishwasher soaps, dish soaps, hand soaps, and bath oils/salts (CIR 2005; http://www.goodguide.com/ingredients/184390-oxybenzone). BP-3 and other benzophenone derivatives often are found as contaminants in boating, residential, and municipal wastewater effluents and are considered "emerging environmental contaminants of concern" by the U.S. Environmental Protection Agency (Eichenseher 2006; Richardson 2006, 2007; Blitz and Norton 2008; Gago-Ferrero et al. 2011; Kameda et al. 2011; Rodil et al. 2012; Aquero et al. 2013).

Between 6000 and 14,000 tons of sunscreen lotion, many of which contain between 1 and 10 % BP-3, are estimated to be released into coral reef areas each year, putting at least 10 % of the global reefs at risk of exposure, and approximately 40 % of coral reefs located along coastal areas at risk of exposure (Shaath and Shaath 2005; UNWTO 2007; Danovaro et al. 2008; Wilkinson 2008). In Okinawa, BP-3 levels on coral reefs that were 300–600 m away from public swimming beaches ranged from 0.4 to 3.8 pptrillion (Tashiro and Kameda 2013); in South America, sediments near coral communities/reefs contained BP-3 concentrations between 54 and 578 pptrillion (Baron et al. 2013). Schlenk et al. (2005) discovered through a Toxicity Identification Evaluation that BP-3 was unequivocally identified as the source of estrogenic activity in marine sediments near wastewater outfalls. Although the half-life in seawater is several months, BP-3 can act as a pseudo-persistent pollutant; its contamination of a site may be constantly renewed, resulting in ecological receptors experiencing persistent exposure (Vione et al. 2013). Concerns regarding the adverse impacts of exposure to BP-3 on coral reefs and other marine/aquatic ecosystems have led to either banning oxybenzone-containing products in marine-managed areas (e.g. Mexico's marine ecoparks; Xcaret 2007; Xel-há 2007) or public relations campaigns by management agencies to encourage reduction of environmental contamination of sunscreen lotions by swimmers (e.g. "Protect Yourself, Protect the Reef" Bulletin U.S. NPS 2012).

BP-3 exhibits a number of toxicological behaviors ranging from the molecular level to multi-organ system pathologies (Gilbert et al. 2012). Benzophenones, including BP-3, are documented mutagens that increase the rate of damage to DNA, especially when exposed to sunlight (Popkin and Prival 1985; Zeiger et al. 1987; Knowland et al. 1993; NTP 2006). BP-3 produced a positive mutagenic response by inducing the *umu* operon (genotoxicity assay Nakajima et al. 2006). Benzophenones, and especially BP-3, either can act directly as genotoxicants or become genotoxicants by bioactivation via cytochrome P450 enzymes (Takemoto et al. 2002; Zhao et al. 2013). The types of damage to genetic material by benzophenones include oxidative damage to DNA, formation of cyclobutane pyrimidinic dimers, single-strand DNA breaks, cross-linking of DNA to proteins, and an increase in the formation of DNA abasic sites (Cuquerella et al. 2012). Benzophenones also exhibit pro-carcinogenic activities (Kerdivel et al. 2013). BP-3 can generate reactive oxygen species, which are potential mutagens, when applied topically to the skin followed by UV light exposure (Hanson et al. 2006).

BP-3 is a reproductive toxicant whose mechanisms of action and its pathological effects are poorly characterized in various model species. In mice studies, BP-3 exposure significantly affected fecundity, as well as inducing unexplained mortality in lactating mothers (Gulati and Mounce 1997). Studies in both mice and rats demonstrated that generational exposure to BP-3 reduced body weight, increased liver (>50 %) and kidney weights, induced a 30 % increase in prostate weight, a reduction in immunocompetence, and significantly increased uterine weight in juveniles (Gulati and Mounce 1997; French 1992; Schlumpf et al. 2008; Rachon et al. 2006). In mammals, BP-3 is renowned for having estrogenic and anti-androgenic activities, causing activation of estrogen receptor proteins and inhibition of androgen receptors (Morohoshi et al. 2005; Suzuki et al. 2005; Kunz et al. 2006; Molina–Molina et al. 2008; Nashez et al. 2010). Topical application of BP-3 to the skin has been shown to be absorbed and transferred to breast milk, creating risk to breast-fed neonates (Hany and Nagel 1995). In addition, an association between exposure to benzophenones and an increased occurrence of endometriosis in women was recently found by Kunisue et al. (2012).

In fish, BP-3 actions are similar to those in mammals, causing an endocrine disruption by modulating estrogen receptor signaling pathways, inducing reproductive pathologies, and reducing reproductive fitness (Kunz et al. 2006; Coronado et al. 2008; Cosnefroy et al. 2011; Bluthgen et al. 2012). Chronic exposure to BP-3 in fish resulted in reduced egg production, induction of vitellogenin protein in males, and a significant reduction in egg hatchings (Nimrod and Benson 1998; Coronado et al. 2008). These findings raise the possibility of "gender shifts" in fish exposed to BP-3 during the entirety of their

life history or during "windows of sensitivity" (Coronado et al. 2008).

A few studies exist that have evaluated the effects of BP-3 exposure in invertebrates. In insects, BP-3 inhibited expression of the *usp* gene (ultraspiracle protein)—a protein that combines with the EcR protein to form the ecdysone receptor, which controls aspects of developmental and reproductive processes (Ozáez et al. 2013). Gao et al. (2013) found that BP-3 exposure resulted in oxidative injuries, reduced glutathione, and adversely affected cell viability in the protozoan ciliate, *Tetrahymena thermophila.*

Since the 1970s, coral reefs have been devastated on a global scale. Regional weather and climate events often are responsible for acute events of mass-mortality of coral reefs (Carpenter et al. 2008). However, the long-term causative processes of sustained demise often are locality specific (Edinger et al. 1998; Rees et al. 1999; Golbuu et al. 2008; Smith et al. 2008; Downs et al. 2011, 2012; Omori 2011). Records of coral recruitment in many areas of the Caribbean, Persian Gulf, Red Sea, Hawaiian Islands, and elsewhere have exhibited precipitous declines (Richmond 1993, 1997; Hughes and Tanner 2000; Rogers and Miller 2006; Williams et al. 2008). This is most apparent in the deterioration of juvenile coral recruitment and survival rates along coastal areas (Dustan 1977; Miller et al. 2000; Abelson et al. 2005; Williams et al. 2008). As with other invertebrate species, coral larvae (i.e., planula) and newly settled coral (i.e., recruits) are much more sensitive to the toxicological effects of pollution compared with adults (Kushmaro et al. 1997). Hence, even small impacts to larval development and survival can have significant effects on coral demographics and community structure (Richmond 1993, 1997). To manage BP-3 pollution and mitigate its effect on the ecological resilience of coral reefs, the toxicological effects of BP-3 on larval survival and development need to be characterized (Fent et al. 2010; US EPA 2012; NRC 2013).

In this study, we examined the toxicological effects of exposures to varying concentrations of BP-3 on the larval form (planula) of the scleractinian coral *Stylophora pistillata,* the most abundant coral species in the northern Gulf of Aqaba, Red Sea (Loya 1972). Many chemical pollutants affect organisms differently when exposed to light, a process known as chemical-associated phototoxicity (Yu 2002; Platt et al. 2008). Because reef-building corals are photosynthetic symbiotic organisms, and many coral species have planulae that are photosynthetically symbiotic (e.g., *S. pistillata*), we examined the effects of BP-3 exposure in planulae subjected to either darkness or to environmentally-relevant light conditions. Histopathology and cellular pathology, planula morphology, coral bleaching, DNA damage as the formation of DNA abasic lesions, and planula mortality were measured in response to BP-3 exposure. Median lethal concentration (LC_{50}), effect concentration (EC_{20}), and no observable effect concentrations (NOEC) were determined for coral planulae exposed to BP-3 in both darkness and in light. Coral planulae are a relatively difficult resource to procure for toxicological studies. Therefore, primary coral cell cultures were used in in vitro toxicological tests of BP-3 to examine their validity as a surrogate model for coral planulae in generating an effect characterization as part of an Ecological Risk Assessment. The confidence in this model was examined by comparisons of the LC_{50} results of BP-3-exposed planulae to the BP-3 LC_{50} of coral cells (calicoblasts) from adult *S. pistillata* colonies. Coral-cell toxicity testing was conducted on six other species that originate from either the Indo-Pacific or Caribbean Sea/Atlantic Ocean basins to provide in vitro data on the species' sensitivity distribution of BP-3. To determine the environmentally relevant concentration of BP-3 in seawater on coral reefs, we measured BP-3 concentrations at various locations in the U.S. Virgin Islands and the U.S. Hawaiian Islands.

Materials and Methods

Planula Collection and Toxicity Exposures

Planula collection and planula-toxicity exposures were conducted at the Inter-University Institute of Marine Sciences (IUI) in Eilat, Israel. *Stylophora pistillata* (Esper 1797) planulae were collected from the wild within the IUI designated research area by placing positively buoyant planula traps over *Stylophora* colonies measuring more than 25 cm in diameter. Permit for collection was given to Y. Loya by the Israel National Park Authority. Traps were set between 17:00 and 18:00 h, and then retrieved at 06:00 h the next morning. Planulae were inspected and sorted by 07:15 h, and toxicity exposure experiments began at 08:00 h.

Experimental design and culture conditions were based on modified (for coral) guidelines set forth in OECD (2013) and described in Downs et al. (2014). This experiment for BP-3 was conducted concurrently with the study conducted in Downs et al. (2014).

All seawater (ASW) was made artificially using Fisher Scientific Environmental-Grade water (cat#W11-4) and Sigma-Aldrich sea salts (cat#S9883) to a salinity of 38 parts per thousand at 22 °C. Benzophenone-3 (BP-3; 2-Hydroxy-4-methoxyphenyl-phenylmethanone; Aldrich cat#T16403) was solubilized in dimethyl sulfoxide (DMSO) and then diluted with ASW to generate stock solutions and exposure solutions. Solutions of BP-3 for toxicity exposures each contained 5 microliters of DMSO

per one liter and were of the following concentrations: 1 mM BP-3 (228 parts per million), 0.1 mM BP-3 (22.8 mg/L; parts per million), 0.01 mM BP-3 (2.28 mg/L; parts per million), 0.001 mM BP-3 (228 μg/L; parts per billion), 0.0001 mM (22.8 μg/L; parts per billion), and 0.00001 mM (2.28 μg/L; parts per billion). For every exposure time-period, there were two control treatments with four replicates each: (a) planulae in ASW, and (b) planulae in ASW with 5 microliters of DMSO per 1 L. There was no statistical difference between the two controls for any of the assays.

Planulae were exposed to different BP-3 concentrations during four different time-period scenarios: (a) 8 h in the light, (b) 8 h in the dark, (c) a full diurnal cycle of 24 h, beginning at 08:00 in daylight and darkness from 18:00 in the evening until 08:00 h the next day, and (d) a full 24 h in darkness. For the 24-h exposure, planulae from all treatments were transferred to new 24-well microplates with fresh ASW/BP-3 media at the end of the 8-h daylight exposure before the beginning of the 16 h dark exposure.

At the end of the 8 and 24-h time points, chlorophyll fluorescence, morphology, planula ciliary movement, and mortality were measured, while at least one planula from each replicate of each treatment was chemically preserved, and the remaining living planulae were flash frozen in liquid nitrogen for the DNA apyrimidinic (AP) site assay.

Chlorophyll Fluorescence as an Estimate of Bleaching

Chlorophyll fluorescence was measured using a Molecular Dynamics microplate fluorometer with an excitation wavelength of 445 nm and an emission wavelength of 685 nm. Fluorescence measurements were taken at the end of the 8-h light and dark periods of BP-3 exposure. All ten planulae in each replicate well were measured in aggregate. Each well was measured independently of the other wells. Justification and caveats for this assay are described in Downs et al. (2014).

DNA Abasic Lesions

DNA abasic or apurinic/apyrimidinic lesions (DNA AP sites) were quantified using the Dojindo DNA Damage Quantification Kit-AP Site Counting (DK-02-10; Dojindo Molecular Technologies, Inc.) and conducted as described in Downs et al. (2014). Four individual planulae (one from each well) from each treatment were individually assayed. Only planulae that were relatively intact were assayed, even if scored as dead. Planulae from 228 ppm BP-3 at 8 h in the light were not collected, because there were no coherent planulae.

Transmission Electron Microscopy

Transmission electron microscopy was used for tissue and cellular pathomorphology assessment on three planulae from each treatment. Methodology for this technique was described in Downs et al. (2014). At least three planula from each treatment were collected and fixed for analysis.

Coral Cell Toxicity Assay

Cultured colonies of *S. pistillata* (Esper 1797) were obtained from Exotic Reef Imports (www.exoticreefimports.com) and did not need a permit for possession. Cultured colonies of *Pocillopora damicornis* (Linnaeus 1758) was provided by the National Aquarium and did not need a permit for possession. *Montastrea annularis*, *Montastrea cavernosa* (Linnaeus 1766), and *Porites astreoides* (Lamarck 1816) were obtained from the Florida Keys National Marine Sanctuary under permit# FKNMS-2011-139. Cultured colonies of *Acropora cervicornis* (Lamarck 1816) and *Porites divaricata* (Lesueur 1821) were provided by Dr. Cheryl Woodley of the U.S. National Oceanic and Atmospheric Administration and did not need a permit for possession. Corals were maintained in glass and Teflon-plumbed aquaria in 36 ppt salinity artificial seawater (Type 1 water using a Barnstead E-Pure filter system that included activated carbon filters) at a temperature of 24 °C. Corals were grown under custom LED lighting with a peak radiance of 288 photosynthetic photon flux density μmol/m^2/s. Light Spectra ranged from 380 to 740 nm. Light was measured using a Licor 250A light meter and planar incidence sensor. Description of coral cell isolation from each species is described in Downs et al. (2010, 2014).

Exposure experiments of cells were conducted in PTFE-Telfon microplates. Cells of all species except *Acropora cervicornis* were exposed to BP-3 concentrations in cell culture media of 570 parts per trillion to 228 parts per million for 4 h in the light, whereas *Stylophora* cells also were exposed for 4 h in the dark. *Acropora cervicornis* cells were exposed to BP-3 concentrations in cell culture media of 570 ng/L (parts per trillion) to 228 mg/L (parts per million) for 4 h in the light. Lighting was from custom LED fixtures that had wavelength emissions from 390 to 720 nm with a light intensity of 295 μmol/m^2/s of photon flux density.

Viability was confirmed using the trypan blue exclusion assay. There were four replicate wells with cells per treatment. Duplicate aliquots of cells from each replicated wells were collected into a microcentrifuge tube, centrifuged at $300 \times g$ for 5 min, and the supernatant aspirated. Cells were gently resuspended in culture media that contained 0.5–1.5 % (w/v) of filtered trypan blue (Sigma-

Aldrich, cat#T6146), and incubated for 5 min. Viable versus dead cells were counted using a modified Neubauer hemocytometer (Hausser-Levy Counting Chamber).

Sampling and analysis of benzophenones in seawater samples via gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS). Dichloromethane, methanol, acetone are pesticide-grade solvents (Fisher Scientific). Analytical standards were purchased from Sigma Aldrich and included: Benzophenone (cat# B9300), Benzhydrol (cat#B4856), 4-hydroxybenzophenone (cat#H20202), 2-hydroxy-4-methoxy benzophenone (cat#H36206), 2,4-dihydroxy benzophenone (cat# 126217), 2-2′-dihydroxy-4-methoxy benzophenone (cat# 323578), 2,3,4-trihydroxy benzophenone (cat# 260576), 2,2′,4,4′-tetrahydroxy benzophenone (cat#T16403). Internal standard solutions (phenanthrene-d10 and chrysene-d12) were purchased from AccuStandard Inc. (New Haven, CT).

Field personnel collecting samples were subject to an Alconox Liqui-Nox detergent decontamination immediately before entering the sampling site and did not apply any sunscreen lotion or nonorganic personal-care products to their body for at least 21 days before sampling. Between 100 and 500 mL of seawater were collected approximately 35 cm below the surface of the water into EPA-certified clean, amber jars. In the field, water samples were extracted using Phenomenex C18 solid phase extractions columns that were first activated with methanol. All columns were capped and then shipped and stored frozen at −80 °C or colder.

Extraction of analytes from seawater samples collected in the U.S. Virgin Islands (under a U.S. National Park Service permit, STT-045-08) followed the methodology described in Jeon et al. (2006). Seawater samples were collected using precleaned 1-L amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Seawater samples were extracted using C-18E cartridges (500 mg, 6 mL Phenomenex Inc.) on a vacuum manifold (Phenomenex Inc.). Cartridges were conditioned with 5 mL of methanol and then 5 mL of water, after which the seawater samples were then added to the column. Following extraction, the cartridges were dried for 10 min, capped, and frozen until processed. The cartridges were eluted with 2 mL of acetone followed by 2 × 5 mL dichloromethane. The extracts were evaporated to dryness under a gentle stream of nitrogen. Then, 50 µL of MSTFA (*N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide, Sigma-Aldrich) was added, capped, vortexed for 30 s, and heated at 80 °C for 30 min. Extracts were transferred to gas chromatography vials with a rinse step to a final volume of 1 mL and the internal standard was added. Percentage recovery for all 8 target analytes using this method with seawater was >95 %.

Seawater samples from Hawaii were collected using precleaned one liter amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Samples were extracted using C-18E cartridges (500 mg, 6 mL Phenomenex Inc.) on a vacuum manifold (Phenomenex Inc.). Cartridges were conditioned as indicated in the previous paragraph and eluted with 5 mL of methanol. For LC–MS analysis, samples were run on an AB_SCIEX 5500 QTRAP Triple Quadrupole Hybrid Linear Ion Trap Mass Spectrometer with a Spark Holland Symbiosis HPLC for analytical separation. The analytes were measured with MRM (multiple reaction monitoring) followed by switching to ion trap functionality (Q3- LIT) to confirm the fragmentation pattern of the MRMs. The source was set at 700 °C and the gasses were set to 60 arbitrary units of nitrogen. The curtain gas was set at 45 arbitrary units, and all MRMs were optimized using infusion based introduction of analytical standards. Analytical separation was performed using a Phenomenex Hydro RP 4.6 × 50 2.6 µm particle size stationary phase, with the mobile phase composed of methanol and water with the addition of 0.1 % formic acid and 5 mM of ammonium acetate in both phases. The flow rate was set at 0.9 mL per min, and a ballistic gradient and re-equilibration was run over 5 min. Percentage recovery for target analytes was >85 %, Limit of Detection was 100 pptrillion, and Quantitative Limit of Measurement was 5 ppbillion (µg/L).

Statistical Methods

OECD (2006) was used as a guidance document for our approach in the statistical analysis of the data. To address different philosophies and regulatory criteria, Effect Concentration response (EC_{20} and EC_{50}) and median Lethal Concentration response (LC_{50}) were determined using three initial methods: PROBIT analysis (Finney 1947), linear or quadratic regression (Draper and Smith 1966), and spline fitting (Scholze et al. 2001). Data were analyzed using linear or quadratic regression and PROBIT methods individually for each experiment, based on model residuals being random, normally distributed, and independent of dosing concentrations (Crawley 1993, Fig. 5.1), as well as having good fit, statistically significant, and biologically interpretable regressors (Agresti 2002; Newman 2013). Spline fitting did not meet these criteria. In several analyses, BP-3 concentrations as $\log_{10}(x + 1)$ were transformed to conform to model assumptions.

Data were tested for normality (Shapiro–Wilk test) and equal variance. When data did not meet the assumption of normality and homogeneity, the no-observed-effect concentration (NOEC) was determined using Kruskal–Wallis one-way analysis of variance, using Dunnett's Procedure (Zar 1996) to identify concentrations whose means differed

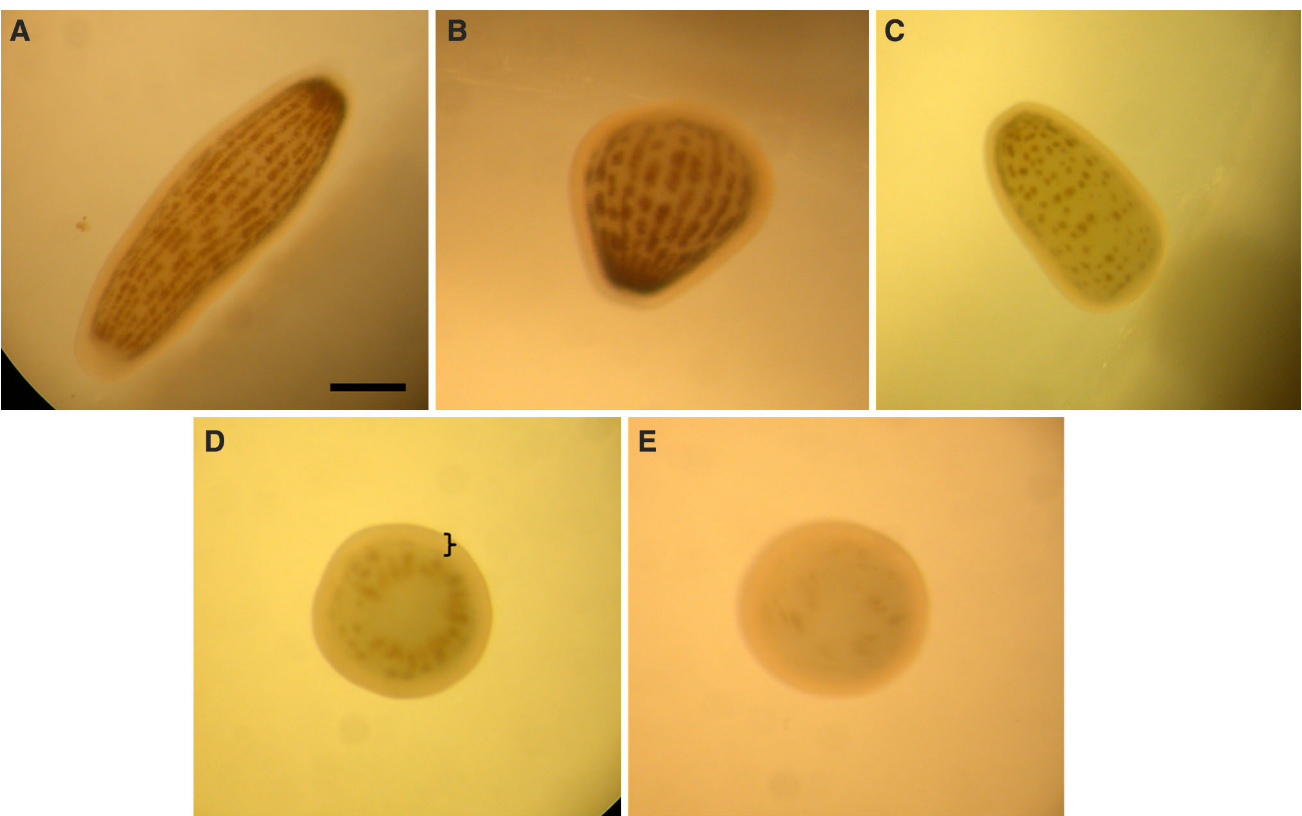


Fig. 1 *Stylophora pistillata* planulae exposed to various treatments of benzophenone-3 (BP-3). **a** Control planula exposed for 8 h in light. **b** Planula exposed to 22.8 parts per billion (µg/L) BP-3 for 8 h in the light. **c** Planula exposed to 228 parts per billion (µg/L) BP-3 for 8 h in the light. **d** Planula exposed to 2.28 parts per million (mg/L) BP3 for 8 h in the light. **e** Planula exposed to 28.8 parts per million (mg/L) BP3 for 8 h in the light. *Scale bar* is 0.5 mm

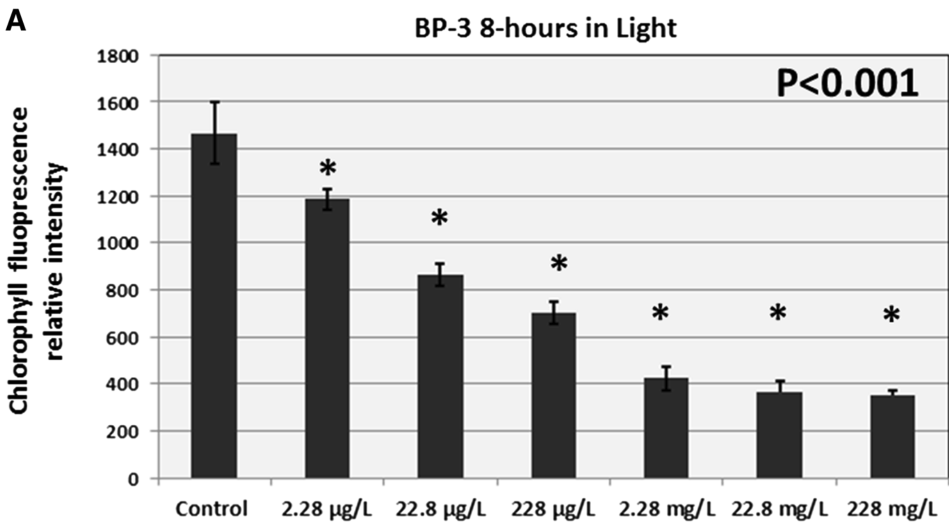
significantly from the control (Newman 2013). When variances among treatments were heterogeneous, we verified these results using a Welch ANOVA. In cases where responses were homogeneous within the control treatment (i.e., all planulae survived) or another concentration (i.e., all planulae died or were deformed), the Steel Method (Steel 1959) was substituted, which is the nonparametric counterpart to Dunnett's Procedure (Newman 2013). Four replicates of each experimental concentration provided good statistical power for parametric analyses, but it is cautioned that the relatively small sample size for the nonparametric Steel Method (Steel 1959) made results of this test less powerful. To facilitate comparisons among other treatment means, figure legends include results of Newman–Keuls Method post hoc test, which compares each concentration to all others.

Parametric (Pearson's r) or nonparametric (Spearman's ρ) regression analyses were used to determine the relationship between mortality of coral planulae and coral cells. Coral planulae are available only immediately after spawning and a strong association between these two responses would allow mortality of coral cells to serve as a surrogate for this reproductive response. JMP version 9.0 or 10.0 (SAS Institute, Inc., Cary, NC), SAS version 9.3 and SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA) were used for analyses.

Results

Toxicopathology

Planulae under control conditions have an elongated, "cucumber-like" morphology with organized rows of zooxanthellae-containing gastrodermal cells running from the aboral pole to the oral pole (Fig. 1a; "brown dots" in the rows are individual zooxanthella cells). Normal planulae are in near-constant motion, being propelled by cilia that cover the elongated body. Within the first 4 h of exposure of planulae to BP-3 in both light and darkness, planulae showed a significant reduction in ciliary movement and the morphology had significantly changed from the elongated form to a deformed "dewdrop" (Fig. 1b). At 228 µg/L BP-3, planulae contain noticeably less zooxanthellae (brown spots) indicative of "bleaching" (Fig. 1c). The mouth of the planula at the oral pole began to increase three- to fivefold in diameter at the end of the 8-h exposure



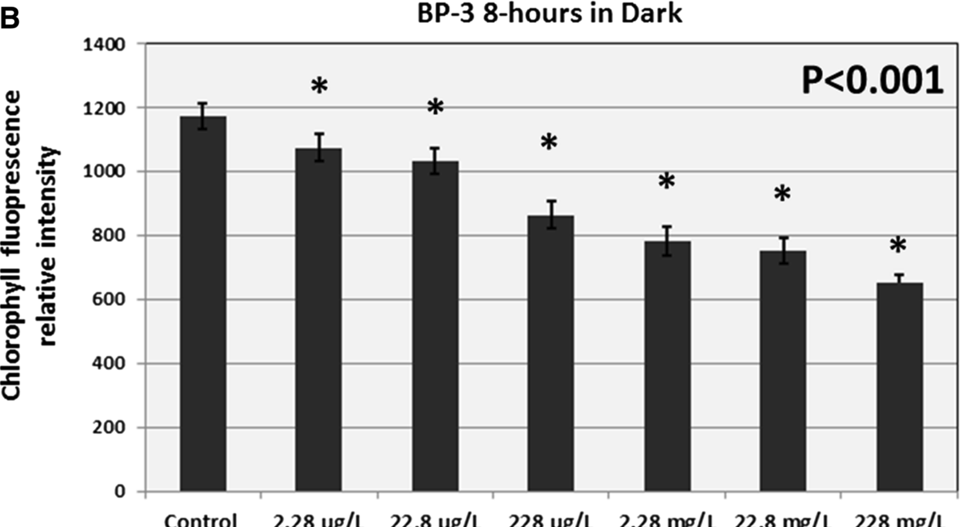


Fig. 2 Relative chlorophyll fluorescence emission at 685 nm with excitation at 445 nm of planulae of *Stylophora pistillata* exposed to various treatments of benzophenone-3 (BP-3). *Bars* show treatment means with whiskers representing ±1 standard error of the mean. $N = 4$ replicates per treatment. **a** Planulae exposed to various BP-3 concentrations for 8 h in the light. Treatment means with *different letters* differed significantly from the control at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Dunnett's Method post hoc test against a control. **b** Planulae exposed to various BP-3 concentrations for 8 h in the dark. Treatment means with different *superscript letters* differed significantly from the control at $\alpha = 0.05$, based on one-way analysis of variance followed by a Dunnett's Method post hoc test against control

(Fig. 1d). By the end of the 8 h of exposure for all BP-3 concentrations, the oral pole was recessed into the body in deformed planulae (Fig. 1b) and the epidermis of all the deformed planulae took on a white opaque hue. For planulae exposed to the higher concentrations of BP-3, it was apparent that the epidermal layer had lost its typical transparency and become opaque (Fig. 1, bracket indicates opaqueness of epidermal layer).

At the end of the 8-h exposure, all planulae exposed to all of the concentrations of BP-3 became sessile. Additionally, there was a positive relationship between exposure to increasing concentrations of BP-3 and planula bleaching (Figs. 1a–e, 2). Bleaching is the loss of symbiotic dinoflagellate zooxanthellae, photosynthetic pigments, or both. Chlorophyll fluorescence as an indicator of the concentration of chlorophyll *a* pigment corroborated these visual observations; exposure to BP-3, whether in light or darkness, caused planulae to bleach (Fig. 2). The Lowest Observable Effect Concentration for inducing chlorophyll-defined bleaching is 2.28 µg/L in the light ($P < 0.001$, Dunnett's Method) and 22.8 µg/L in the dark ($P < 0.01$, Dunnett's Method).

Normal planulae have four layers of organization. At the surface of the planula is the epidermis (Fig. 3a–c). The outer aspect of the epidermis has densely packed ciliated cells (Fig. 3a), spirocysts and nematocysts/blasts (Fig. 3b), and cells containing chromogenic organelles. Between the epidermis and the gastrodermal tissue layers is the mesoglea (Fig. 3c–d). Within the gastrodermal tissue are cells that contain symbiotic dinoflagellate zooxanthellae within an intracellular vacuole (Fig. 3e). Figure 3e depicts a healthy morphology, with the presence of starch granules, coherent chloroplasts, and the presence of a pyrenoid body that interfaces with chloroplasts. Figure 3f illustrates the integrity of chloroplasts (cp) within the dinoflagellate, especially the structure of the tri-partite rows of the

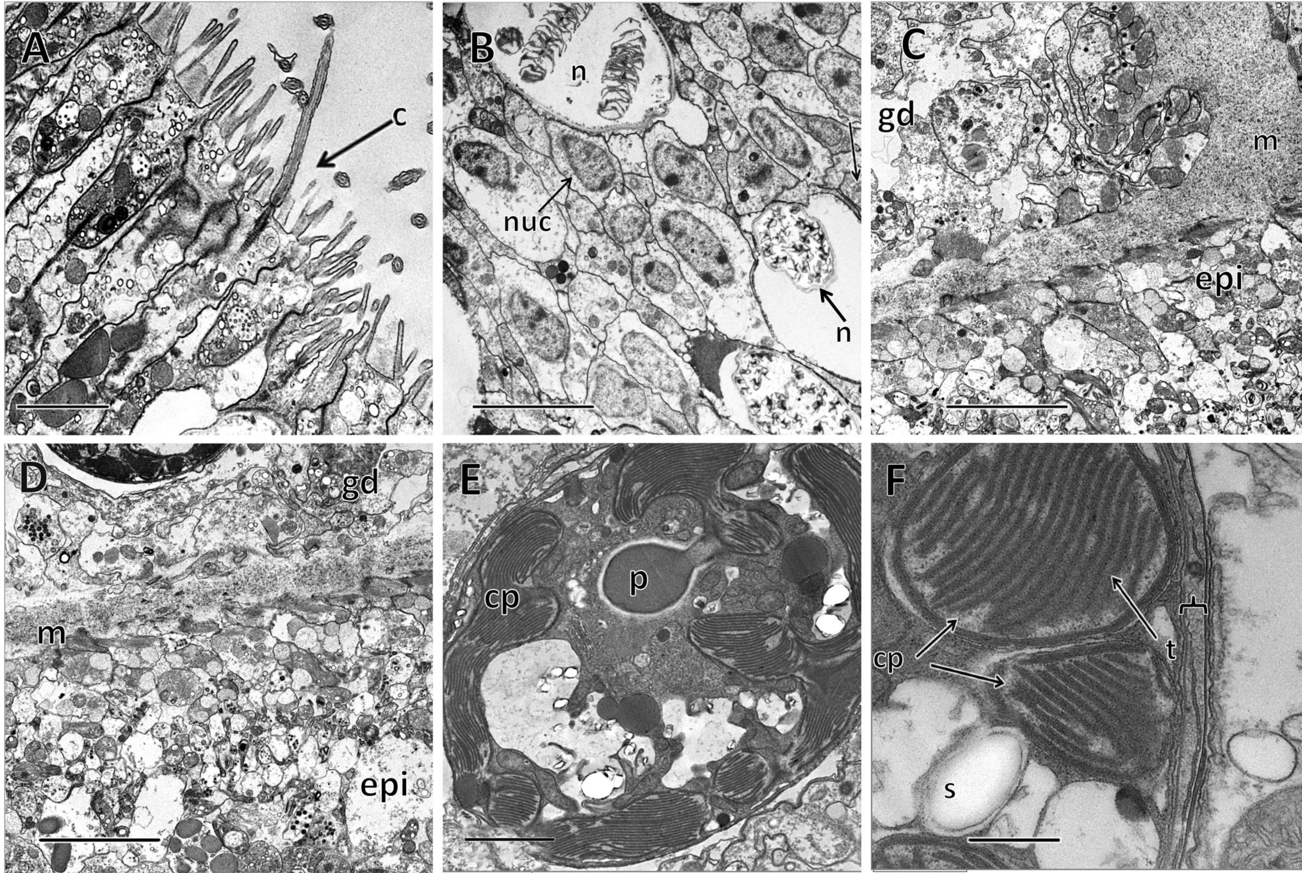


Fig. 3 Transmission electron microscopy of *Stylophora pistillata* planula control treatment. **a** Epidermal surface, indicating the presence of functional cilia (c) and tightly adjoined epidermal cells; *bar* indicates 2000 nm. **b** Epidermal surface indicates intact nematocysts (n) and nuclei (nuc); *bar* indicates 5000 nm. **c** Mesoglea (m) demarks the epidermal tissue (epi) from the gastrodermal tissue (gd); *bar* indicates 5000 nm. **d** Micrograph indicates the interface of the gastroderm (g), mesoglea (m), and epidermis (epi); *bar* indicates 5000 nm. **e** Zooxanthella in the gastrodermal tissue of planula, indicating the presence of intact chloroplasts (cp) and pyrenoid body (p). Notice the absence of a vacuolar space between the coral vacuolar membrane and the thecal plates/membrane of the zooxanthella; *bar* indicates 2000 nm. **f** Close-up of cytosolic structure of zooxanthella. Chloroplasts (cp) exhibit intact chloroplastic membrane and coherent, parallel rows of thylakoid membranes. *Bracket* (]) indicates the absence of vacuolar space between the coral vacuolar membrane and the zooxanthella's thecal plate/membrane; *bar* indicates 500 nm

thylakoid (t) membranes. Dinoflagellates from control planulae contained an abundance of starch granules (S), as well as the absence of vacuolated space between the dinoflagellate's thecal plate and the host's symbiophagic membrane (indicated by "{"; Fig. 3f).

Transmission electron microscopy of planula exposed to 288 parts per billion BP-3 for 8 h in the light (Fig. 4) showed that the planulae experienced catastrophic tissue lysis and cellular degradation in both the epidermis and gastrodermis, as well as partial collapse of the mesoglea (Figs. 3 vs. 4). At the surface of the epidermis, there was a complete loss of ciliated cells (Fig. 4a). The development and extent of cell death and tissue deterioration was greatest at the surface of the epidermis and became less pronounced at the center of the planula. In the middle area of the epidermal tissue, between the outer surface of the epidermis and its boundary with the mesoglea, the incidence of autophagic cell death became more pronounced (Fig. 4b; Tsujimoto and Shimizy 2005; Samara et al. 2008). Individual cells were dense with autophagic bodies, and many of the nuclei exhibited delamination of the nuclear bilayer membrane and vacuolization of the inner nuclear membrane containing chromatin (Fig. 4c; "}" indicates vacuolization; Eskelinin et al. 2011). None of the nuclei observed in the micrographs exhibited any signs of apoptosis, such as condensation of chromatin (Kerr et al. 1972; White and Cinti 2004; Taatjes et al. 2008). Specialized cells, such as spirocysts, also exhibited deterioration (Fig. 4d). The mesoglea exhibited structural deterioration; this vascular space contained an abundance of debris, including detached cells (Fig. 4e). The gastrodermis also exhibited extensive trauma (Fig. 4e–g). Many gastrodermal cells exhibited considerable dense autophagic bodies (Fig. 4f), although there were a few instances of

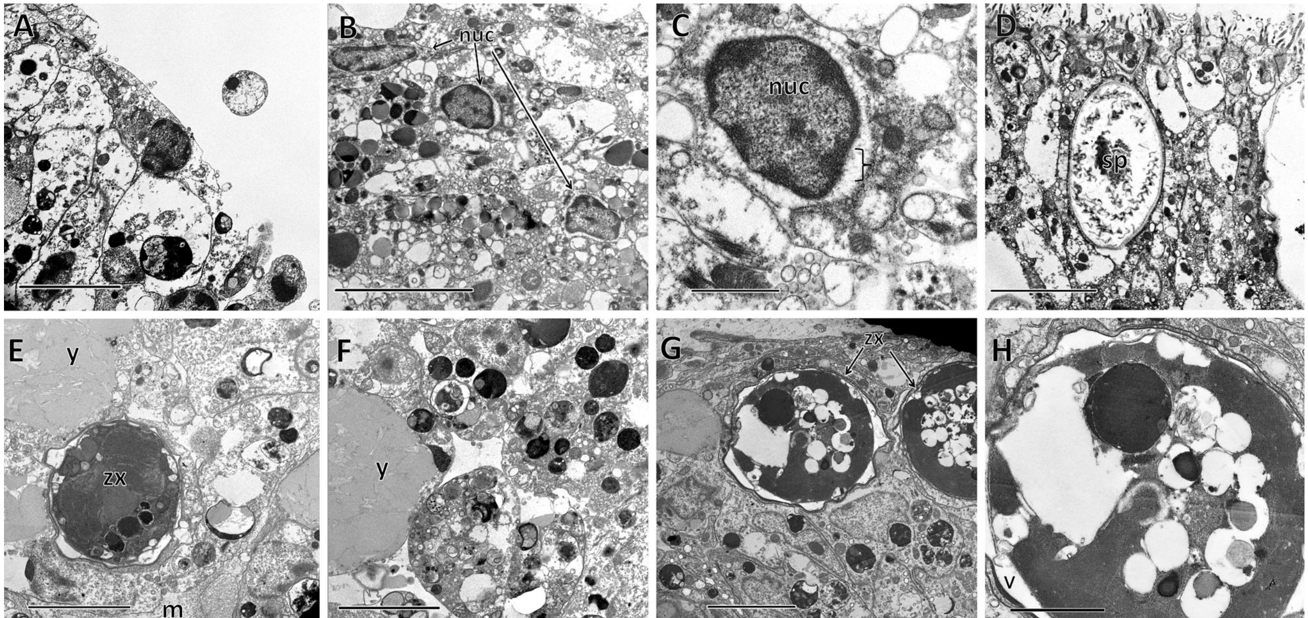


Fig. 4 Transmission electron microscopy of *Stylophora pistillata* planula exposed to 228 parts per billion (μg/L) benzophenone-3 for 8 h in the light. **a** Surface of the epidermal layer; indicating a lack of cilia and cells dying either via necrosis or autophagic cell death; *bar* indicates 5000 nm. **b** Epidermal tissue where cells exhibit an abundance of vacuolated bodies, especially the presence of vacuolated nuclei (nuc); *bar* indicates 5000 nm. **c** Magnification of vacuolated nuclei (nuc) that completely lacks nuclear blebbing (a sign of apoptosis). "}" indicates vacuolization of delaminated nuclear double membrane; *bar* indicates 1000 nm. **d** Epidermal layer with vacuolated ciliated cells, spirocysts (sp) and nematocysts; *bar* indicates 5000 nm. **e** Micrograph depicts intersection of mesoglea (m) and gastrodermal tissue containing both zooxanthella (zx) gastrodermal cells and yolk (y); *bar* indicates 5000 nm. **f** Epidermal tissue adjacent to yolk exhibits extensive autophagic vacuolization; *bar* indicates 5000 nm. **G** Gastrodermal cells containing symbiophagic zooxanthellae. Zooxanthellae have undergone extensive internal vacuolization; *bar* indicates 5000 nm. **h** Increased magnification focused on vacuolated zooxanthella, (v) indicates symbiophagic vacuole; *bar* indicates 2000 nm

nuclear autophagy. Gastrodermal cells containing symbiotic zooxanthella exhibited the early stages of symbiophagy, with vacuolization occurring around the zooxanthella (Fig. 4e–g). None of the zooxanthellae showed "normal" morphologies. They instead displayed extensive internal vacuolization, homogenization of chromatin density, and chloroplast degradation, especially of the thylakoid membranes (Fig. 4g–h).

Transmission electron microscopy of planulae exposed to 228 μg/L BP-3 for 8 h in darkness (Fig. 5) exhibited a similar gradient of cell death and tissue deterioration from the surface of the planula to its center as seen in planulae exposed to BP-3 in the light, although the progression of cellular deterioration was not as severe (Fig. 5a–h). Along the surface of the epidermal tissue layer, ciliated cells were undergoing cellular degradation (Fig. 5a). The cell layer immediately below the ciliated cells was degraded, characterized by an abundance of vacuolated bodies and loss of the plasma membrane (Fig. 5b, c). Many of the nuclei exhibited partial delamination of the bilayer nuclear membrane, but unlike the nuclei observed in planulae exposed to BP-3 in the light, vacuolization was not complete and the bilayer was still partially anchored by nuclear pores (Fig. 5b, c). Deeper into the epidermal layer, along the boundary with the mesoglea, cellular degradation persisted, especially of the spirocysts (Fig. 5d). There is an extracellular matrix that acts as a barrier between the epidermal tissue and mesoglea, and again between the gastrodermal tissue and mesoglea. Under these conditions, the integrity of the boundary layer between the epidermis and mesoglea had severely deteriorated, whereas the boundary layer between the gastrodermis and mesoglea remained intact (Fig. 5e). Within the gastrodermis, a vast majority of the cells were alive, but exhibiting signs of massive autophagy (Fig. 5f; Klionsky et al. 2012). It should be noted that there were almost no instances of delamination of the nuclear membrane in the gastrodermal cells; nuclei looked healthy (Fig. 5f). Many of the cells were dense with autophagosomic bodies, and most of the zooxanthellae were undergoing symbiophagy, as indicated by the vacuolization around the dinoflagellate cell (Fig. 5f; Downs et al. 2009). In zooxanthellae that were not significantly degraded (Fig. 5f vs. h), thylakoids exhibited a pathomorphology similar to that found in zooxanthellae of corals exposed to heat stress (32 °C) in darkness; thylakoid lamellae were diffuse (Fig. 5g; Downs et al. 2013), suggesting that the zooxanthellae were directly affected by the BP-3 exposure. In contrast to the findings of Danovaro

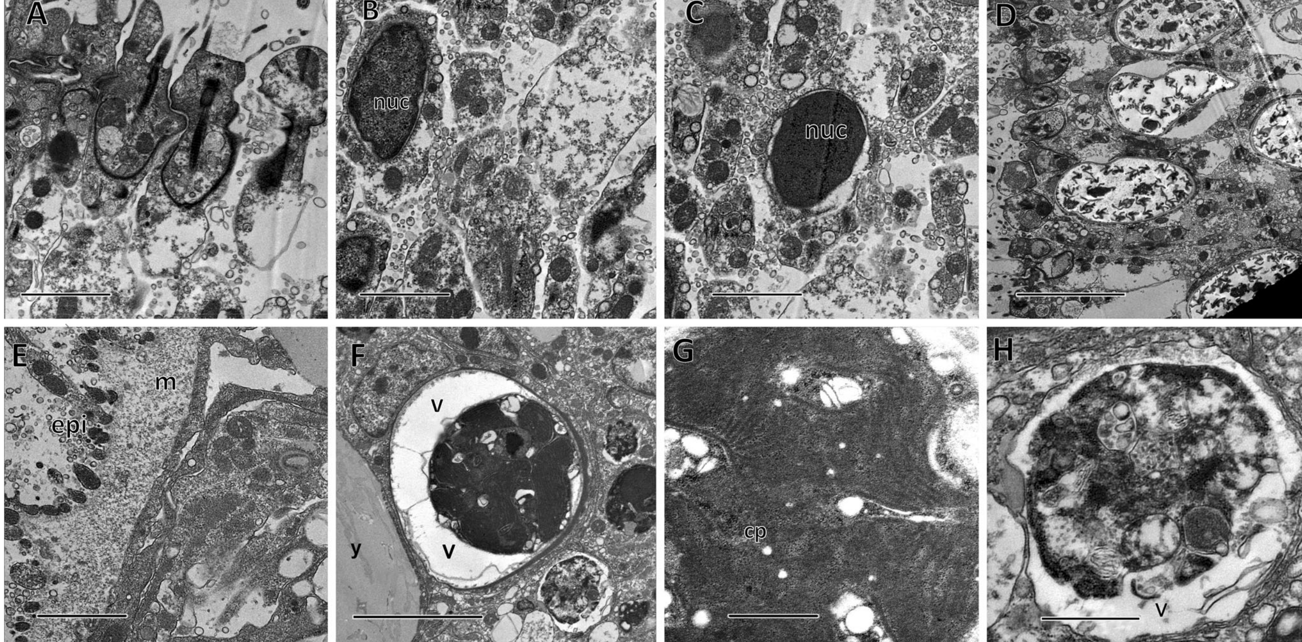


Fig. 5 Transmission electron microscopy of *Stylophora pistillata* planula exposed to 228 parts per billion (μg/L) benzophenone-3 for 8 h in the dark. **a** Surface of the epidermal layer; ciliated cells are present, but undergoing early stages of autophagic cell death. Cells beneath the cilia layer exhibiting late stage autophagic cell death and necrosis. Note scratches in the micrograph; *bar* indicates 2000 nm. **b** Epidermal tissue area between cilia and nematocyst layer showing extensive vacuolization. Early stages of nuclear vacuolization (nuc). Note scratches in the micrograph; *bar* indicates 2000 nm. **c** Epidermal tissue in area exhibiting advanced stages of cell death; nucleus vacuolization (nuc). Note scratches in the micrograph; *bar* indicates 2000 nm. **d** Extensive vacuolization of cells surrounding nematocysts. Note scratches in the micrograph; *bar* indicates 5000 nm. **e** Mesoglea (m), gastrodermal and epidermal tissues. Symbiophagy occurring to zooxanthella (zx) surrounded by extensive vacuolization in neighboring cells; *bar* indicates 2000 nm. **f** Gastrodermal tissue and yolk (y). All cells exhibiting extensive vacuolization (v), especially within the gastrodermal cell surrounding the zooxanthella. Coral cells showing increased level of autophagosome content but no signs of autophagic cell death or necrosis; *bar* indicates 5000 nm. **g** Zooxanthella chloroplast with thylakoid dispersion-pathomorphologies. Chloroplast (cp); *bar* indicates 1000 nm. **h** Zooxanthella exhibiting extensive pyknosis; symbiophagic vacuole (v); *bar* indicates 1000 nm

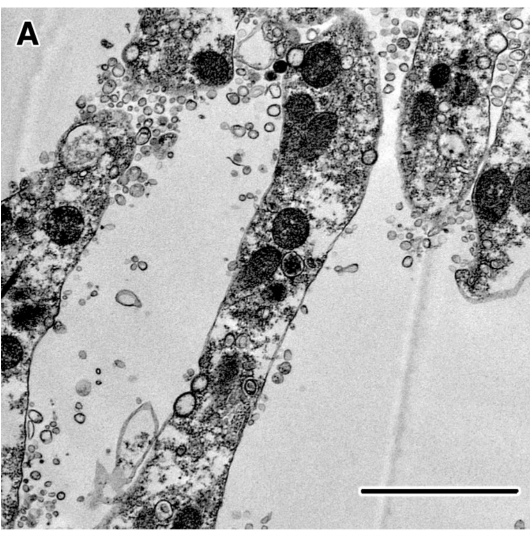
et al. (2008), viral inclusion bodies were not observed in our electron microscopy examination.

During the initial examination of the planulae using transmission electron microscopy, scratches in the microsections under observation were readily apparent (Figs. 5a–c and 6). Scratches to the microsection can arise as a result of hardened particles from the sample that scrape between the diamond blade and micro-sectioned sample (Carson 1997; Crang and Klomparens 1988). This is a common occurrence in biological samples that contain $CaCO_3$ skeleton (coral or vertebrates). These scratches are preventable if the samples are first decalcified before embedding in a resin and sectioned (Crang and Klomparens 1988). Coral planula samples do not normally need to be decalcified, because they should contain no aragonite skeletal matrix. An Alizarin red stain confirmed the presence of a $CaCO_3$ crystal matrix on the surface of the planula (data not shown; Barnes 1972). Decalcifying the fixed coral planulae with EDTA before embedding the sample in resin alleviated the “scratch” artifact and the remaining samples that were processed using a decalcification step were devoid of scratches.

Increasing concentrations of BP-3 induced significantly higher levels of DNA AP lesions in planulae exposed to the light compared to the controls (Fig. 7a, b), as well as planulae exposed to BP-3 in the dark (Fig. 7c, d).

No-Observed-Effect Concentration

Estimating Lowest-observed-effect Concentration (NOECs) for planulae exposed to BP-3 for 8 h was problematic because responses in the control treatment were homogeneous (Shapiro–Wilk; $P < 0.05$); all planulae survived and were not deformed, so analyses defaulted to the less powerful, nonparametric method (Steel 1959). The NOEC for both the proportion of live coral planulae and nondeformed planulae exposed to BP-3 for 8 h in either the light or the dark was 228 ppmillion (mg/L) (Steel Method (Steel 1959), all $Z > 2.32$, $P < 0.0809$; Fig.8a, c). In contrast to the Steel Method, the NOEC for planulae in the light determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks was 228 μg/L (H Statistic = 21.903; $P \leq 0.001$; Dunnett’s Procedure). The NOEC for planulae



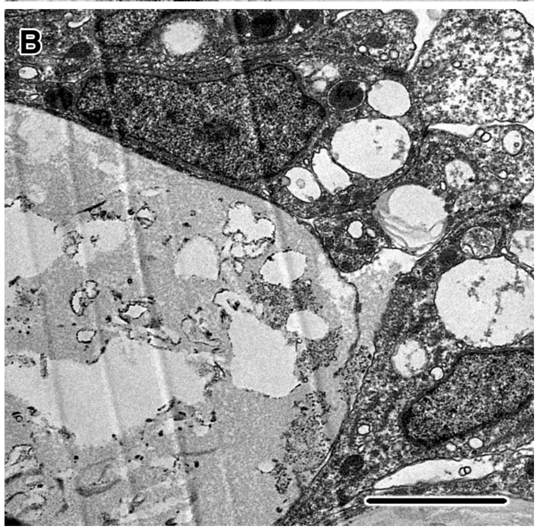


Fig. 6 "Scratch" artifacts in transmission electron microscopy micrographs of *Stylophora pistillata* planula exposed to 288 parts per billion (μg/L) benzophenone-3. When microsectioning planula embedded in a plastic resin without first decalcifying the sample, scratches can manifest on the mounted ultrathin sections. The scratches form as a result of the diamond blade fracturing the aragonite skeleton and pieces of the skeleton adhering to the edge of the diamond blade. As the contaminated blade cuts through the sample block, it scratches the ultrathin sections of the sample. These scratches can be alleviated by cleaning the diamond blade and removing aragonite skeleton in the sample through decalcification before embedding the sample in a resin. **a** Scratches apparent in ultrathin section of epidermal section of a planula; *bar* indicates 2000 nm. **b** Scratches apparent in ultrathin section of gastrodermal section of a planula; *bar* indicated 5000 nm

in the dark determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks was 228 μg/L (H Statistic = 22.402; $P \leq 0.001$; Dunnett's Procedure).

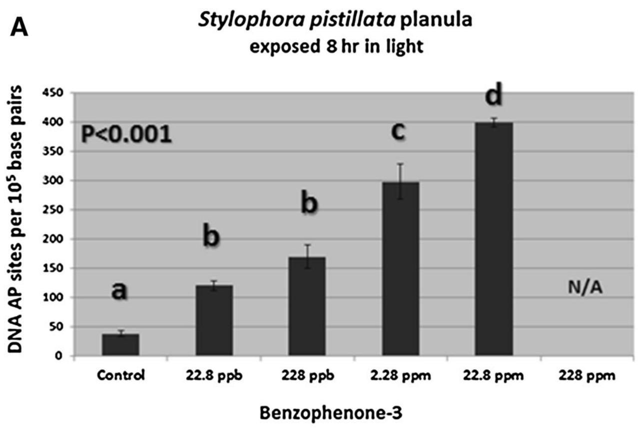
Estimates for NOECs for planulae exposed to BP-3 for 24 h in light or darkness also were problematic because responses in the control and at all concentrations greater than 22.8 μg/L (in certain cases, ≥2.28 μg/L) were homogeneous (Fig. 8b, d); all planulae survived and were not deformed in the control but died at the higher concentrations (Laskowski 1995). Using the nonparametric Steel Method, we determined the NOEC as 2.28 μg/L for the proportion of coral planulae alive after 24 h of exposure to BP-3 in the light and 22.8 μg/L in the dark (both $Z = 2.48$, $P = 0.0543$). The corresponding NOECs for non-deformed planulae were identical to these values (Fig. 9a, c). In contrast, the NOEC for planulae exposed for 24 h in the light, determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks, was 228 μg/L (Fig. 9b; H Statistic = 22.084; $P \leq 0.001$; Dunnett's Procedure). The NOEC for planulae exposed for 24 h in darkness, determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks, was 228 μg/L (Fig. 9d; *H* Statistic = 22.112; $P \leq 0.001$; Dunnett's Method).

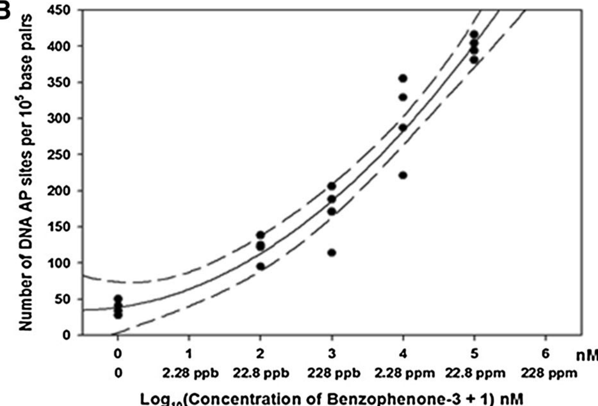
The NOEC for DNA abasic sites in planulae met ANOVA assumptions and was determined as 22.8 μg/L (100 nM; one-way ANOVA $F_{4,15} = 73.1$, $P < 0.0001$, $R^2 = 0.95$; Dunnett's Method for this comparison, $P < 0.0001$) when exposed in the light, and 22.8 μg/L (100 nM) when exposed in the dark (Welch ANOVA $F_{5,7.67} = 142.1$, $P < 0.0001$; Dunnett's Method for this comparison, $P < 0.0001$). The NOEC for mortality of *S. pistillata* calicoblast cells was below the 570 ng/L concentration for cells exposed to the dark for 4 h (Fig. 10a, b). The NOEC for mortality of *S. pistillata* calicoblast cells was 570 ng/L for cells exposed to the light for 4 h (Fig. 10c, d).

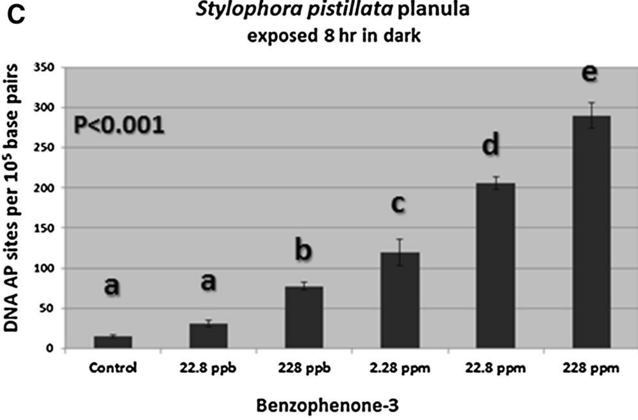
LC_{50}, EC_{50}, and EC_{20} Values

Regression models used to estimate median LC_{50} (concentration expected to cause death in 50 % of the population), EC_{20} and median EC_{50} (effective concentrations, which adversely affect 20 and 50 % of the population, respectively) after 8 h of exposure to BP-3 had coefficients of determination (R^2) between (0.91 and 0.97). Using regression models, the median LC_{50} for the proportion of live coral planulae exposed in the light was 3.1 mg/L, whereas for planulae exposed in the dark, the LC_{50} was 5.4 times higher: 16.8 mg/L (Table 1; Supplemental Fig. 1a, c). PROBIT analysis for LC_{50} in the light was 2.876 mg/L (mg/L), whereas LC_{50} in the dark was 12.811 mg/L (Table 1; Supplemental Fig. 2a, c).

Models used to estimate LC_{50} and EC_{50}, of coral planulae after 24 h of exposure to BP-3 continued to explain the substantial variation ($0.86 < R^2 \leq 0.997$). The 24 h-LC_{50} for the proportion of live coral planulae, after exposure in the light, was just 103.8 μg/L (ppbillion) compared with 873.4 μg/L in the dark exposure (Table 1; Supplemental Fig. 1b, d). PROBIT analysis for 24-h LC_{50} in the light was 139 μg/L, whereas LC_{50} in the dark was 799 μg/L (Table 1; Supplemental Fig. 2b, d).







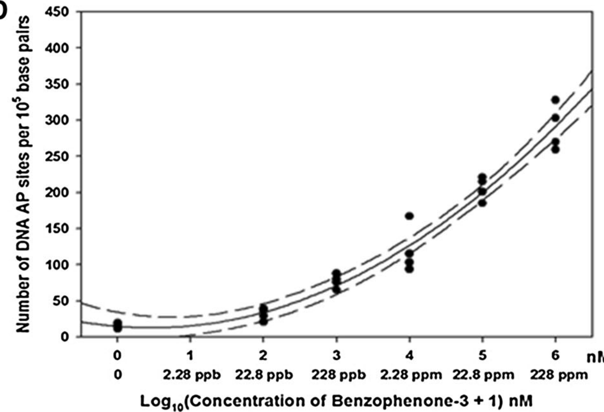


Fig. 7 Number of DNA apyrimidinic lesions in planulae of *Stylophora pistillata* exposed to various concentrations of benzophenone-3 (BP-3). *Bars* show treatment means of four replicates with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Log-linear regression between DNA AP lesions of coral planulae of *Stylophora pistillata* exposed to concentrations of BP-3 for 8 h in the light. Quadratic regression line (*solid*) and 95 % confidence intervals (*dashed lines*) are shown. **c** Planulae exposed for 8 h in the dark. **d** Log-linear regression between DNA AP lesions of coral planulae of *Stylophora pistillata* exposed to concentrations of BP-3 for 8 h in the dark

The 8-h EC_{50} for nondeformed planulae exposed to BP-3 in the light and dark were much lower: 107 and 436 µg/L, respectively using regression modeling (Table 1; Supplemental Fig. 3a, c). PROBIT analysis for 8-h EC_{50} in the light was 133 ppbillion (µg/L), whereas EC_{50} in the dark was 737 µg/L (Table 1; Supplemental Fig. 4a, c). PROBIT analysis for 8-h EC_{20} in the light was 6.3 µg/L, whereas EC_{20} in the dark was 15.5 µg/L (Table 1; Supplemental Fig. 4a, c). The 24-h EC_{50} for nondeformed planulae exposed in the light and dark were much lower: 17 ppbillion and 105 µg/L, respectively using regression modeling (Table 1; Supplemental Fig. 3b, d). PROBIT analysis for 24-h EC_{50} in the light was 49 µg/L, whereas LC_{50} in the dark was 137 µg/L (Table 1; Supplemental Fig. 4a, d). PROBIT analysis for 24-h EC_{20} in the light was 6.5 µg/L, whereas EC_{50} in the dark was 10.4 µg/L (Table 1; Supplemental Fig. 4b, d).

The number of DNA abasic sites increased approximately tenfold across the BP-3 concentration gradient in the light, but nearly 20-fold in the dark (Fig. 7b, d). Similarly, the percentage of dead coral cells increased dramatically with increasing concentrations of BP-3, but the LC_{50} was much lower in the light at 39 µg/L than in the dark at 842 µg/L. PROBIT analysis for 4-h LC_{50} coral cells in the light was 42 ppbillion, whereas LC_{50} in the dark it was 679 µg/L (Table 2; Supplemental Fig. 5a, b).

Species Sensitivity Distribution Using Coral Cell Toxicity Assay

To provide a perspective of the differences in sensitivities of various species of Indo-Pacific and Caribbean coral reefs, the LC_{50}s and LC_{20}s with their corresponding upper

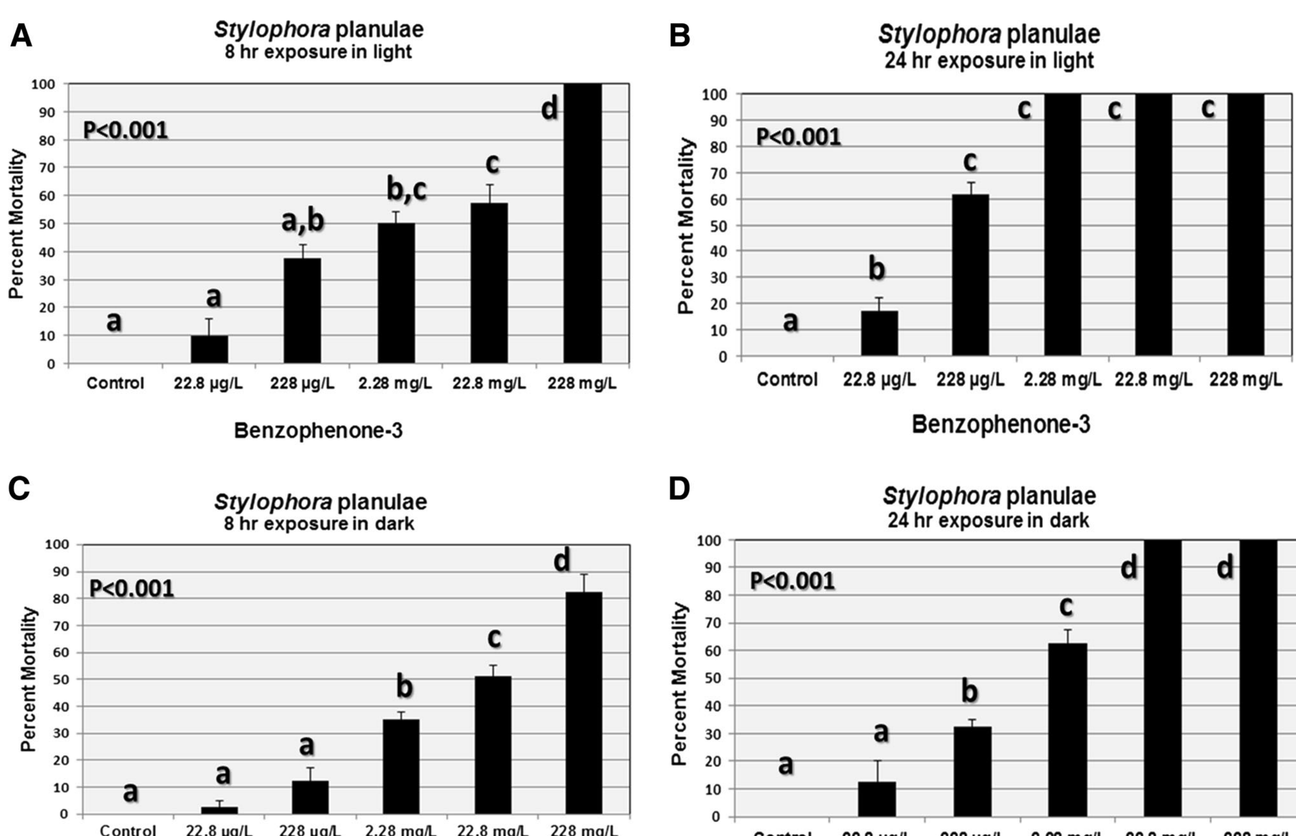


Fig. 8 Percent mortality of planula of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Planulae exposed for 8 h in the light and then 16 h of darkness. **c** Planulae exposed for 8 h in the dark. **d** Planulae exposed for 24 h in the dark

and lower 95 % confidence intervals for the two Indo-Pacific and five Caribbean species are provided in Table 1.

Correction Factor Between Mortality of Coral Planulae and Coral Cells

Coral cells were much more sensitive than coral planulae across a wide range of BP-3 concentrations, which makes cell mortality a potential indicator of reproductive and recruitment failures. To estimate the correction factor needed to translate coral cell mortality into potential mortality of coral planulae, one option is the use of a quadratic regression model to estimate these relationships: In the light ($F_{2,21} = 43.8$, $P < 0.0001$, $R^2 = 0.81$) % mortality of planulae = 2.26 − 0.28 (% mortality of cells) + 0.0107 (% mortality of cells)^2 In the dark ($F_{2,21} = 84.5$, $P < 0.0001$, $R^2 = 0.89$) % mortality of planulae = 0.86 − 0.0007 (% mortality of cells) + 0.0078 (% mortality of cells)^2

Environmental Chemistry Analysis

The purpose of the chemical analysis was to conduct a cursory survey of BP-3 concentrations on coral reefs. Seawater samples were collected from bays in St. John Island, U.S. Virgin Islands: Caneel Bay, Hawksnest Bay, and Trunk Bay in April 2007 (Fig. 11a, b). Caneel Beach is managed by the resort, Caneel Bay. Samples were collected at approximately 16:30 h near the dive platform that adjoins the Caneel Beach and along a large coral community that spans from the edge of Caneel Beach to the edge of Honeymoon Beach. There were 17 swimmers in Caneel Bay in the 48-h period before sampling. Swimmers were monitored from the shore of the resort from dawn to dusk. No benzophenones could be detected in either of the samples collected in Caneel Bay.

Hawksnest Bay is a densely visited beach within the U.S. National Park system on St. John Island. In general, more than 1000 visitors per day can enter into this bay. On the day of sampling, more than 230 people entered the

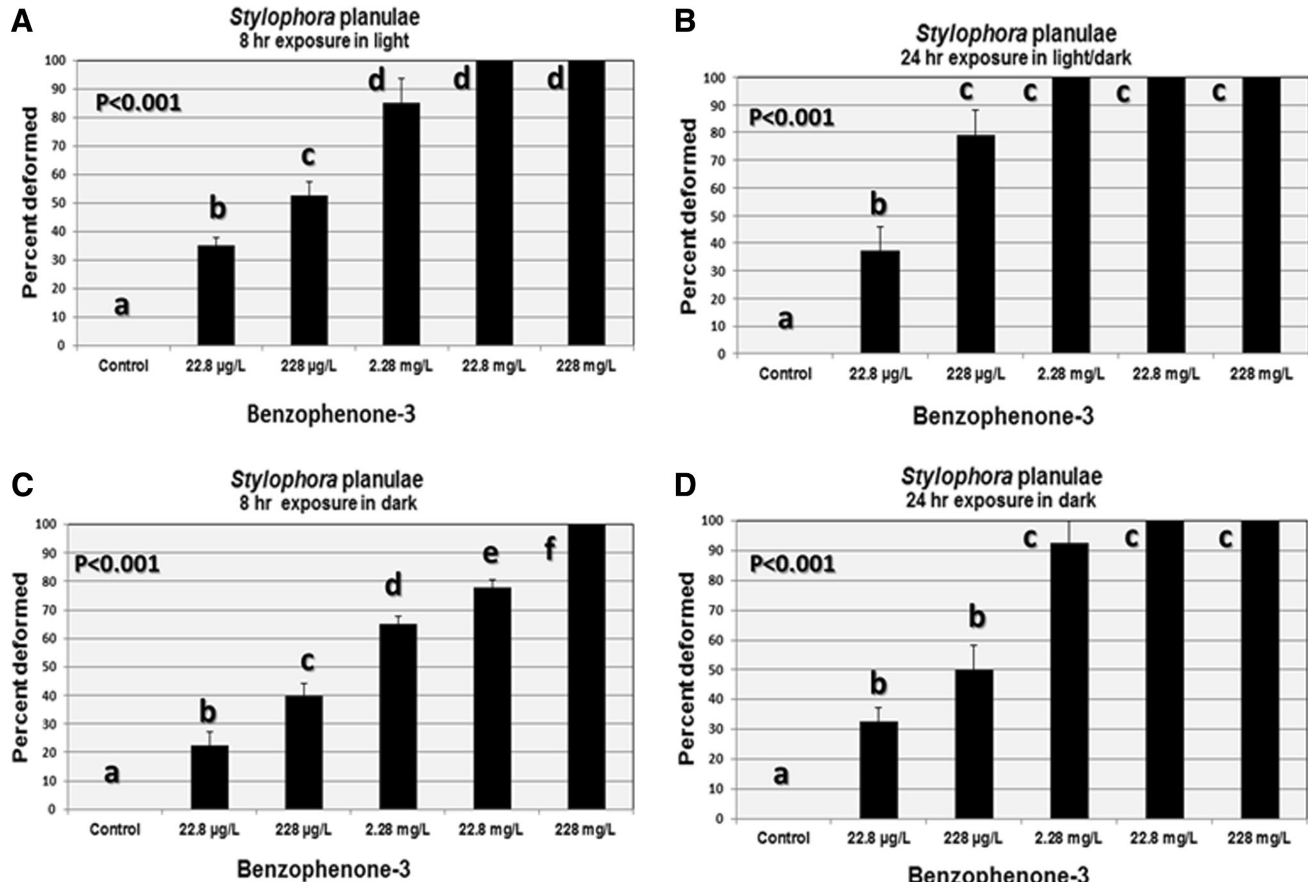


Fig. 9 Percentage of deformed planulae of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Planulae exposed for 8 h in the light, then 16 h of darkness. **c** Planulae exposed for 8 h in the dark. **d** Planulae exposed for 24 h in the dark

water and swam within 20 m of the three large *Acropora palmata* spurs (coral reefs) indicated in Fig. 11c; the majority swam in the sandy grooves that lie between the coral-reef spurs. These spurs are very shallow (1–3 m deep), with live coral often protruding above the surface of the water during low tide. The concentration of BP-3 in the western groove was 75 ppbillion (µg/L), whereas the larger, eastern groove had a BP-3 level of 95 ppbillion (µg/L). Samples were collected between 17:00 and 17:40 h.

Trunk Bay is an iconic landscape and a highly managed natural resource area. Before 2009, there could be more than 3000 visitors on the beach and in the water at Trunk Bay. After 2009, National Park Service policy reduced the number to 2000 visitors per day (personal communication, Rafe Boulon, retired, USVI NP Chief, Resource Management). A coral community surrounds the island in Trunk Bay, as well as an abundance of gorgonians to the west of the island, and there was once a very extensive stand of *A. palmata* corals to the east of the island. At a site near the edge of the Trunk Island coral community, BP-3 levels were 1.395 ppmillion (mg/L) (Fig. 11d). A sampling site 93 m east of the first sampling site contained 580 ppbillion (µg/L) BP-3 (Fig. 11d). Samples were collected at 11:00–11:24 h with more than ~180 swimmers in the water and ~130 sunbathers on the beach within 100 m of the two sampling sites.

Seawater samples were collected at five sites in Maunalua Bay, Oahu Island, Hawai'i on May 30, 2011 between 11:00 and 15:00 h (Fig. 12a, b). ASW samples were collected in public swimming areas in waters that were 1.3 m in depth and 35 cm from the surface of the water. Sites 1–4 had detectable levels of BP-3 (>100 pptrillion; ng/L) but were below the quantitative range of measurement (5 ppbillion (µg/L); Fig. 12b). Site 5 contained measurable levels of BP-3—19.2 ppbillion (µg/L) (Supplemental Fig. 6).

Samples were collected at two sites on June 3, 2011, along the northwest coast of Maui Island, Hawai'i (Fig. 12c). Kapalua Bay is a protected cove and has a

public beach that can often see >500 swimmers/day in the peak tourism season (personal communication, Kapalua Dive Co.; Fig. 12d). A seawater sample was collected 40 m from shore near the center of the bay, immediately above remnants of a coral reef at 09:30 h. The Kapalua sample had detectable levels of BP-3 but was below the quantitative range of measurement (5 ppbillion, 5 µg/L). From 06:30 to 09:30 h on the day of sampling, 14 swimmers had entered Kapaula waters. A seawater sample also was collected at Kahekili Beach Park, Maui Island, Hawai'i (Fig. 12e). Kahekili Beach is a public beach that also serves visitors from a number of nearby hotels and resorts. The sample was collected 30 m from shore, immediately above a coral reef. Unlike Kapalua, Kahekili is an exposed shoreline not protected within a bay, and retention time of contaminants is thought to be minimal because of the prevailing currents. The Kahekili sample had detectable levels of BP-3 but was below the quantitative range of measurement (5 ppbillion). Kahekili is a heavily visited beach and had 71 swimmers within 200 m of the sampling site at the time of sampling (11:45 h).

Table 1 Regression and PROBIT determination of LC_{50} for planulae mortality when exposed to BP-3 in the light and dark, and the EC_{50} for planulae deformity when exposed to BP-3 in the light and the dark

Planulae mortality	LC_{50}
Regression to estimate LC_{50} 8-h light	3.1 mg/L
PROBIT to estimate LC_{50} 8-h light	2.9 mg/L
Regression to estimate LC_{50} 8-h dark	16.8 mg/L
PROBIT to estimate LC_{50} 8-h dark	12.8 mg/L
Regression to estimate LC_{50} 24-h light	103.8 µg/L
PROBIT to estimate LC_{50} 24-h light	1.39 µg/L
Regression to estimate LC_{50} 24-h dark	873.4 µg/L
PROBIT to estimate LC_{50} 24-h dark	799 µg/L
Planulae deformation	**EC_{50}**
Regression to estimate EC_{50} 8-h light	107 mg/L
PROBIT to estimate EC_{50} 8-h light	133 mg/L
Regression to estimate EC_{50} 8-h dark	436 mg/L
PROBIT to estimate EC_{50} 8-h dark	737 mg/L
Regression to estimate EC_{50} 24-h light	17 µg/L
PROBIT to estimate EC_{50} 24-h light	49 µg/L
Regression to estimate EC_{50} 24-h dark	105 µg/L
PROBIT to estimate EC_{50} 24-h dark	137 µg/L
Planulae deformation	**EC_{20} (µg/L)**
PROBIT to estimate EC_{20} 8-h light	6.3
PROBIT to estimate EC_{20} 8-h dark	15.5
PROBIT to estimate EC_{20} 24-h light	6.5
PROBIT to estimate EC_{20} 24-h dark	10.4

PROBIT determination of EC_{20} for planulae deformity when exposed to BP-3 in the light and the dark

Discussion

Toxicopathology

Benzophenone-3 is a phototoxicant and induces different toxicities depending on whether the planulae are exposed to the chemical in light or in darkness. Corals will usually release brooded planulae at night or spawn gametes at night (Gleason and Hofmann 2011). Planulae of broadcasting species (those that spawn eggs and sperm that are fertilized in the water column) are positively buoyant and planktonic, residing at or near the surface of the ocean for 2–4 days before they are able to settle (Fadlallah 1983; Shlesinger and Loya 1985; Harii et al. 2007; Baird et al. 2009). Light levels on a clear sunny day in tropic latitudes can be as high as or higher than 2000 µmol/m^2/s of photosynthetically active radiation—five times more than what the corals experienced in this study, suggesting that actual environmental conditions may aggravate the phototoxicity. Whether the BP-3 pollution comes from swimmers, or from point and nonpoint wastewater sources, planulae will be at

Table 2 Differences in sensitivities of various species of Indo-Pacific and Caribbean coral reefs, the LC_{50}s and LC_{20}s of calicoblast cells exposed in vitro to benzophenone-3 with their corresponding upper and lower 95 % confidence intervals for the two Indo-Pacific and five Caribbean species. (µg/L) = to parts per billion. (ng/L) = parts per trillion

Coral species	LC_{50} (µg/L)	95 % CI	LC_{20}	95 % CI
Indo-Pacific species				
Stylophora pistillata (light)	42	28; 60	2 µg/L	1.14; 3.61
Stylophora pistillata (dark)	671	447; 984	14 µg/L	7; 26
Pocillopora damicornis	8	4.96; 12.15	62 ng/L	24; 136
Caribbean-Atlantic species				
Acropora cervicornis	9	5.4; 14.5	63 ng/L	22; 150
Montastrea annularis	74	40; 126	562 ng/L	166; 1459
Montastrea cavernosa	52	36; 72	502 ng/L	247; 921
Porites astreoides	340	208; 534	8 µg/L	3; 16
Porites divaricata	36	21; 57	175 ng/L	60; 420

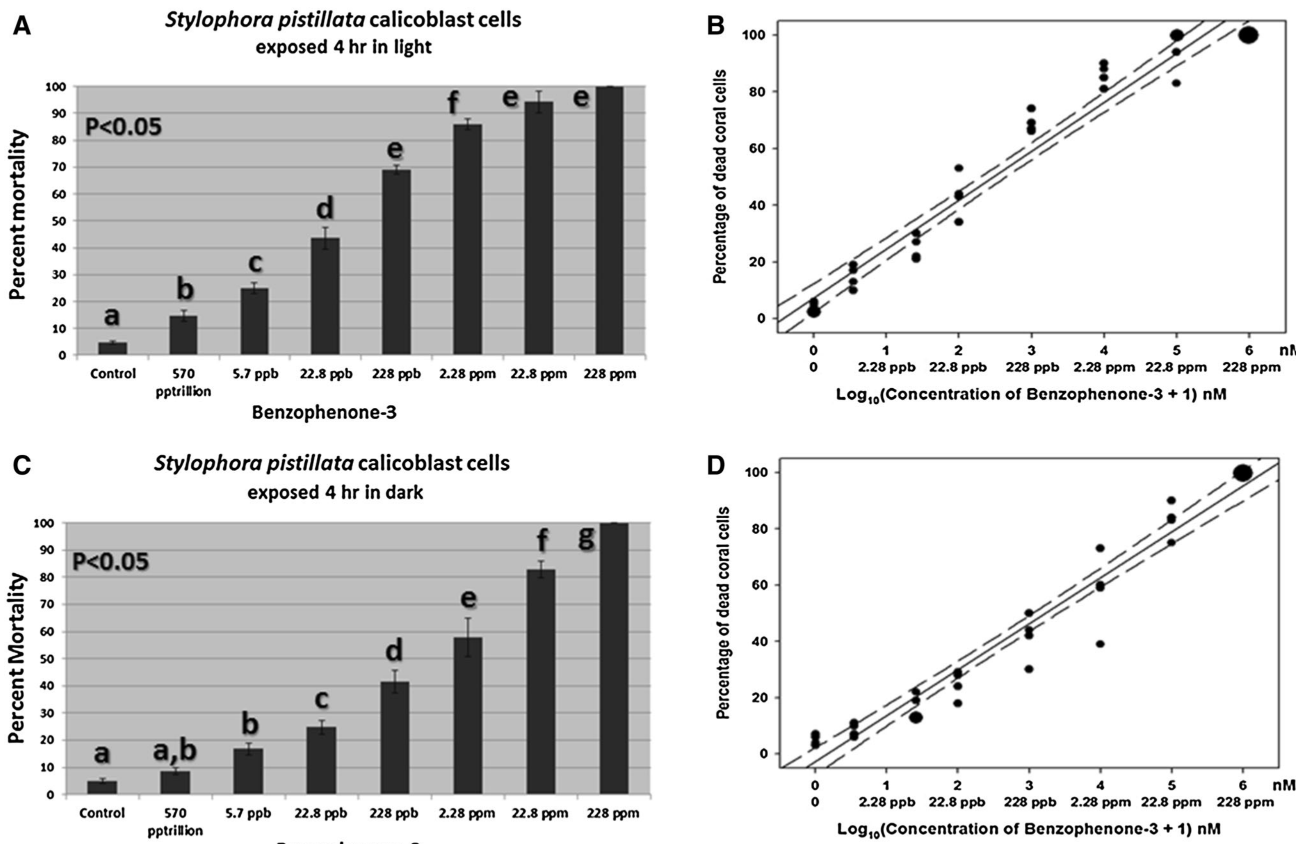


Fig. 10 Percentage mortality of calicoblast cells of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means ($n = 4$) with whiskers representing ± 1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on one-way analysis of variance followed by a Tukey's Honestly Significant Difference Test. **a** Calicoblast cells exposed for 4 h in the light. **b** Log-linear regression between coral cell mortality and concentrations of BP-3 for 4 h in the light. Quadratic regression line (*solid*) and 95 % confidence intervals (*dashed lines*) are shown. *Larger symbols* represent multiple coincident data points, with symbol area proportional to the number of replicates with the same value. **c** Calicoblast cells exposed for 4 h in the dark. **d** Log-linear regression between coral cell mortality and concentration of BP-3 for 4 h in the dark

risk from both forms of toxicities (Brooks et al. 2009; Futch et al. 2010; Pitarch et al. 2010).

As with our previous paper examining benzophenone-2 (Downs et al. 2014), the data in this paper are consistent with the observation by Danovaro et al. (2008) that "sunscreens compounds" cause coral bleaching. In the light, BP-3 caused injury directly to the zooxanthellae, independent of any host-regulated degradation mechanism. Based on the pathomorphology of the thylakoids within the chloroplasts, the most probable interpretation is that BP-3 induces photo-oxidative stress to the molecular structures that form the thylakoid membranes (Downs et al. 2013). In darkness, bleaching resulted from the symbiophagy of the symbiotic zooxanthellae; a process whereby the coral gastrodermal cell "digests" the zooxanthella (Downs et al. 2009). Nesa et al. (2012) demonstrated that following exposure to light, the algal symbionts of corals increased the DNA damage to coral cells in coral planulae. Consistent with the Oxidative Theory of Coral Bleaching (Downs et al. 2002), Nesa et al. hypothesized that the sources of this damage was the production of oxygen radicals. If this is the case, then darkness-associated, BP-3-induced bleaching may reduce the exacerbated morbidity experienced by "bleached" planulae that would occur during the periods of daylight. Regardless of the toxicological mechanism, managing exposure of corals to BP-3 corals will be critical for managing coral reef resilience in the face of climate-change pressures associated with coral bleaching (West and Salm 2003).

Autophagy was the dominant cellular response to BP-3 exposure (Figs. 4a–f, 5b–d; Yla-Antilla et al. 2009). Micro-autophagosomes were abundant in all cell types and larger vacuolated bodies of specific organelles were readily observed. None of the nuclei in any coral cell-types exhibited any of the classic signs of apoptosis, such as pyknosis or karyorrhexis of the nucleus (Krysko et al.

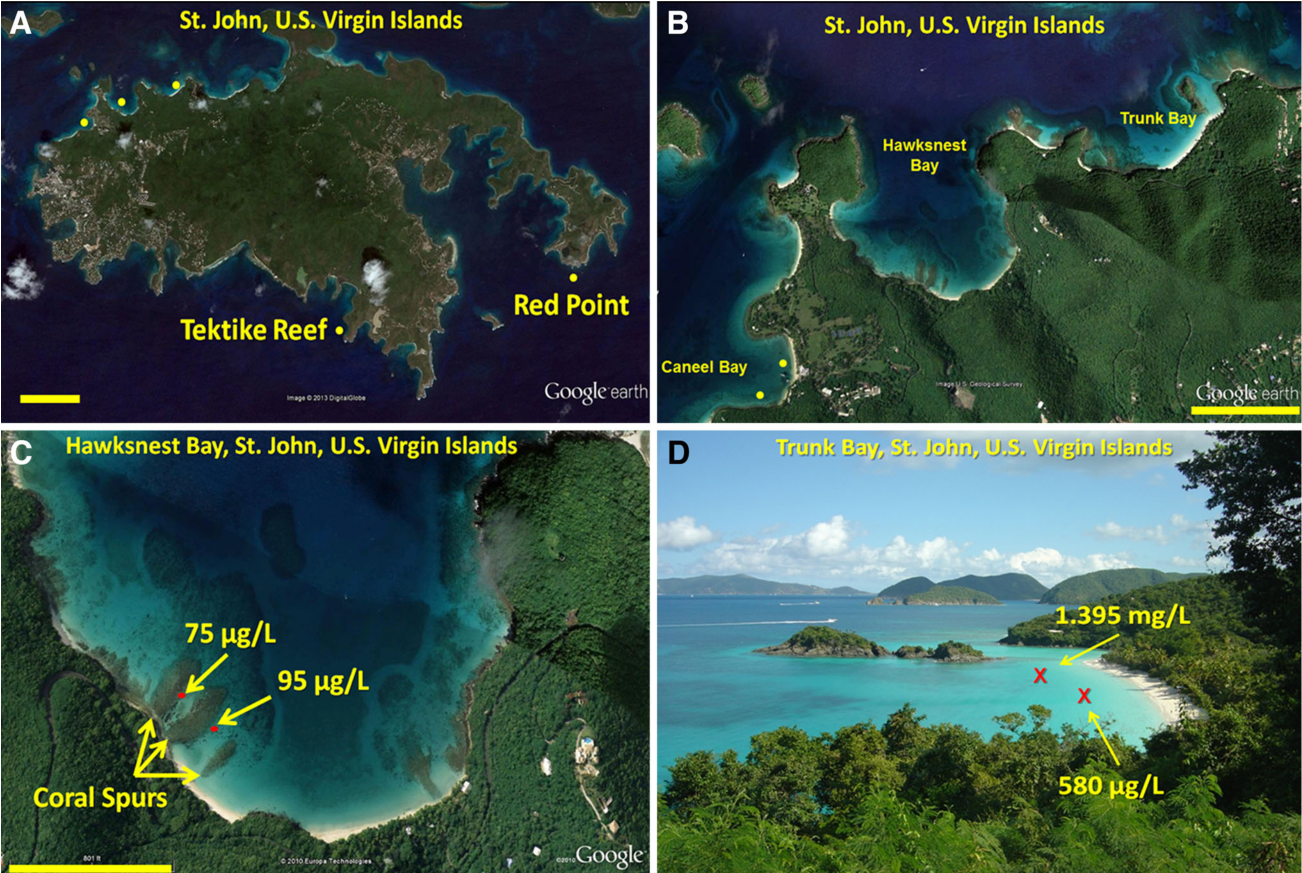


Fig. 11 Seawater analysis of benzophenone-3 (BP-3) in coral reef areas in St. John Island, U.S. Virgin Islands. **a** Aerial view of St. John indicating the five sampling sites, indicated by a *yellow dot*. No benzophenones were detected in samples from *Red Point* or at Tektite Reef. All samples were taken between 12:00 and 14:00 h. *Scale bar* is 1.5 km. **b** Aerial view of the three northwestern sites within St. John National Park: Trunk Bay, Hawksnest Bay, and Caneel Bay. The two sampling sites at Caneel Bay are indicated by *yellow dots*. No benzophenones were detected in samples from Caneel Bay. *Scale bar* is 500 m. **c** Aerial view of the two sampling sites in Hawksnest Bay, St. John Island. *Yellow arrows* indicate three coral reef spurs that are dominated by the U.S. Threatened Species, *Acropora palmata*. *Yellow arrows* pointing at *red dots* indicate the sample site. *Values* indicate the concentration of BP-3 in the water column. *Scale bar* is 245 m. **d** Elevated view of Trunk Bay, St. John Island. *Yellow arrows* pointing to *red* "X" indicate the sample site. The *values* indicate the concentration of BP-3 in the water column at those two sites

2008). The most fascinating aspect of these autophagic events were the delamination of the nuclear bilayer membrane (Figs. 4b, c, 5b, c), a classic hallmark of autophagic cell death and further evidence arguing against apoptosis as a regulated mechanism of cnidarian cell death (Tasdemir et al. 2008; Yla-Antilla et al. 2009; Klionsky et al. 2012). In both the light and the dark, there was a gradation of vitiated cells beginning at the surface of the epidermis to "non-morbid" cells in the gastrodermis that surrounded the yolk. In Figs. 4a and 5a, the cells are severely degraded; it is difficult to distinguish any mechanism of cell death, and the cells could easily be labeled as necrotic. Going 20,000 nm into the planula from the surface, cells exhibited the hallmarks of autophagic cell death. This tissue transect of the gradation of cell death is evidence for a model of cell death, first demonstrated in *C. elegans*, that requires autophagic degradation of cells for the manifestation of necrosis (Samara et al. 2008; Eskelinin et al. 2011).

BP-3 is a genotoxicant to corals, and its genotoxicity is exacerbated by light. Based on the current literature, this was not unexpected, but our data do underscore the threat that BP-3 may pose to not only corals but also to other coral-reef organisms (Hanson et al. 2006; Cuquerella et al. 2012). DNA AP lesions can be produced in response to oxidative interaction or alkylation events (Fortini et al. 1996; Drablos et al. 2004). Accumulation of DNA damage in the larval state has implications not only for the success of coral recruitment and juvenile survival, but also for reproductive effort and success as a whole (Anderson and Wild 1994; Depledge and Billinghurst 1999). Surviving planulae exposed to BP-3 may settle, metamorphose, and

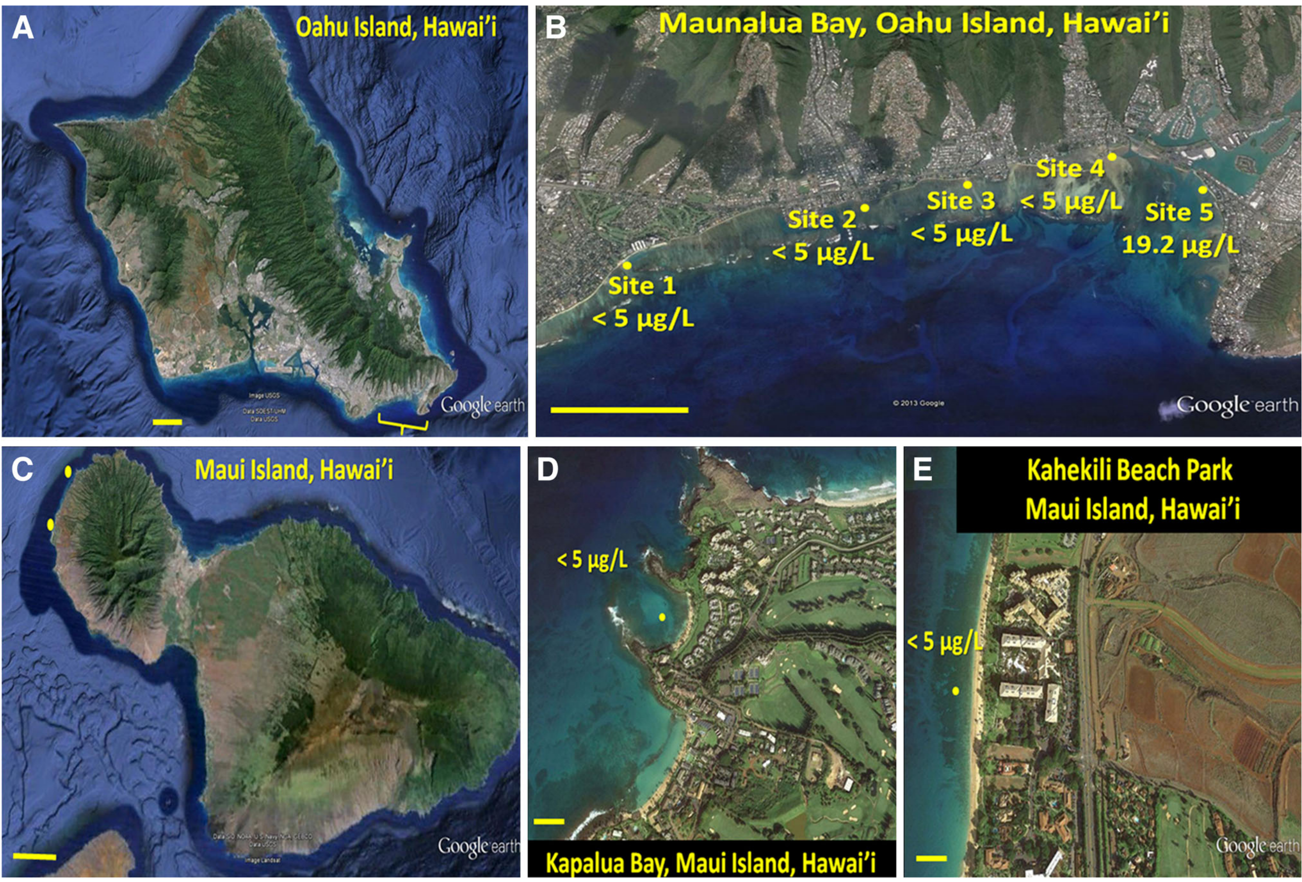


Fig. 12 Seawater analysis of benzophenone-3 (BP-3) in coral reef areas in Oahu and Maui islands, Hawai'i. *Yellow dots* indicate the sampling location in the panels. **a** Aerial view of Oahu indicating the five sampling sites. *Scale bar* is 5 km. **b** Aerial view of the five sampling site along the coast of Maunalua Bay, Oahu. Sites 1–4 had levels of BP-3 that were detectable, but below the quantitative range. *Scale bar* is 1.5 km. **c** Aerial view of the two sampling sites in Maui, Hawai'i. *Scale bar* is 6 km. **d** Elevated view of Kapalua Bay, Maui. *Scale bar* is 100 m. **e** Elevated view of Kahekili Beach, Maui. *Scale bar* is 100 m

develop into colonial adults, but they may be unfit to meet the challenges of other stressor events, such as increased sea-surface temperatures. Cnidarians are rather unusual in the animal kingdom in that the germline is not sequestered away from the somatic tissue in early stages of development; the somatic tissue gives rise directly to the germline during seasonal reproductive cycles. Damage to the genomic integrity of coral planulae therefore may have far-reaching and adverse impacts on the fitness of both the gametes in adults.

The ossification of the planulae from exposure to BP-3 is one of the strangest cases of developmental endocrine disruption to wildlife, although skeletal endocrine disruption in vertebrates is only now being recognized (Colburn et al. 1993; Depledge and Billinghurst 1999; Golub et al. 2004; Lind et al. 2004; Doherty et al. 2004; Agas et al. 2013). In mammals, estrogen and estrogenic compounds may influence different estrogen and thyroid hormone receptors, which affect bone growth and composition (Rickard et al. 1999; Lindberg et al. 2001; Golub et al. 2004). In classic vertebrate physiology, estrogen plays a complex role in ossification and skeletal maintenance, affecting both bone anabolism and catabolism (Simmons 1966; Väänänen and Härkönen 1996). In vertebrates, exposure to high levels of estrogen can result in skeletal hyperossification (Pfeiffer et al. 1940; Rickard et al. 1999). For "classic" endocrine disruptors, such as tributyltin and dioxin, ossification is inhibited, not induced (Birnbaum 1995; Jamsa et al. 2001; Tsukamoto et al. 2004; Finnila et al. 2010; Agas et al. 2013). Osteo-endocrine disruption is both complex and complicated; different compounds affect different cell types within the skeletal tissue differently (Hagiwara et al. 2008a, b; Agas et al. 2013). Benzophenones as endocrine disruptors are no exception; BP-3 and BP-2 showed contradictory effects on estrogen and aryl hydrocarbon receptors, and both compounds induced "…a kind of endocrine disruption that is not assessed by 'classical' estrogenic markers" (Schlecht et al. 2004; Seidlová-Wuttke et al. 2004; Ziolkowaska et al. 2006).

The ossification-induced opacity of the epidermal tissue layer of planulae was readily observed at the three highest

concentrations of BP-3 exposure but was not visually obvious at the lower concentrations, although we know from the electron microscopy sample processing that ossification was present to a lesser extent in the lower BP-3 exposures. Many endocrine disruptors do not exhibit a "classic" monotonic exposure–response curve, but instead exhibit nonmonotonic behaviors (vom Saal et al. 1995; Conolly and Lutz 2004; http://epa.gov/ncct/edr/non-monotonic.html). Ossification of planulae can be assayed by a variety of methods, including alizarin staining and calcein fluorescence. This study was not designed to be an exhaustive characterization of exposure–response behavior (i.e., regulatory toxicology); hence lower BP-3 exposure concentrations were not attempted. More comprehensive studies that examine the ossification response of both acute and chronic exposure of BP-3 in the lower pptrillion and ppquadrillion need to be conducted to determine accurately this endocrine behavioral response.

Ecotoxicology and Species Sensitivity

To conduct a relevant and accurate ecological risk or threat assessment, it is imperative that the species chosen reflects the structure of the specific coral-reef ecosystem being affected (Suter 2007). *Stylophora pistillata* used in this study, is indigenous to specific regions in the Indo-Pacific basins, and hence may not be a valid representative for coral-reef communities in Hawaii or the Atlantic/Caribbean basins. The use of coral planulae in research studies is a relatively difficult resource to obtain. It requires access to healthy coral colonies that are reproductively viable, spawning in specific dates and specific moon phases, and in addition, obtaining the necessary collection and transport permits. We therefore applied an in vitro primary cell toxicity methodology using a specific coral cell type that has been proposed as a surrogate for either planula or colonial polyp studies (Downs 2010). Comparison of LC_{50}s of coral cells in the light (42 ppbillion; µg/L) and coral planula in the light for 8 and 24 h [2.876 ppmillion (mg/L) and 139 ppbillion (µg/L), respectively] exhibits a similar response. The increased sensitivity of in vitro cell models versus whole organism models is a common phenomenon and accepted principle (Blaauboer 2008; Gura 2008). Diffusion of BP-3 across the epidermal boundary layer and reaching concentrations that are toxic in the interior of the planula (e.g., gastroderm) versus direct exposure by cultured cells could likely be the major factor influencing the variation in LC_{50} rate. Although there are obvious caveats to using in vitro models, this may be the only way to conduct ecotoxicological research and ecological risk assessments on coral species that are currently endangered with extinction, such as the species on the IUCN's Red List or species proposed/listed for protection under the U.S. Endangered Species Act.

When an environmental stressor impacts a community of organisms, different species may respond (tolerate) dissimilarly to one another; some species may tolerate the stressor at a particular level, whereas other species may succumb (Johnston and Roberts 2009; Maloney et al. 2011). This species sensitivity distribution is a crucial concept for ecological risk assessments and a predictor of the species composition of a community (community phase-shift) in reacting to a pollution stressor, as well as defining the probability of success for community/ecological restoration (Posthuma et al. 2002; van Woesik et al. 2012). This concept readily applies to corals and coral reefs. Coral bleaching in response to heat stress or freshwater input is an excellent example of this community behavior; some species have high tolerance to stress-induced bleaching, whereas others are highly susceptible, resulting in species-specific extinctions in localized areas (Goreau 1990; Loya et al. 2001; Jimenez and Cortes 2003). Species sensitivity distribution in response to pollutants in corals is also well documented, including synergisms between pollutants and heat stress (Loya 1975; Brown 2000; Fabricius 2005). For the Caribbean, the species sensitivity to BP-3 toxicity is consistent with the model for coral tolerance to general stress as set forth by Gates and Edmunds (1999): corals with slower growth rates, such as massive or boulder coral species, are inherently more tolerant than coral species with higher growth rates (e.g., branched species such as *A. cervicornis* and *P. divaricata*). In fringing reefs that have been impacted by anthropogenic stressors, especially fringing reefs near tourist beaches, *Acropora* species are the first to experience localized extinction. Species that tentatively endure a decade or longer of sustained stress, but are intermediate in their persistence, are the large boulder corals found in the genus *Montastrea* (synomym *Orbicella*). Coral cell toxicity data indicated that *P. astreoides* was at least 4.5× more tolerant to BP-3 toxicity than the second more tolerant coral species and at least 38× more tolerant than the most sensitive species. This is consistent with observations that *P. astreoides* is usually the last to become extinct in a polluted-impacted locality and one of the first to recruit once water quality parameters reach a minimum level of habitability (Peters 1984; Lirman et al. 2003; Alcolado-Prieto et al. 2012). From a management perspective, these data can be used to predict the changes in coral-reef community structure when challenged with BP-3, regarding which species will become extinct, as well as the species that will persist in areas that are adjacent to tourist beaches, popular mooring sites, or near sewage discharges. These data also can be integrated directly into reef resilience management

plans against climate change, acting as both a measurable endpoint for management effectiveness and as a target (concentration of BP-3 in seawater on a reef) for establishing action values for reef management.

Management of BP-3 Pollution for Coral Reef Conservation and Restoration

What do these pathological toxicities induced by BP-3 mean demographically and ecologically for corals and coral reefs? Trunk Bay in St. John Island, the U.S Virgin Islands, may represent an example of this effect. Ecologically, this area has been severely degraded in the past 25 years, despite the limited input from human activities in the watershed and from marine sources. The most obvious input is recreational swimming at Trunk (Downs et al. 2011). During our monitoring of this site from 2005 to 2010, settlement of planulae and recruitment/survival of juvenile coral was almost 0 %. Established coral colonies in this area were assayed for regeneration of tissue over experimentally induced lesions (laceration-regeneration assay, a single diagnostic test for the general health of a coral; Fisher et al. 2007); not a single colony exhibited any regeneration of any of the lesions during the 5-year investigation (Downs et al. 2011). This was in contrast with Caneel Bay, which had undetectable levels of BP-3 resulting from a much lower density/rate of swimmers and has a flourishing coral community on its southern bank with an abundance of recruitment. These demographic-level pathologies are consistent with the pathologies that manifest from BP-3 exposure. The pathologies exhibited at this site can be seen at other coral reef swimming areas the world over: Eilat, Israel (degraded with an abundance of sunscreen lotion users) versus Aqaba, Jordan (thriving coral reefs with swimmers that do not use sunscreen lotion; Fuad Al-horani, personal communication), Honolua Bay in Maui, Hawaii, Hanauma Bay Beach in Oahu, Hawaii, Seven Mile Beach in Grand Cayman, Bathway Beach in Grenada, Playa Langosta, and Playa Tortugas Beaches in Cancun, Mexico. At Okinawa, Tashiro and Kameda (2013) demonstrated that BP-3 contamination from beaches can travel over 0.6 km in distance from the pollution source. The threat of BP-3 to corals and coral reefs from swimmers and point and non-point sources of waste-water could thus be far more extensive than just a few meters surrounding the swimming area.

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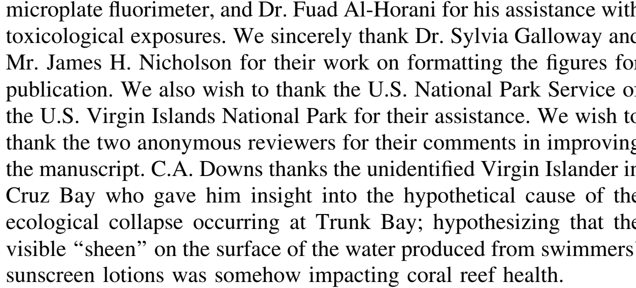

microplate fluorimeter, and Dr. Fuad Al-Horani for his assistance with toxicological exposures. We sincerely thank Dr. Sylvia Galloway and Mr. James H. Nicholson for their work on formatting the figures for publication. We also wish to thank the U.S. National Park Service of the U.S. Virgin Islands National Park for their assistance. We wish to thank the two anonymous reviewers for their comments in improving the manuscript. C.A. Downs thanks the unidentified Virgin Islander in Cruz Bay who gave him insight into the hypothetical cause of the ecological collapse occurring at Trunk Bay; hypothesizing that the visible "sheen" on the surface of the water produced from swimmers' sunscreen lotions was somehow impacting coral reef health.

Compliance with Ethical Standards

Conflict of Interest The authors can identify no potential conflicts of interest, neither financial nor ethically, involved in the writing or publication of this manuscript.

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Environmental occurrence and ecological risk assessment of organic UV filters in marine organisms from Hong Kong coastal waters

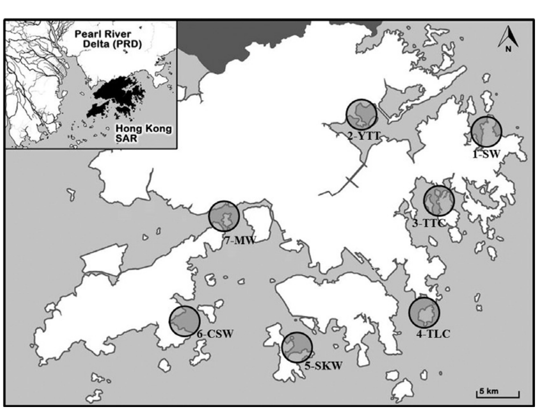
Ziye Sang [a], Kelvin Sze-Yin Leung [a,b,c,*]

[a] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region
[b] HKBU Institute of Research and Continuing Education, Shenzhen Virtual University Park, Shenzhen, PR China
[c] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, PR China

HIGHLIGHTS

- The occurrence of seven organic UV filters in marine organisms was investigated.
- UV filters showed higher detection frequencies and levels in mussels than in fish.
- Their spatial distribution implied a positive correlation with the direct sources.
- EHMC and BP-3 could pose significant risks to marine aquatic ecosystem.
- UV filters showed higher risks to marine aquatic ecosystem than freshwater.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic UV filters, now considered to be emerging contaminants in aquatic ecosystems, are being intensively tracked in environmental waters worldwide. However, their environmental fate and impact of these contaminants on marine organisms remains largely unknown, especially in Asia. This work elucidates the occurrence and the ecological risks of seven UV filters detected in farmed fish, wild mussels and some other wild organisms collected from local mariculture farms in Hong Kong. For all of the organisms, ethylhexyl methoxycinnamate (EHMC) and octyl dimethyl *p*-aminobenzoic acid (OD-PABA) were the predominant contaminants with the highest concentrations up to 51.3 and 24.1 ng/g (dw), respectively; lower levels were found for benzophenone-8 (BP-8), octocrylene (OC) and benzophenone-3 (BP-3) from <LOQ to <14.4 ng/g (dw); 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene camphor (3-BC) were rarely detected. Additionally, the detection frequencies and measured concentrations of all targets were clearly higher in mussels than in fish. Spatial distribution of studied UV filters indicated a positive correlation between their measured concentrations and the anthropogenic activities responsible for their direct emission. The ecological risk assessment specific to the marine aquatic environment was carried out. The risk quotient (RQ) values of EHMC and BP-3 were calculated as 3.29 and 2.60, respectively, indicating these two UV filters may pose significant risks to the marine aquatic environment.

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* Corresponding author.
E-mail address: s9362284@hkbu.edu.hk (K.S.-Y. Leung).

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1. Introduction

Organic ultraviolet (UV) filters, as the active ingredients in sunscreen products, block solar radiation by absorbing UV-A (320–400 nm) and UV-B (280–320 nm) (Nakata et al., 2009). Nowadays, the growing concern over the risks associated with UV radiation exposure have popularized their inclusion in a wide array of personal care products in addition to sunscreens, such as cosmetics, skin lotions, hair dye and shampoos as well as in some packing materials and plastics products (Li et al., 2007; Coltro et al., 2003; Zenker et al., 2008). As a consequence of their extensive consumption, these chemicals inevitably enter the aquatic ecosystem not only directly through recreational activities in water or by the shore but also indirectly via wastewater treatment plants (WWTPs) release (Groz et al., 2014) sourced from water used for showering, laundry and even excretion (Asimakopoulos et al., 2014).

Preliminary animal tests have indicated that at least a few commonly used UV filters such as BP-3, 4-MBC, 3-BC and EHMC (Inui et al., 2003; Kunz and Fent, 2006; Kunz et al., 2006a; Schlumpf et al., 2004) can disrupt endocrine function. Current toxicity information suggests widespread application of these substances could be risky to the aquatic ecosystem. Moreover, due to their high lipophilicity, UV filters are readily concentrated and accumulated in living aquatic organisms, such as fish and mussels, which has been documented by several research groups in Europe (Gago-Ferrero et al., 2015; Gomez et al., 2012; Fent et al., 2010). Bioaccumulation of EHMC and OC has been observed in Gomez's study (Gomez et al., 2012). With the exposure concentrations of around 11.0 μg/L, EHMC was accumulated in the soft tissue of marine mussels (*Mytilus galloprovincialis*) from 15 ng/g (dw) to 38 ng/g (dw) over a period of 48 h, and OC was accumulated from <2 ng/g (dw) (below LOD) to 60 ng/g (dw). Another study found that when *Pimephales promelas*, a type of minnow, was exposed to 3-BC for 21 days, an average bioconcentration factor was found up to 313 (Kunz et al., 2006b). In the long term, UV filters could bring even greater damage to aquatic ecosystem since their exceptional bioavailability may magnify their environmental impact as these xeno-estrogens accumulate along the aquatic food web (Fent et al., 2010; Gago-Ferrero et al., 2015).

As far as we know, knowledge of occurrence for UV filters in biota samples is still limited, especially in Asia. 4-MBC was firstly reported in the occurrence study focusing on various marine organisms collected from the Ariake Sea, in Japan, with an undetectable level in any tested samples (Nakata et al., 2009). Recently in South China, BP-3, 4-MBC and OP-PABA were detected in the marine fish and other wild organisms, which were collected from the Pearl River Estuary, within a concentration range of 0.1–41.5 ng/g (dw), while EHMC and OC were not detected in this study (Peng et al., 2015). Contrarily, relatively ampler studies and information of UV filters found in aquatic organisms were reported in Europe. BP-3, EHMC, OC and OD-PABA are the predominant sunscreen compounds that frequently detected in the marine organisms collected along the coasts of Norway, Portugal and France, with much higher concentration levels up to >800–1000 ng/g (dw); these organisms mainly include cod, shrimp, and mussels (Bachelot et al., 2012; Groz et al., 2014; Langford et al., 2015). Besides in marine organisms, EHMC, OC, BP-3 and 4-MBC have also been detected in aquatic organisms collected from rivers and lakes, mostly in Spain and Switzerland (Balmer et al., 2005; Buser et al., 2006; Fent et al., 2010; Gago-Ferrero et al., 2015), albeit with relatively lower concentrations (mainly below 700 ng/g (lipid weight)) compared to those in marine organisms. Additionally, OC was once detected in the liver tissue of Franciscana dolphins – a kind of aquatic mammals collected in Brazilian coast, within the concentration range of 89–782 ng/g (lipid weight) (Gago-Ferrero et al., 2013a). Combining the environmental risks of UV filters along with the limited information of their occurrence associated with aquatic organisms in Asia, a comprehensive study is necessary so as to support good environmental management, both in Hong Kong and worldwide.

The objectives of this study were to (i) make an investigation on the occurrence of seven UV filters in marine organisms, namely BP-3, BP-8, 4-MBC, 3-BC, OD-PABA, EHMC and OC; (ii) perform their spatial distribution in farmed fish, wild mussels and other organism samples collected in Hong Kong coastal environment; and (iii) evaluate the ecological risks of the present UV filters pose to the marine aquatic environment.

2. Materials and methods

2.1. Chemicals and reagents

Chemical standards for BP-3 (CRM), BP-8 (98%), 4-MBC (>99%), EHMC (CRM) and OC (CRM) were obtained from Sigma-Aldrich (St. Louis, MO, USA); OD-PABA (95%) was purchased from TCI (Shanghai, China); 3-BC (90–100%) was obtained from MP Biomedical (Irvine, CA, USA). 1000 mg/L individual stock solution of each UV filter was prepared in pure methanol and then stored in sealed amber glass vessels at 4 °C. A mixture of standard solutions of seven UV filters at the concentration of 10 mg/L was prepared daily and diluted to 1 mg/L for spiking when needed.

Solvents including Milli-Q water (Millipore, Bedford, MA, USA) LC-MS grade methanol, dichloromethane (DCM), ethyl acetate (AcEt) (Duksan Pure Chemicals, Seoul, Korea), absolute ethanol (Uni-Chem, Orientalab, China) and formic acid (98% purity, Merck, Rahway, NJ, USA) were employed in this study. Diatomaceous earth and cellulose filter papers (19.8 mm) were purchased from Dionex (Sunnyvale, CA, USA).

2.2. Sample collection and preparation

Farmed fish, wild mussels and some other wild organisms (including prawns, conch and sea urchin) were collected in June to July 2015, from seven local mariculture farms located around the coast of Hong Kong. The farms were selected such that they geographically represent the entire mariculture area of this region. Hong Kong is situated on the southern coast of China; it is surrounded by sea on three sides. Currently, there are 26 mariculture zones distributed on the eastern and southern coasts of the city (AFCD, 2006). In all of these zones, mariculture farms are usually and preferably placed in sheltered areas, far from pollution sources. The seven sampling locations are shown in Fig. 1, namely Sham Wan (1-SW), Yim Tin Tsai (2-YTT), Tai Tau Chau (3-TTC), Tung Lung Chau (4-TLC), Sok Kwu Wan (5-SKW), Cheung Sha Wan (6-CSW) and Ma Wan (7-MW).

Among these locations, 1-SW and 4-TLC are the most remote from urban areas; while 2-YTT and 3-TTC are located in Tai Mei Tuk and Sai Kung, respectively, which are two areas famous for seaside and water recreational activities. 5-SKW is close to the pier on Lamma Island, near a popular tourist fishing village with a population of >6000 residents. 7-MW is also near a small traditional fishing village on the shore near Tuen Mun but the fishery there is on the wane. 6-CSW is situated in a secluded bay on the southeastern corner of Lantau Island, the largest inland in Hong Kong.

The farmed fish samples collected from these seven locations were: mangrove snappers (*Lutjanus argentimaculatus*) from 1-SW, 2-YTT and 4-TLC; white blotched snappers (*Lutjanus stellatus*) from 5-SKW and 6-CSW; and Sabah giant groupers (*Epinephelus lanceolatus*) from 3-TTC and 7-MW. For each location, three samples of fish reared in different floating rafts were randomly collected (except 3-TTC and 7-MW, where only two fish from each could be caught) with the body length between 30 and 40 cm; triplicate measurements were carried out for each fish sample. Most of these cultured fish are mainly fed with moist or dry pellet feed; trash fish are also sometimes used as feed. The wild mollusk species including green mussels (*Perna viridis*) and clams (*Mactra antiquata* and *Corbicula* sp.) were collected in five locations (i.e., all except 5-SKW and 7-MW). A mixture of 15 individuals

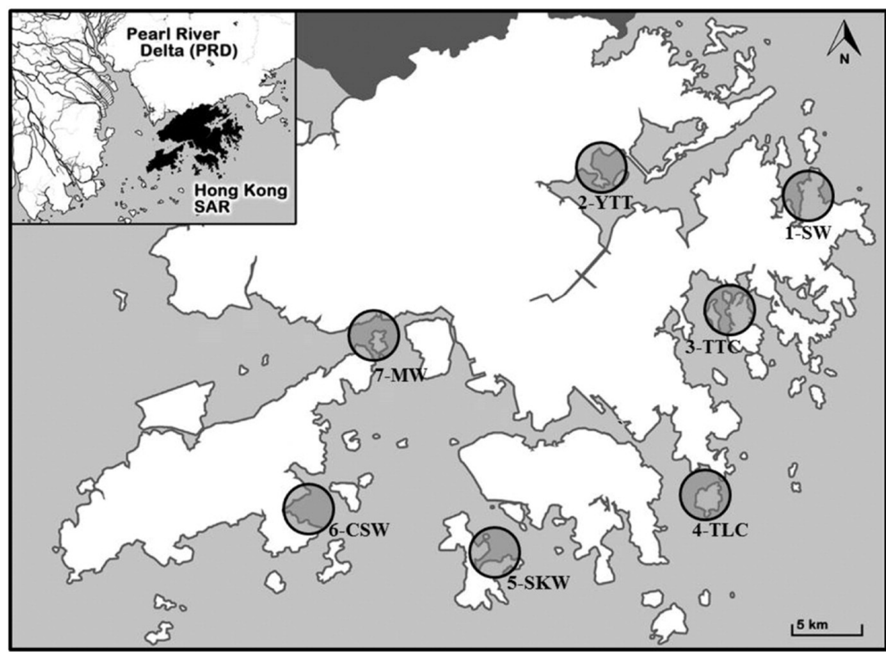


Fig. 1. Map of Hong Kong showing sampling locations of seven local mariculture farms.

with similar body length (5–8 cm) was regarded one complete sample, representative for one location. Triplicate analyses of one complete mussel sample (15 individuals) were carried out for each location as well. Other wild marine organisms were also collected occasionally such as tiger prawns (*Penaeus monodon*), conch (*Babylonia* sp.) and purple sea urchin (*Anthocidaris crassispina*), since it was not always possible to find them. Totally, there were 28 marine samples collected in this study.

Samples were individually packaged in single-used plastic bags and kept in a portable insulated container with ice packs to maintain the organisms alive and fresh during the one-hour transportation from the field to the laboratory. Treatment of samples was begun as soon as they arrived in the laboratory. For fish samples, evisceration and removal of skin, tail, head, fins and bones was carried out, such that only filets were used for testing. Mussels and other non-scaly marine organisms were cleaned of sedimentary materials and deshelled so that the soft tissues of the whole body were used. Filets and soft tissues were then homogenized individually and stored at −20 °C/−80 °C for no <24 h before lyophilization. After that, the totally dried samples were ground into fine powder that was individually packed in zip-lock bags and placed in an opaque desiccator for further use.

2.3. Analytical procedures

The extraction method for biota samples was modified from Gago-Ferrero et al. (2013b). After accelerated solvent extraction (ASE) (ASE 200, Dionex Corporation, Sunnyvale, CA, USA), the sample solution was further purified using Supelclean LC-18 solid-phase extraction (SPE) cartridges (500 mg/3 mL, Supelco, PA, USA). Each cartridge was conditioned with 3 mL × 3 of methanol follow by 3 mL × 3 of Milli-Q water. Then the sample solution was loaded onto the cartridge at the speed of 1 drop/s. After drying for 30 min under vacuum, the extractant was eluted with 3 mL methanol followed by two aliquots of 2.5 mL ethanol at a rate of 1 drop/2 s. Finally, the eluent was evaporated under a gentle nitrogen stream with purity of 99.9% until totally dried and then reconstituted with 1 mL methanol. Before instrumental analysis, the reconstituted eluent was syringe-filtered via 0.22 μm PTFE (Grace, IL, USA).

2.4. Instrumental analysis

Instrumental analysis was performed using an Acquity Ultra Performance LC system hyphenated to a Quattro Ultima triple quadrupole mass spectrometer (Waters, MA., USA). The UPLC system comprises an auto-injector with a 10-mL sample loop and a temperature-controlled column compartment. Chromatographic analysis for UV filters was carried out with an Acquity BEH C18 column (2.1 i.d. × 50 mm, 1.7 μm, Waters); an injection volume of 4 μL was used for all samples. The mobile phases consisted of a mixture of milliQ water (A) and LC-MS grade methanol (B), both with 0.1% (v/v) formic acid added. The elution program protocol entailed a total run time of 5.5 min as follows: elution started with 65% of mobile phase B for 0.5 min, followed by 100% for 1.7 min; elution gradient was held at 100% for 1 min, then returned to 65% for 0.3 min, and initial conditions were reached in the final 2 min. The constant flow rate of 0.4 mL/min was applied throughout the elution program.

MS/MS detection was performed with electrospray interface operated in positive ionization mode (ESI+) at the capillary voltage of 1.6 kV. The source and desolvation temperatures were set at 150 °C and 500 °C, respectively. Nitrogen was used with a cone gas flow of 150 L/h nebulization and of 1000 L/h for desolvation. The quantitative analysis was performed in selected reaction monitor (SRM) mode. Two major characteristic fragments of the protonated molecular ion $[M + H]^+$ were monitored for each analyte to enhance the sensitivity and selectivity. The retention time (t_R), SRM transition pairs, cone voltages and collision energies are shown in Table 1. Instrument control, data acquisition and processing were performed using MassLynx software.

2.5. Analytical method validation

Five-point standard calibration curves were plotted within the concentration range of 0–100 ng/g, by spiking the standard solutions of UV filters into the sample-free blank solutions comprising 5 mL ethanol and 45 mL Milli-Q water before SPE. The linearities were calculated using the least square method. Recovery test was performed by spiking UV filter standard solutions at three concentrations, namely 8, 40 and 400 ng/g (dw), in the market samples, and the sample-free blanks as

Table 1
SRM conditions used in UPLC-MS/MS determination of UV filters.

UV filters	Chemical structure	Log Kow[a]	Retention time (min)	Molecular mass	SRM transition (m/z)	Cone voltage (V)	Collision energy (eV)
BP-8		4.31	0.89	244.24	245.08 → 121.00	24	18
					245.08 → 151.03		20
BP-3		3.52	1.34	228.25	229.09 → 151.03	26	20
					229.09 → 105.04		20
3-BC		4.49[b]	1.79	240.34	241.18 → 91.01	26	36
					241.18 → 97.01		18
4-MBC		5.47	2.01	254.37	255.15 → 105.02	26	30
					255.15 → 97.01		18
OC		6.88	2.25	361.47	362.25 → 250.13	24	8
					362.25 → 232.10		20
OD-PABA		5.77	2.33	277.40	278.18 → 150.97	30	30
					278.18 → 166.09		22
EHMC		5.80	2.38	290.40	291.18 → 179.11	12	8
					291.18 → 161.00		18

[a] Kameda et al. (2011).
[b] Zenker et al. (2008).

well as triplicate tests were carried out to test repeatability. Recoveries were expressed as the ratio of concentrations calculated from the spiked samples to spiked blanks. LOD and LOQ were determined by spiking the UV filter standard solutions into the sample-blanks and then using the signal-to-noise ratios of 3 times and 10 times for calculations.

3. Results and discussion

3.1. Method validation

3.1.1. Validation results

Validation results including linearity (R^2), recovery, repeatability (RSD), LOD and LOQ for each UV filter are summarized in Table 2. In previous studies, wide concentration ranges for UV filters detected in aquatic organisms have been reported. For example, Peng et al. reported 0.11–1.52 ng/g (dw) of BP-3 in wild marine organisms (Peng et al., 2015), while Fent et al. reported up to 151 ng/g lipid, also in marine organisms (Fent et al., 2010). OC was detected in mussels in a wide concentration range of <2–7112 ng/g (dw) (Bachelot et al., 2012). Therefore, in order to accommodate a wider working range, three standard calibration curves covering the low (0–100 ng/g (dw)), medium (0–500 ng/g (dw)) and high (0–1000 ng/g (dw)) concentration ranges were validated. In our study, most of the detected UV filters were in a low concentration, a five-point standard calibration curve with a linear range of 0–100 ng/g (dw). $R^2 > 0.995$ was obtained for all targets. Recovery was tested by spiking the standard solutions at three concentration levels which were 8 (low), 40 (medium) and 400 (high) ng/g (dw) so as to ensure a high accuracy of the method. For all of the analytes, recoveries were in the range of 80.4–114.8% and the variation was determined to be random, which means no bias was observed in any of the UV filters at the three concentrations. Also in this step, triplicate measurements were performed for these three concentration levels. Good repeatability of RSD ≤ 8% (n = 3) for all was obtained. LOD was calculated as 3 times the signal-to-noise ratio, and found to be in the range of 0.9–1.9 ng/g (dw). LOQ was calculated as 10 times the signal-to-noise ratio and found to be in the range of 2.9–6.2 ng/g (dw). These results are comparable to those reported in previous studies (Gago-Ferrero et al., 2013a, 2015; Groz et al., 2014), and it is reasonable to apply this method for the analysis of real samples.

3.1.2. Matrix effect study

Due to the complexity of the organisms' matrices, which may affect the analytical performance of the developed method, evaluation of the matrix effect was carried out using imported frozen aquatic products brought from the local market, namely fish of red bigeye (*Priacanthus macracanthus*), blue mussels (*Mytilus edulis*) and tiger prawns (*Penaeus*

Table 2
Summaries of the analytical method validation test results.

UV filters	Linearity (R^2) (range: 0–100 ng/g dw)	LOD (ng/g dw)	LOQ (ng/g dw)	Recoveries (RSD%)			
				Spiked levels (ng/g dw)	Fish	Mussels	Prawns
EHMC	0.9975	1.2	4.0	8	96.9% (1.7)	93.7% (6.7)	92.7% (4.3)
				40	94.6% (5.9)	80.4% (3.5)	112.3% (7.3)
				400	99.8% (5.5)	82.1% (2.5)	112.2% (2.4)
OD-PABA	0.9976	1.9	6.2	8	89.2% (5.9)	90.0% (3.1)	94.4% (5.6)
				40	108.2% (2.8)	88.8% (4.1)	111.4% (2.9)
				400	110.0% (8.0)	109.8% (3.0)	99.3% (0.5)
BP-8	0.9968	0.9	3.0	8	97.6% (1.8)	93.5% (2.3)	91.0% (5.3)
				40	88.1% (1.9)	89.9% (5.1)	89.7% (4.7)
				400	91.2% (2.4)	95.7% (0.7)	102.4% (0.7)
OC	0.9985	1.4	4.5	8	94.6% (6.3)	98.0% (4.7)	95.7% (7.4)
				40	110.3% (7.5)	86.6% (6.2)	106.8% (7.0)
				400	113.1% (7.4)	83.7% (8.0)	96.1% (6.9)
BP-3	0.9958	0.9	2.9	8	96.4% (1.1)	95.5% (1.0)	98.3% (2.0)
				40	113.0% (3.4)	102.9% (1.5)	92.2% (3.9)
				400	94.8% (4.2)	99.0% (3.2)	114.8% (2.4)
4-MBC	0.9989	1.8	6.0	8	88.6% (6.0)	95.0% (3.5)	94.8% (4.8)
				40	91.5% (7.2)	94.0% (4.9)	111.4% (7.5)
				400	96.4% (5.7)	90.9% (1.7)	113.8% (1.9)
3-BC	0.9975	1.8	6.0	8	91.4% (1.1)	90.9% (1.6)	96.6% (6.5)
				40	88.6% (5.2)	95.5% (6.3)	114.3% (7.9)
				400	86.6% (5.0)	89.6% (0.7)	94.5% (2.5)

monodon). The matrix effect of the marine organisms on each procedure throughout the whole analytical method was investigated step by step, including ASE extraction, SPE purification and instrumental analysis. Standard solution of UV filters at a concentration of 400 ng/g (dw) was pre- and post-spiked into the market samples and into the sample-free controls for each investigated analytical procedure. The matrix effects were expressed as the ratio of the pre-spiked signal abundance to relevant post-spiked signal abundance. Afterwards, the results were compared with those obtained from the sample-free controls (e.g. $\text{sample}_{[\text{pre-ASE spiked/post-ASE spiked}]}$ vs. $\text{sample-free}_{[\text{pre-ASE spiked/post-ASE spiked}]}$), and the calculated values <1 or >1 represented the signal suppression or enhancement, respectively, caused by the matrix effects corresponding to each analytical step.

The results indicated that the matrices of fish, mussels and prawns had no significant influence on the extraction efficiencies of ASE. At the same time, the signal suppression and enhancement in the instrumental analysis (UPLC-MS/MS) caused by the matrix effects of the three species was acceptable, ranging from 81.2% to 118.4%. However, with respect to the matrix effects on SPE purification efficiencies, the recoveries for three species calculated as pre-SPE spiking/post-SPE spiking of OD-PABA, EHMC and OC were $<78\%$ and generally lower than the values for the other UV filters. To solve this, the standard calibration curves were finally created by spiking the relevant concentrations into 50-mL solvent blanks (5 mL ethanol and 45 mL Milli-Q water) and subsequently loading those solutions onto the SPE cartridges using the same process for the sample solutions.

During the measurement of real samples, three groups of fishes including mangrove snappers, white blotched snappers and Sabah giant groupers were collected from seven local mariculture farms and were further analyzed to present the spatial distribution of seven UV filters in Hong Kong marine environment. Thus, it is necessary to evaluate the matrix effects sourced from three different fishes, since it is not always available to collect same species during the sampling. Standard solutions of UV filters were spiked into the fish samples and the sample-free controls before ASE. After analysis, the signal abundances for all seven UV filters obtained from each group of fish were compared to those from the sample-free controls. As the result, the variation ranged from 88 to 117% for three fishes collected from all seven locations, and no bias was found for any UV filters. Therefore, it is reasonable to use the measurement of our fish samples to present the distribution of target UV filters.

3.2. Environmental occurrence of UV filters in marine organisms

3.2.1. Concentrations in marine organisms

In this study, almost none of the seven targets UV filters were detected in the other wild organisms that occasionally collected (including prawns, conch and sea urchin), except EHMC, OD-PABA and BP-8 were only once detected in a conch, at the concentration levels below LOQ. For the fish and mussels, five sunscreen compounds namely EHMC, OD-PABA, BP-8, OC and BP-3 were commonly detected in the collected samples, whereas 4-MBC and 3-BC were rarely found (specifically, 4-MBC was only detected in one mussel sample at the concentration below LOQ and 3-BC was never observed in this study). Among all the UV filters studied, EHMC was determined at the highest concentrations both in fish and mussels, and it had the highest detection frequency of 75% in all samples (see Tables 3A and 3B). The concentration range of EHMC from the mean value to the maximum detected in fish was 4.2–12.7 ng/g (dw) and in mussels it was measured as 21.9–51.3 ng/g (dw) (detection frequency of 100% for mussels). OD-PABA was also measured at relatively higher concentrations compared to other UV filters; specifically, it was detected in a range of 14.2–24.1 ng/g (dw) in mussels, while from below LOQ to 10.3 ng/g (dw) in fish. Lower concentrations were similarly observed for BP-8, OC and BP-3, which were as high as 4.9, 5.4 and 3.1 ng/g (dw) in fish and 14.4, 11.6 and 12.4 ng/g (dw) in mussels, respectively. However, for these three UV filters, the

Table 3A
Mean concentrations $\pm$ S.D. of UV filters detected in marine organisms (ng/g dw), triplicate measurements ($n = 3$) with RSD $\leq 8\%$.

Sampling location	Species	EHMC			OD-PABA			BP-8			OC			BP-3			4-MBC			3-BC		
1-SW	Fish	n.d.	n.d.	N.Q.	n.d.	n.d.	N.Q.	N.Q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mussel	N.Q.			N.Q.			N.Q.			n.d.			n.d.			n.d.			n.d.		
	Conch	N.Q.			N.Q.			N.Q.			n.d.			n.d.			n.d.			n.d.		
2-YTT	Fish	5.5 $\pm$ 0.2	6.2 $\pm$ 0.3	N.Q.	6.4 $\pm$ 0.2	N.Q.	N.Q.	4.2 $\pm$ 0.1	4.9 $\pm$ 0.1	N.Q.	5.4 $\pm$ 0.1	N.Q.	N.Q.	N.Q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Queen clam	24.6 $\pm$ 0.6			18.7 $\pm$ 0.4			10.7 $\pm$ 0.4			N.Q.			9.7 $\pm$ 0.3			n.d.			n.d.		
	Sand clam	33.1 $\pm$ 0.7			22.6 $\pm$ 0.6			13.5 $\pm$ 0.4			11.6 $\pm$ 0.5			12.4 $\pm$ 0.5			n.d.			n.d.		
3-TTC	Fish	9.3 $\pm$ 0.2	12.7 $\pm$ 0.2	-	7.2 $\pm$ 0.2	10.3 $\pm$ 0.2	-	4.8 $\pm$ 0.1	N.Q.	-	N.Q.	N.Q.	-	3.1 $\pm$ 0.1	N.Q.	-	n.d.	n.d.	-	n.d.	n.d.	-
	Mussel	51.3 $\pm$ 1.5			24.1 $\pm$ 0.8			14.4 $\pm$ 0.4			8.8 $\pm$ 0.3			10.3 $\pm$ 0.4			N.Q.			n.d.		
	Prawn	n.d.			n.d.			n.d.			n.d.			n.d.			n.d.			n.d.		
4-TLC	Fish	n.d.	n.d.	N.Q.	n.d.	N.Q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mussel	N.Q.			n.d.			n.d.			n.d.			n.d.			n.d.			n.d.		
5-SKW	Fish	8.8 $\pm$ 0.2	6.4 $\pm$ 0.1	N.Q.	7.1 $\pm$ 0.2	9.7 $\pm$ 0.2	N.Q.	3.8 $\pm$ 0.1	4.7 $\pm$ 0.2	N.Q.	N.Q.	N.Q.	n.d.	n.d.	3.0 $\pm$ 0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Sea urchin	n.d.			n.d.			n.d.			n.d.			n.d.			n.d.			n.d.		
6-CSW	Fish	n.d.	4.1 $\pm$ 0.1	N.Q.	n.d.	N.Q.	N.Q.	N.Q.	N.Q.	3.2 $\pm$ 0.1	n.d.	n.d.	N.Q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mussel	17.5 $\pm$ 0.3			16.2 $\pm$ 0.3			8.9 $\pm$ 0.2			N.Q.			N.Q.			n.d.			n.d.		
7-MA	Fish	N.Q.	5.1 $\pm$ 0.1	-	7.3 $\pm$ 0.2	6.8 $\pm$ 0.1	-	4.1 $\pm$ 0.2	N.Q.	-	n.d.	4.7 $\pm$ 0.1	-	n.d.	N.Q.	-	n.d.	n.d.	-	n.d.	n.d.	-

n.d.: <LOD.
N.Q.: <LOQ.
-: no sample collected.

Table 3B
Detection frequencies of detected UV filters in marine organisms.

Sample size	EHMC	OD-PABA	BP-8	OC	BP-3	4-MBC	3-BC
All samples (28)	75.0% (21/28)	71.4% (20/28)	71.4% (20/28)	46.4% (13/28)	32.1% (9/28)	3.6% (1/28)	0.0% (0/28)
Fish (19)	73.7% (14/19)	73.7% (14/19)	73.7% (14/19)	47.4% (9/19)	26.3% (5/19)	0.0% (0/19)	0.0% (0/19)
Mussels and clam (6)	100% (6/6)	83.3% (5/6)	83.3% (5/6)	66.7% (4/6)	66.7% (4/6)	16.7% (1/6)	0.0% (0/6)
Others organisms (3)	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

mean values were all measured below LOQ in fish, while they were calculated as 8.2, 5.1 and 5.9 ng/g (dw), respectively, in mussels. Beyond that, the detection frequencies for BP-8 in both fish and mussels were as high as those of OD-PABA, namely 73.4% and 83.3%, respectively, and they were much higher than the detection frequencies of OC and BP-3. In general, the concentrations of each UV filter in both fish and mussels showed the same descending order of concentration, as EHMC > OD-PABA > BP-8 > OC ≈ BP-3.

EHMC was the most frequently detected UV filter in environment samples (such as wastewater, surface water, seawater, sediment and biota) and was always found to be at high concentration levels (Zenker et al., 2008; Tsui et al., 2014a, 2014b; Tsui et al., 2015; Gago-Ferrero et al., 2015; Gago-Ferrero et al., 2013b). These high concentrations are likely due to its chemical properties—EHMC has good stability and high lipophilicity—as well as to the high consumption of products containing it and the failure of WWTPs to remove it (Li et al., 2007; Balmer et al., 2005). The concentration limits of EHMC contained in cosmetics and personal care products are as high as 10–20% depending on the different regulations worldwide, which are the highest limitations among all the organic UV filters (Sánchez-Quiles and Tovar-Sánchez, 2015). Moreover, the percentage of the personal care products containing EHMC reached 90% in China (Zhang et al., 2008; Tsui et al., 2015). At the same time, much higher loading of EHMC in WWTPs (as high as 2.3–119 g per 10,000 persons per day) was reported in Zurich, when compared to other commonly used UV filters (Balmer et al., 2005). Furthermore, it has been reported that the removal efficiencies of EHMC in most WWTPs in Hong Kong were mainly below 60% (Tsui et al., 2014b). Therefore, the large consumption in daily life along with the limited removal efficiencies in the WWTPs contribute to the massive amount of EHMC discharged into the environmental waters. In our previous study on the occurrence of UV filters in the seawaters in Hong Kong, EHMC was detected at the highest concentration of all filter studied, namely 191.67 ng/L in coastal water (Sang, 2016. Table A2). In addition, its relatively higher lipophilicity—with log Kow value of 5.80 (Kameda et al., 2011)—makes it much easier for biota to absorb and accumulate this UV filter from the aqueous phase. As a result, high concentrations of EHMC were observed in marine organisms.

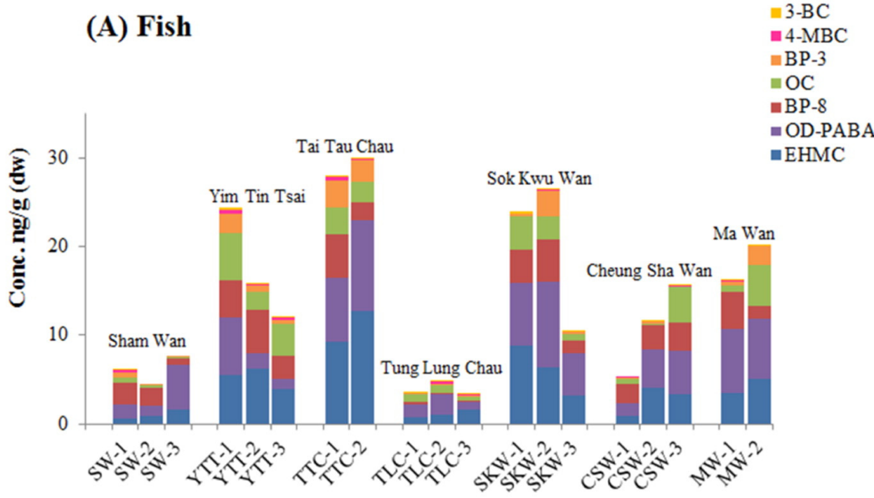
Similar to EHMC, OD-PABA also has a high log Kow value (5.77) and is permitted at high contents in commercial products. Formulations with concentrations as high as 8% are permitted in Europe and China, while up to 10% are allowed in Japan. Moreover, the detection frequencies higher than 75% for OD-PABA along with a concentration up to 224 ng/L in the effluents of WWTPs in Hong Kong indicated the incomplete elimination of this compound and its inevitable emission into the local water environment (Tsui et al., 2014b). Thus, the relatively higher concentrations of OD-PABA measured in the marine organisms in this study may be related to its high usage and its higher log Kow value (Kameda et al., 2011) as well. It has been reported that EHMC and OD-PABA are the predominant UV filters in sediments in Hong Kong (Tsui et al., 2015). In contrast, OC, which is also frequently detected in environmental waters, also has high lipophilicity and good stability, and is also widely used in personal care products, was not found in high concentrations in marine organisms in this study. The reasons for this phenomenon remain unclear at this stage and further studies are still needed. Coincidentally, similar circumstance for OC was also observed in Tsui's study (Tsui et al., 2015), in which lower concentrations of OC was detected in the sediment samples collected in marine of Hong Kong when compared to the other UV filters with similar log Kow values. An observation of extremely low detection frequencies for OC in the influents and effluents of five local WWTPs (Tsui et al., 2014b) may imply a rather lower emission of this compound into the environmental water. Moreover, Bachelot has indicated that OC may be not as persistent as EHMC when investigated in mussels collected from French coastal regions (Bachelot et al., 2012). He reported that OC was detected up to >1000 ng/g (dw) in June to August in mussel samples, while dropping to as low as total absence in September and November; in contrast, EHMC remained present in mussels even after the summer. Therefore, to acquire better knowledge on the environmental behaviors of these sunscreen compounds in aquatic biota, further studies on their uptake, metabolism and excretion mechanisms in the organisms are highly demanded.

BP-3 is another UV filter, widely detected in environmental waters, widely consumed and with a high content in personal care products and cosmetics—up to 10% allowed by regulations in both Europe and China (Sánchez-Quiles and Tovar-Sánchez, 2015). However, due to its lower log Kow value of 3.52 (Kameda et al., 2011), meaning it is less lipophilic than other UV filters, the concentrations of BP-3 detected in the biota samples were not as high as those in environmental waters compared with EHMC and OD-PABA. Another aspect to consider is the metabolism of this UV filter. Many studies have proved that BP-3 is readily metabolized (Kunz and Fent, 2006). As a major metabolic product of BP-3, BP-8 has a higher log Kow value of 4.31 and higher bio-concentration factor (BCF = 524) compared to its parent compound (BCF = 502) (Kim and Choi, 2014). This high lipophilicity could explain why BP-8 is often found in the sediments. To the best of our knowledge, 3-BC has never been detected in marine organisms. 4-MBC was often reported as not detected or in extreme low concentrations in aquatic organisms for those previous studies in USA, Spain and Japan (Gago-Ferrero et al., 2015; Mottaleb et al., 2009; Nakata et al., 2009).

3.2.2. Spatial distribution in Hong Kong

Seven sampling locations along the coastline of Hong Kong were briefly introduced in Section 2.2. Fig. 2A and B show the spatial distributions of the UV filters detected in the fish and mussels, respectively, with regard to the local mariculture farms from which the samples were taken. The maximum total concentrations of seven investigated UV filters in the farmed fish were observed at the location 3-TTC, followed by 5-SKW and 2-YTT (Fig. 2A). The mussels showed the same concentration pattern (Fig. 2B), with the highest concentrations of all detected UV filters being reported at 3-TTC. Relatively higher levels were also detected in sand clams and queen clams both collected in 2-YTT. Relevant information for 5-SKW was absent since no mollusk species was collected there. In contrast, the lowest concentration levels of the target compounds were found at 4-TLC and 1-SW for both fish and mussels. Especially at 4-TLC, only EHMC and OD-PABA were occasionally detected there at concentrations even lower than LOQ. Additionally, the median levels of UV filters measured in the marine organisms were observed at 7-MW and 6-CSW.

Since all the sampling locations in this study were at official mariculture farms in Hong Kong, they were as far away from sewage discharge outlets as possible in order to avoid pollution via the effluent of WWTP. Thus, any indirect input of UV filters from incomplete treatment in the WWTP may not be a focus of concern in this study. Even so, there is always some chance of indirect input, and this should not be ignored.



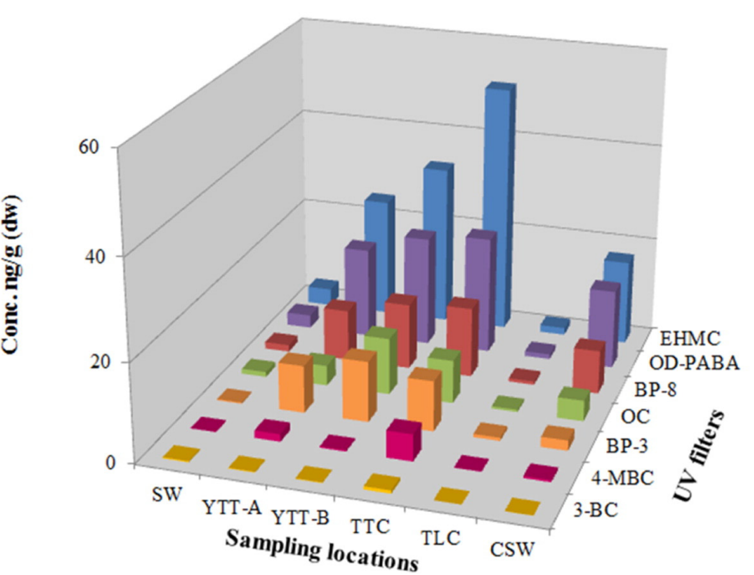


Fig. 2. Distribution of detected UV filters in (A) fish and (B) mussels and clams along the coast of Hong Kong. SW, TTC, TLC and CSW: Mussels. YTT-A: Queen clams. YTT-B: Sand clams.

Nevertheless, assuming that input is negligible, further discussion here will focus on the direct input of UV filters into the ecosystem. As previously described, 4-TLC and 1-SW, where the minimum concentrations of UV filters were detected, are remote from urban areas and sparsely populated. As a result, the anthropogenic activities and influence there is less intensive compared to the other locations and thus the pollution caused by the directly input of UV filters should be limited. Conversely, 3-TTC, 2-YTT and 5-SKW, where the relatively larger amounts of UV filters were measured, are famous places for intensive recreational activities such as water sports, seaside leisure activities and tourism, not only in summer but all year round. These activities represent enormous opportunities for the substances contained in personal care products such as UV filters to enter the ecosystem directly and easily. We did indeed find a positive correlation between the measured concentrations and the anthropogenic activities; from this it can be inferred that the direct input of the UV filter compounds related to the anthropogenic activities significantly affects the their occurrence in marine organisms. Such direct input could result in rapid and even serious effects compared to indirect input way because the substances might directly and rapidly interact with the biota once entering the ecosystem.

3.2.3. Global comparison

So far, many studies have focused on the occurrence of UV filters in environmental waters especially coastal water, rivers, lakes, and WWTP effluent (Zenker et al., 2008; Tsui et al., 2014a, 2014b; Tsui et al., 2015). Studies of the occurrence of UV filters in marine organisms, however, are very few. Up to now, most of the studies have come from Europe, with only a few from Asia. In Europe, BP-3, EHMC, OC and OD-PABA are the predominant UV filters detected, and they were reportedly determined in coastal biota samples collected from Norway, France and Portugal at concentrations higher than thousands ng/g (dw) (Langford et al., 2015; Bachelot et al., 2012; Groz et al., 2014). In Oslo, Norway, the concentration levels and detection frequencies of BP-3, OC and EHMC were found to be higher in cod liver than in shrimp. BP-3 and OC were measured at levels as high as 1037 and 11,875 ng/g (ww), whereas EHMC was determined at comparatively lower concentrations, in the range of 30–36.9 ng/g (ww). None of the UV filters were

detected in any wild shore crab samples in this study (Langford et al., 2015). In France, in a study of mussels collected both from the coastal regions of the Atlantic and Mediterranean, 100% of the samples had quantifiable EHMC, ranging from 3 to 256 g/g (dw), whereas 55% of the samples had detectable amounts of EHMC, ranging from <2 to 7112 ng/g (dw). In summary, EHMC was the most frequently detected UV filter while OC was at its highest concentration in mussels. Interestingly, OD-PABA was never detected in this study (Bachelot et al., 2012). Also in mussels collected from the coasts of Portugal, OC was measured at the maximum concentration of 3992 ng/g (dw), while EHMC and OD-PABA reached levels up to 1765 and 833 ng/g (dw), respectively (Groz et al., 2014). Viewing the studies in freshwater biota, all the UV filters mentioned above (BP-3, EHMC, OC and OD-PABA) together with 4-MBC were detected at comparable or a little lower concentration levels in aquatic organisms sampled from the rivers and lakes, mainly in Switzerland and Spain (Gago-Ferrero et al., 2015; Fent et al., 2010; Zenker et al., 2008; Buser et al., 2006). Another similar study on fish samples collected from rivers and creeks in the USA reported non-detectable levels of 4-MBC and OC (Mottaleb et al., 2009). Additionally, along the Brazilian coast, OC was detected at concentrations from 89 to 782 ng/g (lipid weight) in the liver tissue of dolphins (Gago-Ferrero et al., 2013a). Even less information on the occurrence of UV filters in marine biota is available for Asia. In the Ariake Sea of Japan, 4-MBC was not found in any of 55 marine organisms nor in sediments (Nakata et al., 2009). In south China, BP-3 and OD-PABA were detected in farmed fish and wild marine organisms collected from the Pearl River Estuary at extremely low levels, within ranges of 0.106–1.520 ng/g (dw) and 0.239–0.360 ng/g (dw), respectively. Surprisingly, the highest concentration of 4-MBC, up to 14.7 ng/g (dw), was measured in the filets of farmed fish. EHMC and OC were not detected in this study (Peng et al., 2015). In our study in Hong Kong, which is geographically located adjacent to the Pearl River Estuary, six UV filters were detected in marine fish and mussels with frequencies from 3.6% to 75% in the concentration range of <6.0–50 ng/g (dw).

Comparing the occurrence in marine organisms in Europe and Asia (Table A1), in general, the measured total concentrations of UV filters in East Asia are lower than those in Europe by at least 1–2 orders of magnitude; detection frequencies are also much lower. Probably the differences in habits associated with the use of skin care products between the two studied areas are the main reason for this observation. It has been reported that consumption of sunscreens is much higher in Europe than Asia. In 2012, the average regional consumption in Europe was reported as 52 mL, while the value in Asia Pacific was only 4 mL (Osterwalder et al., 2014). As a result of extremely high consumption, UV filters would inevitably enter the aquatic ecosystem as well as accumulate in aquatic organisms. Another factor contributing to regional differences could be the higher annual average temperature in East Asia, especially in South China, when compared with Europe. Higher temperature may accelerate the degradation and metabolism for the UV filters in the aquatic ecosystem and even inside organisms' bodies. More investigations are needed to determine the underlying reasons. Even so, there are some similarities between the two regions. The UV filters that predominate in both Europe and Asia are EHMC, BP-3 and OD-PABA. The occurrence of OC differs in these two areas. It was detected at relatively higher frequencies and concentrations in Europe whereas it was rarely detected, or detected only at limited levels, in East Asia.

3.3. Ecological risk assessment

According to the Technical Guidance Document (TGC) of European Commission on risk assessment (EC, 2003), the basic approaches to risk assessment for marine aquatic environments are very similar from those for freshwater environments; there are numerous common principles and objectives. Beyond that, the modification specific for the assessment of the marine environment is focusing on the assessment factor (AF) (10–10,000). However, the ecotoxicity data of compounds specific to the marine organisms are sometimes limited and not always available. Then, the ecotoxicity data derived from freshwater species are used (EC, 2003).

In this study, the measured environmental concentrations (MECs) of several UV filters detected in marine environment of Hong Kong (Sang, 2016; Table A2) are adopted to perform the risk assessment using approaches for both inland aquatic compartment and marine aquatic environment. The aim was to compare the differences in the assessment results derived from two series of AFs, and also in the ecological risks of UV filters posed to the freshwater and marine aquatic systems. The median and maximum MECs of BP-3, 4-MBC, EHMC and OC were used to calculate the $RQ_{general}$ and RQ_{worst}, respectively. For these four detected UV filters, the ecotoxicity information according to the aquatic species in one to three tropic levels both in freshwater and seawater are collected to get the predicted no effect concentrations (PNECs) via being divided by the AFs which are depended on the relevant data of ecotoxicity for each substance. Results and related information for the ecological risk assessment are given in Tables 3A and 3B.

Table 4A gives the results of ecological risk assessment for the marine aquatic environment. The ecotoxicity data with the highest sensitivity tested via the freshwater and saltwater organisms were used and the AFs of 1000 for BP-3, 4-MBC and EHMC while 10,000 for OC are used respectively for the risk assessment specific to marine system. The calculated $RQ_{general}$'s of four UV filters are as follows: (a) $RQ_{general}$ of OC < 0.001. This means, at the measured concentrations, OC poses an extremely low risk to the marine aquatic system. (b) $RQ_{general}$ of 4-MBC is 0.33. This is between 0.1 and 1, which means, at the measured concentrations, 4-MBC poses a medium potential risk to the marine environment. (c) $RQ_{general}$ of BP-3 is 2.60, while $RQ_{general}$ of EHMC is 3.29. Values > 1 indicate that both these UV filters are highly suspected of having an adverse effect on the marine aquatic environment (Hernando et al., 2006; Rodríguez et al., 2015). Considering its widespread occurrence and relatively high measured concentrations in the environmental waters as well as its stronger ecotoxicity, EHMC may present the highest environmental risk to the marine aquatic system compared with the other tested UV filters.

Table 4A
Ecotoxicological information from three trophic levels of freshwater and saltwater organisms of UV filters and the risk assessment for marine aquatic environment.

UV filters	Ecotoxicity data for freshwater organisms (mg/L)			Ecotoxicity data for marine organisms[d] (mg/L)				AF	NOEC (mg/L)	PNEC (ng/L)	MEC_{max} (ng/L)	MEC_{median} (ng/L)	RQ_{worst}	$RQ_{general}$
	Algae[a]	Invertebrates[a]	Fish	Autotrophs	Herbivores		Carnivores							
BP-3	0.560	1.670	0.191[b]	0.014	3.473	3.280	0.711	1000	0.014	13.87	82.35	36.07	5.94	2.60
4-MBC	0.210	0.100	–	0.171	0.587	0.854	0.193	1000	0.100	100	74.50	33.32	0.75	0.33
EHMC	0.240	0.040	10000[c]	0.075	3.118	0.284	0.199	1000	0.040	40	191.67	131.74	4.79	3.29
OC	–	–	10000[c]	–	–	–	–	10,000	10,000	1,000,000	63.63	36.64	<0.001	<0.001

[a] IC10 of *D. subspicatus*, EC50 and NOEC of *D. magna* (Sieratowicz et al., 2011).
[b] NOEC of *D. rerio* (Kinnberg et al., 2015).
[c] LC50 of *B. rerio* and *L. idus* (Brooke et al., 2008).
[d] EC50 of *I. galbana*, *M. galloprovincialis*, *P. lividus* and *S. armata* (Paredes et al., 2014).

Table 4B
Ecotoxicological information from three trophic levels of freshwater organisms for UV filters and the risk assessment for freshwater environment.

UV filters	Ecotoxicity data for freshwater organisms (mg/L)			AF	NOEC (mg/L)	PNEC (ng/L)	MEC_{max} (ng/L)	MEC_{median} (ng/L)	RQ_{worst}	$RQ_{general}$
	Algae[a]	Invertebrates[a]	Fish							
BP-3	0.560	1.670	0.191[b]	100	0.191	1910	82.35	36.07	0.04	0.02
4-MBC	0.210	0.100	–	100	0.100	1000	74.50	33.32	0.07	0.03
EHMC	0.240	0.040	10000[c]	100	0.040	400	191.67	131.74	0.48	0.33
OC	–	–	10000[c]	1000	10,000	10,000,000	63.63	36.64	<0.001	<0.001

[a] IC10 of *D. subspicatus*, EC50 and NOEC of *D. magna* (Sieratowicz et al., 2011).
[b] NOEC of *D. rerio* (Kinnberg et al., 2015).
[c] LC50 of *B. rerio* and *L. idus* (Brooke et al., 2008).

In Table 4B, the same ecotoxicity data from the freshwater organisms and the same MECs of four UV filters are used for the risk assessment on freshwater environment, in which the AFs of 100 for BP-3, 4-MBC and EHMC while 1000 for OC are employed respectively for calculation. As the result shows, all the $RQ_{general}$ values of the four UV filters are <1, which means all these tested UV filters do not show the significant potential risk to the freshwater environment according to the assessment methodologies generally used for the inland aquatic compartment. For BP-3, 4-MBC and OC, the $RQ_{general}$ values are even lower than 0.1 which indicate only slight potential risk to the inland aquatic system, whereas only for EHMC the $RQ_{general}$ value is between 0.1 and 1 which indicates only slightly higher potential risks to the freshwater environment.

These results are similar in both evaluation methodologies in that EHMC always shows relatively higher potential risks than the other three UV filters, and OC shows the lowest $RQ_{general}$ values (<0.001). However, comparing the evaluation criteria with those for marine aquatic environment, the $RQ_{general}$ values of four tested UV filters are one to two orders of magnitude lower when using inland aquatic environment assessing methods. For example, for BP-3 in particular the $RQ_{general}$ value in freshwater risk assessment is 0.02 (<1, no significant potential risk) while in marine risk assessment it is 2.60 (>1, adverse effect). In other words, the prediction is totally different based on the same MECs. Thus, the potential risks posed to the marine aquatic environment of the detected UV filters could be largely underestimated when simply using inland aquatic environment methodologies. Underestimating potential risks can mislead both scientists and legislative bodies seeking to protect water resources; further studies may not be done and appropriate safeguards may not be enacted. As a result, it may not be able to get sufficient attention and controls for the substances which are potentially harmful to the marine aquatic environment, such as the emerging contaminants contained in the personal care products with quite large consumptions.

Another difference in the ecotoxicity to each level of organisms may also reflect that UV filters pose the environmental risks to the marine aquatic system distinct from those to the freshwater environment. Comparing the ecotoxicity data of marine organisms and fresh water organisms, it can be seen that the lowest EC50 values for three UV filters tested via marine organisms are all reported from the alga *Isochrysis galbana* (Paredes et al., 2014), whereas in freshwater organisms this is not true. For 4-MBC and EHMC in freshwater, the lowest ecotoxicity data are the no observed effect concentrations (NOECs) tested in the freshwater flea, *Daphnia magna*, rather than in an alga (*Desmodesmus subspicatus*) (Sieratowicz et al., 2011), while for BP-3 in freshwater, the lowest ecotoxicity data are observed from the zebrafish (*Danio rerio*) (Kinnberg et al., 2015). This may indicate that organisms at lower trophic levels such as alga might be the most affected species due to the existence of UV filters in marine ecosystems, whereas in freshwater systems the organisms at higher trophic levels may be more sensitive to UV filters than the alga species. Therefore, UV filters are likely to express inconsistent ecotoxicities in different ecosystems, such as marine versus freshwater, and thus it is unreasonable to assess their ecological risks by simply using general criteria without any specific adjustment.

In previous reports, the RQ values for BP-3 and OC detected in surface waters in Norway were calculated as <1 and their risks were reported as low (Langford et al., 2015). Similarly, the occurrence of BP-3 and several other UV filters found in the urban groundwater in Barcelona posed no acute risk according to their risk assessment, in which the RQs were all below 4×10^{-3} (Molins-Delgado et al., 2016). For the study in Gran Canaria Island, Spain, the risk assessment was performed on BP-3, 4-MBC and EHMC with the RQ values of 0.0–6.6, 0.1–10.4 and 0.4–18.9, respectively (Rodríguez et al., 2015). Significant adverse effects were found for 4-MBC and EHMC, consistent with the fact that RQ values higher than 10 were reported for both compounds. Lower potential risk for BP-3 was obtained. The RQ values of BP-3, 4-MBC and EHMC were also calculated as higher than 1 using the MECs of the UV filters detected in the seawater samples collected in Hong Kong (Tsui et al., 2014a), indicating the potential risks to the aquatic ecosystem posed by UV filters are becoming both significant and ubiquitous.

4. Conclusion

The present report describes the occurrence study of seven UV filters in farmed and wild marine organisms. It is based on a study of organisms collected in Hong Kong coastal fish farms. The report not only provides information on the occurrence and regional distribution of these filters, but also provides a global comparison of these figures with similar figures from East Asia and Europe. Higher concentrations were universally detected in wild mussels than in farmed fish collected from the same locations. The highest total amount of UV filters were found in samples from Sai Kung, reflecting the direct impact of anthropogenic activities on their occurrence in aquatic environments. Comparison of these results with those from studies in Europe indicates that the predominant UV filters detected in aquatic organisms are almost same worldwide; these filters are EHMC, OD-PABA and BP-3. Compared with Europe, however, whereas the concentrations measured in Hong Kong are much lower. Additionally, risk assessment indicates that long-term persistence of these UV filters at present levels poses significant potential risks to the marine aquatic environment. These risks may be amplified through the food chain and ecological cycle. Therefore, further study, possibly further controls, and alternative chemicals should all be explored as approaches to the environmental management of these emerging contaminants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.05.120.

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Author information
1 UNESCO-IHE, Institute for Water Education, Westvest 7, 2611, AX, Delft, Netherlands.
2 Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, H2O Building, c/Emili Grahit 101, E17003, Girona, Spain.
3 UNESCO-IHE, Institute for Water Education, Westvest 7, 2611, AX, Delft, Netherlands. g.ferrero@unesco-ihe.org.
4 Water and Soil Quality Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council for Scientific Research (CSIC), Jordi Girona 18-26, E-08034, Barcelona, Spain.
5 Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, 08010, Barcelona, Spain.
6 Delft University of Technology, Stevinweg 1, 2628, CN, Delft, Netherlands.
7 Laboratory of Chemical and Environmental Engineering (LEQUIA), Institute of the Environment, University of Girona, E17071, Girona, Spain.

Abstract
The occurrence of 32 pharmaceuticals and 14 UV filters in swimming pools and spas was studied. Fifty-one water samples were collected from 17 pools located in sport centres and hotels in Catalonia, Spain. The samples were analysed by liquid chromatography-tandem mass spectrometry. The pharmaceuticals atenolol, carbamazepine, hydrochlorothiazide, metronidazole, ofloxacin, sulfamethoxazole, acetaminophen, ibuprofen, ketoprofen and phenazone were measured in water samples at concentrations higher than their limit of quantification (LOQ). The highest concentration of any individual pharmaceutical was measured for the diuretic hydrochlorothiazide (904 ng/L). The most frequently detected pharmaceutical was carbamazepine, as it was observed in more than half of all the water samples measured (53 %, 27/51). The UV filters at concentrations higher than LOQ in water samples were BP1, BP2, BP3, BP8, THB, 4DHB, 4MBC, OD-PABA, 1HBT, MeBT and DMeBT. The highest concentration of UV filter observed was 4MBC (69.3 ng/L) while the most frequent UV filters in the samples were 1HBT (59 %, 30/51). The results also showed that pharmaceuticals and UV filters were most frequently found in spas. Finally, from a water treatment technology perspective, the lowest occurrence of pharmaceuticals was in the pools applying sand filters followed by disinfection by sodium hypochlorite, while the lowest occurrence of UV filters was in the pools applying coagulation, sand filtration, UV and salt electrolysis.

7) Analytical and Bioanalytical Chemistry

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Analysis of UV filters in tap water and other clean waters in Spain

M. Silvia Díaz-Cruz, Pablo Gago-Ferrero, Marta Llorca, Damià Barceló

Abstract

The present paper describes the development of a method for the simultaneous determination of five hormonally active UV filters namely benzophenone-3 (BP3), 3-(4-methylbenzylidene) camphor (4MBC), 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA), 2-ethylhexyl 4-methoxycinnamate (EHMC) and octocrylene (OC) by means of solid-phase extraction and gas chromatography–electron impact ionization–mass spectrometry. Under optimized conditions, this methodology achieved low method limits of detection (needed for clean waters, especially drinking water analysis), between 0.02 and 8.42 ng/L, and quantitative recovery rates higher than 73% in all cases. Inter- and intraday precision for all compounds were lower than 7% and 11%, respectively. The optimized methodology was applied to perform the first survey of UV absorbing compounds in tap water from the metropolitan area and the city of Barcelona (Catalonia, Spain). In addition, other types of clean water matrices (mineral bottled water, well water and tap water treated with an ion-exchange resin) were investigated as well. Results evidenced that all the UV filters investigated were detected in the water samples analyzed. The compounds most frequently found were EHMC and OC. Maximum concentrations reached in tap water were 290 (BP3), 35 (4MBC), 110 (OD-PABA), 260 (EHMC), and 170 ng/L (OC). This study constitutes the first evidence of the presence of UV filter residues in tap water in Europe.

Oxybenzone HEL Monograph - 3 of 7

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11 attachments (3 MB)

8 da Silvia Occurrence UVF Drinking Water Sao Paulo Brazil.pdf; 9 Jiang Absorption of sunscreens across human skin- an evaluation of commercial products for children and adults.docx; 10 Janjua J Invest Dermatol.docx; 11 Benzophenone urine Anal methods.pdf; 12 Meeker et al EnvSciTech 2013 urine_conc_UV_filt_in_Puerto_Rican_women.pdf; 13 Gonzalez Percutaneous absorption of benzophenone-3, a common component of topical sunscreens Clin Exp Dermatol.docx; 13 Gonzalez Percutaneous absorption of benzophenone-3, a common component of topical sunscreens Clin Exp Dermatol.docx; 14 Wang Benzophenone-3 USA China.docx; 15 and 16 Calafat Oxy in 97 percent samples.pdf; 17 Zhang Sci total Environment 461-462() 49-55[2013] BP urine and blood China.pdf; 19 Rodríguez-Gómez Determination of benzophenone-UV Filters in human milk.docx;

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,
Craig Downs – Executive Director – Haereticus Environmental Laboratory
Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:
- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.
- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.
- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:

1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone
2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

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RESEARCH ARTICLE

The occurrence of UV filters in natural and drinking water in São Paulo State (Brazil)

Claudia Pereira da Silva[1] · **Elissandro Soares Emídio**[1] · **Mary Rosa Rodrigues de Marchi**[1]

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Abstract Ultraviolet (UV) filters are widely used in the formulation of personal care products (PCPs) to prevent damage to the skin, lips, and hair caused by excessive UV radiation. Therefore, large amounts of these substances are released daily into the aquatic environment through either recreational activities or the release of domestic sewage. The concern regarding the presence of such substances in the environment and the exposure of aquatic organisms is based on their potential for bioaccumulation and their potential as endocrine disruptors. Although there are several reports regarding the occurrence and fate of UV filters in the aquatic environment, these compounds are still overlooked in tropical areas. In this study, we investigated the occurrence of the organic UV filters benzophenone-3 (BP-3), ethylhexyl salicylate (ES), ethylhexyl methoxycinnamate (EHMC), and octocrylene (OC) in six water treatment plants in various cities in Southeast Brazil over a period of 6 months to 1 year. All of the UV filters studied were detected at some time during the sampling period; however, only EHMC and BP-3 were found in quantifiable concentrations, ranging from 55 to 101 and 18 to 115 ng L^{-1}, respectively. Seasonal variation of BP-3 was most clearly noticed in the water treatment plant in Araraquara, São Paulo, where sampling was performed for 12 months. BP-3 was not quantifiable in winter but was quantifiable in summer. The levels of BP-3 were in the same range in raw, treated and chlorinated water, indicating that the compound was not removed by the water treatment process.

Keywords UV filters · Endocrine disruptors · Brazil · River water · Drinking water · Occurrence

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✉ Mary Rosa Rodrigues de Marchi
mssqam@iq.unesp.br; maryrmarchi@gmail.com

Claudia Pereira da Silva
claudiaufs@yahoo.com.br

Elissandro Soares Emídio
elissandro_se@yahoo.com.br

[1] Analytical Chemistry Department, Chemistry Institute, São Paulo State University—UNESP, Araraquara, SP 14801-970, Brazil

Introduction

Because of concern regarding the depletion of the ozone layer and the consequential increase of UV radiation in the stratosphere that results in a higher skin cancer risk, UV filters have become an important constituent in everyday product formulations (e.g., skin moisturizing cream, body lotion, spray and hair dye, shampoo, and lipstick; Brausch and Rand 2011). UV filters have other applications, such as stabilizers in plastics, paints, textiles, and in other materials, to prevent the photodegradation of polymers and pigments (Ozáez et al. 2013). These products can act as an environmental source of UV filters, primarily from degradation in landfills (Fent et al. 2008).

UV filters are colourless or yellow substances that absorb almost no visible light but that significantly absorb light in the UVA-UVB range. A common feature of all UV filters is the presence of an aromatic ring with side chains that have various degrees of unsaturation (Díaz-Cruz and Barcelo 2009). The majority of UV filters are lipophilic; however, the presence of phenol groups in their structures can give them some mobility in the aquatic environment (Negreira et al. 2009; Richardson 2009).

UV filters are classified as inorganic (physical) or organic (chemical) according to their chemical nature. The inorganic UV filters (e.g., titanium dioxide and zinc oxide), basically act

by reflecting, scattering, and absorbing UV light, whereas the organic UV filters (e.g., benzophenone, octocrylene) act mainly by absorbing UV light (Cuderman and Heath 2007).

Some of these UV filters protect from UVB radiation, others offer protection against wavelengths in the UVA range, and few are efficient against both UVA and UVB. Thus, to ensure an efficient sun protection factor (SPF), high concentrations of UV filters have been used in personal care products (PCPs) and, in general, a combination of UV filters is used. Consequently, consumers can be exposed to high concentrations of several UV filters simultaneously (Fent et al. 2010; Manová et al. 2013). To protect the public health, the UV filters used in PCPs are regulated in some countries, mainly in the European Union and North America.

However, no worldwide environmental regulatory rules exist. In Brazil, this input to the environment is probably excessive. Considering that Brazil is a tropical country and has a high incidence of solar radiation, there is high consumption of products that contain UV filters. According to the Brazilian Association of Toiletries, Perfumes & Cosmetics, Brazil is the third largest consumer of cosmetics in the world, trailing only the USA and Japan (ABIHPEC 2014), and it still has an unreliable sanitary sewer system. According to the Brazilian Institute of Geography and Statistics, only 28.5 % of Brazilian cities collect and treat sewage, which leads to the hypothesis that the environmental levels of these compounds in Brazil can be higher than those found in other countries.

Although several papers in the literature report on the estrogenic activity of UV filters (Fent et al. 2008; Kunz and Fent 2006; Schlumpf et al. 2004), as well as on their occurrence and environmental fate (especially regarding water resources; Liu et al. 2011; Magi et al. 2012; Zhang and Lee 2012) and tap water (Basaglia et al. 2011; Díaz-Cruz et al. 2012; Zhang and Lee 2013), papers about Brazil could not be found. Only, an extraction method development for the determination of PCPs from drinking-water treatment sludge including benzophenone-3 (BP-3; Cerqueira et al. 2014). Therefore, this study aims to provide information about the occurrence and fate of the UV filters BP-3, ES, EHMC, and OC in Brazil, which are widely used in cosmetics. The study is focused on the occurrence and concentrations of these UV filters in aqueous samples from water treatment plants (WTP). The WTPs selected for study are located in the central region of São Paulo. Sao Paulo State has the better basic sanitary conditions in Brazil, collecting 87 % and treating 53 % of the sewage (SNIS 2013).

Materials and methods

Chemicals and reagents

The compounds selected for this study were four UV filters used with high frequency in sun creams largely sold in Brazil. In Brazil, there is not available information about retail marketing for cosmetics, including sunscreens. Thus, we conducted an informal survey based on the more traditional cosmetics brands sold in Brazil, by multinational and Brazilian companies. This survey included several types of sunscreen formulation and protection factors (n=52). Among the substances found most frequently in the formulation, as described in the products labels, those with the greatest estrogenic potential, according to the literature, were selected to be included in this study (BP-3, ES, EHMC, and OC).

The UV filters (BP-3, ES, EHMC, and OC), the surrogate benzophenone-d10 (BP-d10) and the internal standard, and benzyl cinnamate (BC), all of high purity (>97 %), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Names, abbreviations, chemical structures, and some physicochemical data for the UV filters are shown in Table 1. High-purity solvents (methanol [MeOH] and ethyl acetate [AcOEt] were of HPLC grade) were obtained from Mallinckrodt Baker Inc. (Paris, KY, USA). Hydrochloric acid (HCl, 37 % m/v) was purchased from the JT Baker Chemical Co. (Phillipsburg, NJ, USA) to adjust the pH of the water samples.

Standard solutions of the individual UV filters were prepared at a concentration of 1,000 mg L^{-1} in AcOEt and then diluted (with the same solvent) to achieve a stock solution mixture at 5 mg L^{-1} for each compound. All the standard solutions were stored at −20 °C. Working solutions of different concentrations were prepared daily by appropriate dilution (v/v) of the stock solution in AcOEt.

Cleaning of glassware

Considering that UV filters are ingredients of PCPs (shampoo, lipstick, soap, and sunscreen), laboratory contamination appeared to be imminent. To avoid contamination from this source, nitrile gloves were worn during all procedures during the breakup of the glassware and materials to be used, sampling, sample pre-treatment, and analysis. Glassware cleaning consisted of the following steps: a running tap water rinse, immersion in neutral Extran 5 % detergent (Merck, Darmstadt, Germany) overnight, a tap water rinse followed by a distilled water rinse and finally, an acetone rinse. The clean glassware was air dried for a minute or two, then transferred to an oven at >100 °C. Volumetric glassware was air dried. All glassware was rinsed with AcOEt immediately prior to use.

Sampling sites description

The area selected for study was the Unit of Water Resources Management number 13 (UWRM-13), located in southeastern Brazil, in the central region of São Paulo State (Fig. 1). São Paulo is divided into 22 Units of Water Resources

Table 1 Analytes, abbreviations, chemical structures, molecular weights, Log K_{ow} and pK_a values for the UV filters included in this work

Analyte (abbreviations)	Chemical structure	Molecular weight (g mol^{-1})	Log K_{ow}	pKa
Benzophenone-3 (BP-3)		228.24	3.79	7.56
Ethylhexyl methoxycinnamate (EHMC)		290.40	5.80	-
Ethylhexyl salicylate (ES)		250.33	5.97	8.13
Octocrylene (OC)		361.49	6.88	-
Benzyl cinnamate[a] (BC)		238.28	4.06	-
Benzophenone -d_{10}[b] (BP-d_{10})		192.28	3.18	-

[a]internal standard; [b]surrogate

Management (UWRM). UWRM-13, also called Hydrographic Basin Tietê-Jacaré, is composed of 34 municipalities that contain approximately 3.5 % of the São Paulo State population, about 1.5 million inhabitants (CETESB 2012) and was selected as the study area. The municipalities included in this study were Araraquara, Bueno de Andrada (Araraquara district), Bauru, Jau, São Carlos, and Trabiju. In the municipalities of Araraquara, Bauru, Jau, and São Carlos, the water treatment steps are coagulation, flocculation, sedimentation, filtration, chlorination, and fluoridation. In Bueno de Andrada and Trabiju, the water source is groundwater and the treatment consists only of disinfection by chlorination and fluorination (which occurs directly in the piping).

Sampling and sample preparation

Water samples were collected from October 2012 to February 2014, in the Water Treatment Plants (WTP)

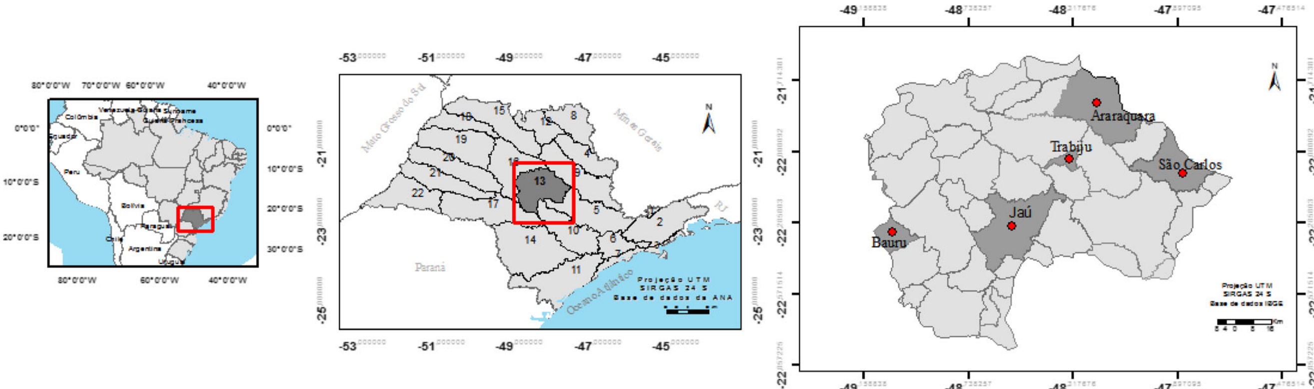

Fig. 1 Water sampling cities. São Paulo State at Brazil map and the Unit of Water Resources Management (UWRM), on the São Paulo State map, showing the cities where water samples were collected

Table 2 Concentrations of UV filters in natural, treated, and chlorinated water (ng L^{-1})

	Analyte	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water
Araraquara		March/2013			April/2013			May/2013		
	BP-10[a]	102(5)	100(2)	110(10)	76(9)	83(3)	84(8)	89(5)	86(2)	98(11)
	ES	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	19(10)	18(9)	18(9)	23(3)	25(4)	21(8)	n.d.	n.d.	n.d.
	EHMC	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013			July/2013			August/2013		
	BP-10[a]	59(6)	63(5)	71(2)	80(5)	92(1)	85(4)	89(9)	90(11)	97(10)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	59(67)	<LOQ
		September/2013			October/2013			November/2013		
	BP-10[a]	111(6)	109(11)	109(12)	68(11)	82(7)	85(4)	77(8)	73(8)	86(5)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	22(1)	19(2)	20(4)	33(2)	28(15)	25(7)	38(2)	29(9)	31(4)
	EHMC	n.d.	n.d.	75(11)	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		December/2013			January/2014			February/2014		
	BP-10[a]	58(8)	61(12)	69(8)	59(3)	63(15)	65(5)	106(9)	106(16)	141(5)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	26(4)	23(3)	23(10)	36(5)	32(4)	28(3)	30(6)	25(13)	23(7)
	EHMC	<LOQ	<LOQ	57(64)	65(19)	55(11)	70(58)	55(10)	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bauru		April/2013			May/2013			June/2013		
	BP-10[a]	107(15)	105(5)	131(3)	48(6)	81(17)	115(23)	59(6)	63(5)	71(2)
	ES	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	28(15)	33(14)	115(1)	20(36)	26(27)	72(54)	n.d.	n.d.	n.d.
	EHMC	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	<LOQ	<LOQ	87(57)	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
		July/2013			August/2013			September/2013		
	BP-10[a]	45(12)	57(3)	73(8)	93(6)	100(18)	111(9)	123(16)	106(9)	105(3)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	669(5)[b]	707(6)[b]	309(6)[b]	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	188(2)[b]	211(4)[b]	199(4)[b]	n.d.	n.d.	n.d.
São Carlos		April/2013			May/2013			June/2013		
	BP-10[a]	110(8)	121(7)	93(3)	93(9)	85(5)	81(11)	80(2)	82(10)	82(4)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	44(10)	60(31)	n.d.
	EHMC	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	101(10)	<LOQ	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		July/2013			August/2013			September/2013		
	BP-10[a]	86(11)	84(5)	80(8)	94(10)	94(15)	92(40)	105(6)	104(9)	114(6)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2 (continued)

	Analyte	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water
Jau		March/2013			April/2013			May/2013		
	BP-10[a]	96(5)	100(3)	105(6)	79(5)	79(11)	82(5)	65(23)	73(10)	74(17)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	18(8)	18(1)	n.d.	24(8)	26(6)	28(15)	n.d.	n.d.	n.d.
	EHMC	<LOQ	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013			July/2013			August/2013		
	BP-10[a]	60(1)	59(7)	66(4)	83(6)	84(1)	92(6)	115(6)	123(6)	135(13)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	21(20)	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	755(8)[b]	629(16)[b]	292(3)[b]
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	208(12)[b]	176(10)[b]	183(24)[b]

BP-10 was used as a surrogate. Average recoveries and relative standard deviations (RDS) for $n=3$

n.d. not detected, *<LOQ* below the limit of quantification

[a] BP-10 used as surrogate at 200 ng L^{-1}

[b] Quantification disregarded (contamination was suspected)

at the municipalities included in this study at three points: the entrance of the WTP, before any treatment (*river water*), after treatment without chlorination (*treated water*), and after chlorination (*chlorinated water*). Sampling in Bauru, Bueno de Andrada, Jau, São Carlos, and Trabiju was performed over a period of 6 months, while in Araraquara, the sampling was performed over a period of 12 months to evaluate the influence of seasonality on the occurrence of the UV filters.

All samples were collected inside the water treatment plant, except in Bauru, where there was no collection point for one of the sample types (river water or raw water) inside the station, so it had to be directly collected from the river. In Trabiju and Bueno de Andrada, the water is collected underground and is disinfected directly in the pipe, so only two types of water samples were analyzed: groundwater and chlorinated water.

The samples were collected in the morning between 07:00 and 10:30 in amber Pyrex glass bottles with screw caps of 4 L capacity. After collection, the samples were transported to the laboratory under refrigeration at 4 °C (ice packs) and protected from light. In the laboratory prior to extraction, the samples were filtered through a glass fibre filter with a pore size of 0.6 μm (Macherey-Nagel GF3), acidified to pH 3, spiked to 200 ng L^{-1} with a surrogate (BP-d10) and immediately extracted, and analyzed according to the previously developed and validated solid-phase extraction (SPE) method and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS; Silva et al. 2013; Silva et al. 2015).

SPE extraction and GC-MS/MS analysis

The SPE method employed was a previously published method by Silva et al. (2013). The extraction of the sample analytes was performed using 200 mg/6 mL, Strata X SPE cartridges obtained from Phenomenex (Torrance, USA). The SPE cartridges were first conditioned with 5 mL of AcOEt, 5 mL of MeOH and 5 mL of deionized water. Sample volumes of 500 mL at pH 3 were passed through the cartridge. The cartridge was then dried under total vacuum for 5 min and 500 μL of MeOH was added. The analytes were eluted with 3×2 mL of AcOEt. Then, 500 μL of an internal standard solution of 100 μg L^{-1} BC was added to the eluate. The eluate was evaporated to 1 mL under a gentle flow of nitrogen gas prior to analysis by GC-MS/MS.

GC-MS/MS analysis was performed on a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA, USA) that was equipped with a Saturn 2000 ion trap mass spectrometer . The chromatography was performed under the following conditions: helium was the carrier gas (maintained at a constant flow rate of 1.2 mL min^{-1}); an injector temperature of 300 °C, operating in splitless injection mode with a sampling time of 1 min. The separation of the analytes was performed with a fused-silica ZB-5MSi capillary column (30 m length × 0.25 mm i.d. and 0.25 mm film thickness; Phenomenex, Torrance, CA, USA). The temperature program was as follows: 60 °C for 1 min; ramped at 25 °C min^{-1} to 160 °C, which was maintained for 1 min; and ramped at 10 °C min^{-1} to 300 °C, which was maintained for 2 min. The total run time was 22.0 min. The transfer line, trap, and manifold temperatures were maintained at 300, 50, and 220 °C, respectively.

Results and discussion

Quality parameters of the method

The analytical method used to determine UV filters in water, in this work, was based on SPE and GC-MS/MS. The detailed study of multivariate optimization procedure for this method was published previously (Silva et al. 2013), as well as, the validation parameters, including matrix effect (Silva et al. 2015).

For all samples (6 months to 1 year) for all cities (Araraquara, Bauru, Jau, São Carlos, Trabiju and Bueno de Andrada), the recovery of the surrogate (BP-d10) ranged 70–120 %, and the relative standard deviations (RSDs) was below 20 %, as shown in Tables 2 and 3. Therefore, the method satisfied the requirements for precision and accuracy for quantification.

Sampling aspects

The most favorable Brazilian scenario (south-eastern Brazil) regarding basic sanitation, where the sewage collection far exceeds the national average, was used as the study area. However, note that the treatment is performed in only 48 % of collector municipalities. This absence of sewage treatment in more than half of the collector municipalities in the region of the country that has the best sanitary conditions clearly shows the seriousness of the national environmental situation. The poor collection and treatment of sewage in Brazil implies serious negative consequences to public health and the environment. In this context, the quantitative determination of UV filters in the raw water and the drinking water under the "best" conditions (south-east) is of importance.

The state of São Paulo in the Southeast Brazil is divided into 22 UWRM. UWRM-13 was selected as the study area

Table 3 Concentrations of UV filters in ground and chlorinated water (ng L^{-1})

	Analyte	Ground water	Chlorinated water	Ground water	Chlorinated water	Ground water	Chlorinated water
Bueno de Andrada		March/2013		April/2013		May/2013	
	BP-10[a]	133(7)	143(11)	107(10)	99(9)	108(5)	112(10)
	ES	n.d.	<LOQ	n.d.	n.d.	<LOQ	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
	EHMC	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013		July/2013		August/2013	
	BP-10[a]	71(8)	72(23)	105(13)	122(3)	130(40)	80(30)
	ES	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	BP-3	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trabiju		March/2013		April/2013		May/2013	
	BP-10[a]	105(21)	113(3)	88(8)	88(9)	59(14)	66(14)
	ES	n.d.	105(258)	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	137(148)	<LOQ	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013		July/2013		August/2013	
	BP-10[a]	60(5)	61(6)	93(3)	85(2)	115(15)	112(6)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	724(4)[b]	273(2)[b]
	OC	n.d.	n.d.	n.d.	n.d.	175(22)[b]	174(19)[b]

BP-10 was used as a surrogate. Average recoveries and relative standard deviations (RDS) for $n=3$

n.d. not detected, *<LOQ* below the limit of quantification

[a] BP-10 used as surrogate at 200 ng L^{-1}

[b] Quantification disregarded (contamination was suspected)

because it is a populous region in which the sewage collection is nearly 100 %, although in some municipalities, the sewage collected is not treated. For example, Bauru, the most populous municipality in this basin, treats only 10 % of the sewage collected (CETESB 2012).

Study of the occurrence of UV filters in environmental samples

The concentrations of the UV filters in samples from the six WTPs in the UWRM-13 are presented in Tables 2 and 3. All UV filters were detected in the samples from the WTPs, however, quantifiable concentrations were found only for BP-3 and EHMC. The concentrations of BP-3 and EHMC ranged 18–115 and 55–101 ng L^{-1}, respectively. Although the other analytes were present in some samples, they were not present at levels that could be reliably quantified.

Both BP-3 and EHMC, among other UV filters, have been found throughout the world. They have been detected in water, soil, sediment, sludge, and biota. BP-3 and EHMC have been detected in river and tap water worldwide (Table 4). The maximum level of BP-3 detected in river water was 114 ng L^{-1} in Slovenia (Cuderman and Heath 2007), according to a review by Kim and Choi (2014). EHMC has been found in slightly higher concentrations; Kameda and collaborators found concentrations up to 1,040 ng L^{-1} in highly polluted rivers in Japan (Kameda et al. 2011). It was reported by Díaz-Cruz et al. (2012) that concentration of BP-3 and EHMC were up to 295 and 256 ng L^{-1}, respectively, in the public water supply. These literature values are comparable to the values obtained for river water and drinking water in this study.

BP-3, EHMC, and OC are the UV filters most used in PCPs. BP-3 is the most polar, which is possibly why it occurs with the highest frequency in the samples studied. BP-3 was quantified in Araraquara during almost every sample period (March 2013 to February 2014) except only May, June, July, and August 2013. BP-3 was quantified in Bauru during April and May 2013; in São Carlos, only in June 2013; and in Jau during March, April, and June 2013. EHMC was quantified in Araraquara only in September 2013, January 2014, and February 2014; and it was quantified in São Carlos in June 2013. The other two UV filters (ES and OC) were below quantifiable levels, although they were detected. Figure 2 shows (1) a chromatogram of the SPE products and the GC-MS/MS analysis for the UV filters at the limit of quantification and (2) a chromatogram of the SPE products and the GC-MS/MS analysis for the UV filters in river water from the Araraquara WTP in January 2014.

The high concentrations of EHMC and OC in August in Bauru, Jau, and Trabiju might be explained by contamination during the collection and/or extraction of the sample. Although these compounds were detected (below the LOQ) in previous months, these values appear to be discordant with others from the same sampling sites Even if their coefficients of variation are excellent, the values are suspect because these analytes were detected in Bauru, Jau, and Trabiju at similar concentrations. Because the samples from the three municipalities were collected on the same day and also processed together, the possibility of contamination cannot be discarded. Contamination was also not confirmed because an entire cleaning procedure of glassware and materials was conducted,

Table 4 Summary of the measured concentrations of UV filters in river water and tap water (ng L^{-1})

Sample	Maximum concentration of BP-3 (ng L^{-1})	Maximum concentration of EHMC (ng L^{-1})	Country	Reference
River water	44	101	Brazil	This article
Drinking water	105	75	Brazil	This article
River water	114	88	Slovenia	Cuderman and Heath (2007)
River water	30	21	Spain	Rodil and Moeder (2008)
River water	52	–	Spain	Negreira et al. (2009)
River water	<LOQ	<LOQ	Australia	Liu et al. (2011)
River water	12	1040	Japan	Kameda et al. (2011)
River water	n.d.	n.d.	Singapore	Zhang and Lee (2012)
Tap water	295	256	Spain	Diaz-Cruz et al. (2012)
River water	69	10	Italy	Magi et al. (2012)
River water and groundwater	38	–	Spain	Gago-Ferrero et al. (2013)
River water	15	–	Taiwan	Wu et al. (2013)
Tap water	n.d.	n.d.	Singapore	Zhang and Lee (2013)
Groundwater	19	–	Spain	Jurado et al. (2014)

<LOQ below the limit of quantification, *n.d.* not detected, – not studied

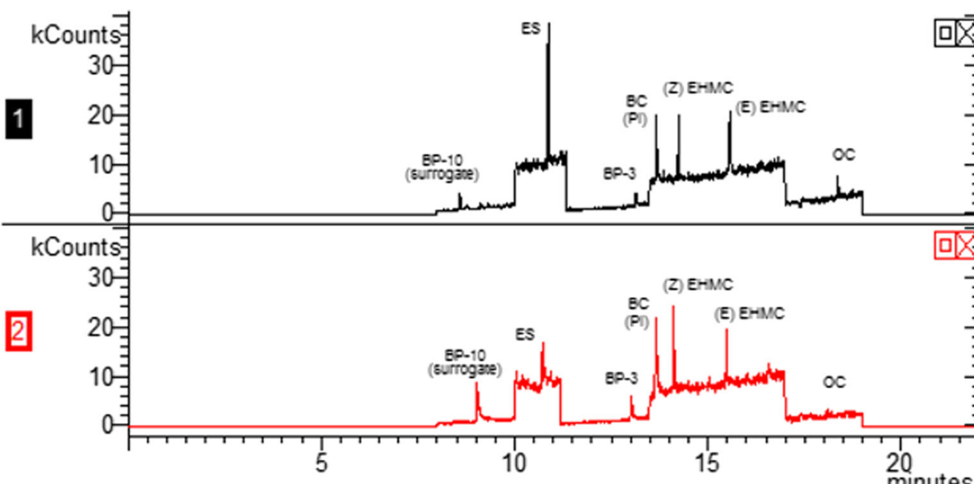

Fig. 2 SPE and GC-MS/MS chromatogram in MRM mode of the analysis of *1* WTP (treated water—Araraquara) spiked at 10, 100, 10, 50, and 50 ng L^{-1} to BP10, ES, BP3, EHMC, and OC, respectively. The internal standard BC was spiked at 50 μg L^{-1}. *2* WTP (river water—Araraquara—January) the surrogate BP-10 and the internal standard BC was spiked at 100 μg L^{-1}. EHMCs are present as both (*E*) and (*Z*) isomer

in addition to care with the use of gloves during the procedure for separation of the glassware for the collection until the moment of injections.

In Araraquara, the sampling was performed over one year period (March 2013 to February 2014), so in that municipality, the seasonal occurrence of BP-3 was evident, as shown in Fig. 3. It should be noted that during winter time (May–August) UV filters were not quantifiable probably because of the lower consumption of sunscreens in the winter season.

Figure 3 indicates a lower concentration of BP-3 in chlorinated water samples compared to treated and river samples, probably because of the chlorination of the aromatic ring of the BP-3 (Negreira et al. 2008). However, the concentration in chlorinated water is only slightly lower than in treated and river water. It is suggested that the water treatment procedure used was not sufficient to eliminate these substances from the drinking water. This finding is disturbing because BP-3 has often been reported as an endocrine disruptor (Fent et al. 2008), inducing hormonal activity in vitro and in vivo. Benzophenones have been associated with the induction of vitellogenin, alterations in the gonads, a reduction in fertility and reproduction, and feminization of sexual characteristics in male fish (Díaz-Cruz and Barcelo 2009).

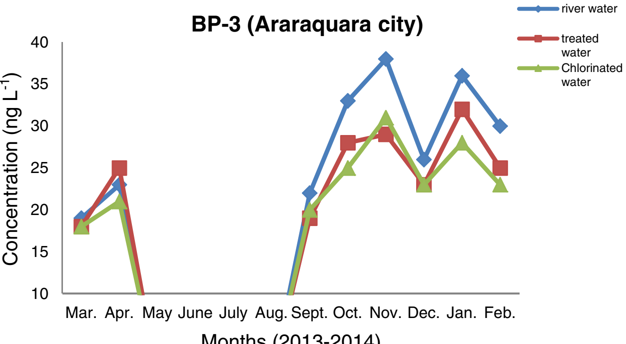

Fig. 3 Average monthly concentration (*n*=3) of BP-3 in river water (◆), treated water (●), and chlorinated water (▲) in the Araraquara WTP

Despite the low concentration found in this work for natural water analysis, to achieve a more complete environmental risk analysis discussion is mandatory to include UV filters determination in other environmental compartments as sediment and water particulate matter. On the other hand, in the literature, conclusive studies regarding the temporal effects of environmentally relevant concentrations are absent. However, even at very low concentrations, long-term exposure can present deleterious effects (Díaz-Cruz and Barcelo 2009). Additionally, the propensity for rapid accumulation of these substances and their potential combined effects should not be neglected because they are introduced into the environment daily in a mixture with other compounds. All these issues clearly indicate the need for further studies to evaluate the environmental occurrence and fate of UV filters, as well as toxicological and ecotoxicological studies (which are currently lacking) that can support the worldwide environmental regulation of these compounds.

Conclusions

UV filters were found in low concentrations in raw water and in the public water supply from treatment plants in the UWRM-13. All of UV filters studied were detected at some point during the study, both in the raw water and the drinking water, but only BP-3 (18–115 ng L^{-1}) and EHMC (55–101 ng L^{-1}) were found in quantifiable concentrations.

The concentrations of BP-3 increased during the summer, most likely due to direct and indirect environmental input. Although the concentrations found were low (in nanograms per liter level), it does not indicate that these substances are not liable to cause environmental or human damage because safe environmental or human exposure levels for these compounds have not yet been determined. Additionally, this is the first report of these compounds in Brazil, but only water samples were analyzed. The physicochemical properties of some of

these compounds indicate a high potential for accumulation in particulate matter, sediments and even in the biota.

The occurrence and quantification of UV filters in drinking water raises the question about the safe levels of these substances, indicating the need for improved water treatment processes for their removal. Thus, our results contribute to a discussion about the improvement of the water and sewage treatment in Brazil, as well as the development of global environmental legislation regarding emerging contaminants.

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Absorption of sunscreens across human skin: an evaluation of commercial products for children and adults.

Jiang R1, Roberts MS, Collins DM, Benson HA.
Author information
1 School of Pharmacy, University of Queensland, Brisbane, Australia.

Abstract
AIMS:
Topical sunscreens are routinely applied to the skin by a large percentage of the population. This study assessed the extent of absorption of a number of common chemical sunscreen agents into and through human skin following application of commercially available products.
METHODS:
Sunscreen products were applied to excised human epidermis in Franz diffusion cells with the amount penetrating into and across the epidermis assessed by h.p.l.c. for 8 h following application.
RESULTS:
All sunscreen agents investigated penetrated into the skin (0.25 g m-2 or 14% of applied dose), but only benzophenone-3 passed through the skin in significant amounts (0.08 g m-2 or 10% of the applied dose). With one exception, suncreen agents in corresponding products marketed for adults and children had similar skin penetration profiles.
CONCLUSIONS:
Whilst limited absorption across the skin was observed for the majority of the sunscreens tested, benzophenone-3 demonstrated sufficiently high penetration to warrant further investigation of its continued application.

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Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans.

Janjua NR1, Mogensen B, Andersson AM, Petersen JH, Henriksen M, Skakkebaek NE, Wulf HC.
Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark.
NRJ01@bbh.hosp.dk

Abstract
Recent in vitro and animal studies have reported estrogen-like activity of chemicals used in sunscreen preparations. We investigated whether the three sunscreens benzophenone-3 (BP-3), octyl-methoxycinnamate (OMC), and 3-(4-methylbenzylidene) camphor (4-MBC) were absorbed and influenced endogenous reproductive hormone levels in humans after topical application. In this 2-wk single-blinded study 32 healthy volunteers, 15 young males and 17 postmenopausal females, were assigned to daily whole-body topical application of 2 mg per cm(2) of basic cream formulation without (week 1) and with (week 2) the three sunscreens at 10% (wt/wt) of each. Maximum plasma concentrations were 200 ng per mL BP-3, 20 ng per mL 4-MBC, and 10 ng per mL OMC for females and 300 ng per mL BP-3, 20 ng per mL 4-MBC, and 20 ng per mL OMC for men. All three sunscreens were detectable in urine. The reproductive hormones FSH, LH were unchanged but minor differences in testosterone levels were observed between the 2 wk. A minor difference in serum estradiol and inhibin B levels were observed in men only. These differences in hormone levels were not related to sunscreen exposure.

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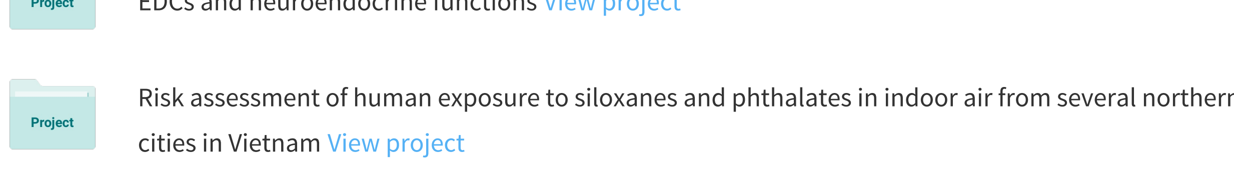

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Analysis of five benzophenone-type UV filters in human urine by liquid chromatography-tandem mass spectrometry

Tatsuya Kunisue,[a] Qian Wu,[a] Shinsuke Tanabe,[b] Kenneth M. Aldous[a] and Kurunthachalam Kannan*[ac]

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Benzophenone (BP)-type UV (ultraviolet) filters, especially 2-hydroxy-4-methoxybenzophenone (2OH–4MeO–BP), are widely used in the U.S., to protect the skin and hair from UV irradiation. Despite human exposures to UV filters through the dermal application of products containing sunscreen agents, few studies have examined the occurrence of UV filters in humans. Thus far, few sensitive methods are available for the determination of 2OH–4MeO–BP in human urine. Furthermore, methods for the determination of other BP derivatives, including 2,4-dihydroxybenzophenone (2,4OH–BP), which is formed from 2OH–4MeO–BP *via* metabolic activities of the cytochrome P450 enzymes, have not been available. In this study, we have developed a method for the analysis of five BP derivatives: 2OH–4MeO–BP, 2,4OH–BP, 2,2′-dihydroxy-4-methoxybenzophenone (2,2′OH–4MeO–BP), 2,2′,4,4′-tetrahydroxybenzophenone (2,2′,4,4′OH–BP), and 4-hydroxybenzophenone (4OH–BP) in human urine, using liquid–liquid extraction and liquid chromatograph (LC)-tandem mass spectrometer (MS/MS) analysis. The instrumental calibration range for each of the BP derivatives ranged from 0.05 to 100 ng ml^{-1}, and showed excellent linearity ($r > 0.99$). The respective limits of detection (LODs) and limits of quantification (LOQs) were determined to be 0.082 and 0.28 ng ml^{-1} for both 2,4OH–BP and 4OH–BP; 0.13 and 0.44 ng ml^{-1} for 2,2′OH–4MeO–BP; and 0.28 and 0.90 ng ml^{-1} for both 2OH–4MeO–BP and 2,2′,4,4′OH–BP. Recoveries of BP derivatives through the entire analytical procedure were between 85.2 and 99.6%. The coefficients of variation (CVs) of five replicate analyses within 1 day, and across 5 days, were respectively 1.4 and 3.7% for 2,4OH–BP; 1.7 and 3.0% for 2OH–4MeO–BP; and 2.8 and 4.5% for 4OH–BP. When BP derivatives were determined in urine samples from 23 U.S. (Albany, New York) and 22 Japanese (Matsuyama, Ehime) volunteers, higher concentrations of 2,4OH–BP, 2OH–4MeO–BP, and 4OH–BP were found in samples collected from females in the Albany cohort, probably reflecting great usage by U.S. females. The urine sample from a known sunscreen user contained very high concentrations of 2,4OH–BP and 2OH–4MeO–BP. 2,2′,4,4′OH–BP and 2,2′OH–4MeO–BP were not detected in any of the urine samples analyzed. Our results indicate considerable exposure to highly estrogenic 2,4OH–BP and 2OH–4MeO–BP by females in the U.S., and suggest the need for further studies on potential health effects.

1. Introduction

For the protection of skin and hair from ultraviolet (UV) irradiation, organic chemicals that can absorb the UV radiation and attenuate the negative effects have been used in a variety of personal care products; those chemicals are commonly referred to as UV filters. UV filters enter the aquatic environment either directly, *via* wash-off from skin and clothes during water recreational activities, or indirectly, *via* discharges of sewage and swimming pool waters. UV filters have been detected in environmental matrices such as water, soil, and sediment samples.[1] A few investigations on experimental animals have indicated that some organic UV filters possess significant estrogenic effects.[2]

In the U.S., 2-hydroxy-4-methoxybenzophenone (2OH–4MeO–BP or BP-3), a benzophenone (BP) derivative, is widely used in a variety of cosmetic products as a UV filter, and it is also used as a UV stabilizer in plastic surface coatings, to prevent photodegradation of polymers or foods.[3] Human exposures to 2OH–4MeO–BP can be through the dermal and oral routes. When sunscreen lotion containing 2OH–4MeO–BP was applied to human skin, significant amounts of this chemical penetrated the epidermal barrier, and 1–2% of the amount applied on the skin was absorbed in the bloodstream within 10 h.[4] In addition, 2OH–4MeO–BP has been shown to penetrate human skin more readily than do other sunscreen agents.[5,6] Further, 2OH–4MeO–BP has shown weak estrogen-like activity *in vitro* and *in vivo*[2,7–10] and antiandrogenic activity *in vitro*;[10,11] thus it is a potential endocrine-disrupting compound.

[a]*Wadsworth Center, New York State Department of Health, Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York, 12201-0509, USA. E-mail: kkannan@wadsworth.org; Fax: +518-473-2895; Tel: +518-474-0015*
[b]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama, 790-8577, Japan*
[c]*State Key Laboratory of Urban Water Resources & Environment, IJRC PTS, Harbin Institute of Technology, Harbin, 150090, China*

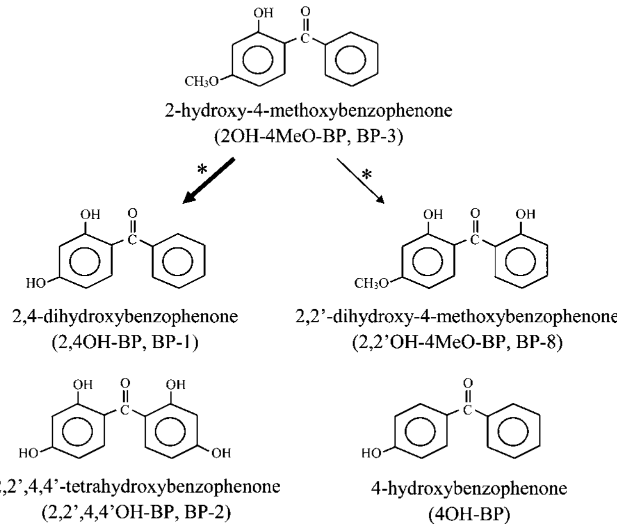

Fig. 1 Chemical structures of the benzophenone-type UV filters analyzed in this study. *Metabolic pathway of BP-3 was suggested from some experimental studies (The thickness of arrows indicates that BP-1 is mainly formed from BP-3).

It has been reported that, in rats and piglets, 2OH–4MeO–BP is metabolized to 2,4-dihydroxybenzophenone (2,4OH–BP or BP-1) and 2,2′-dihydroxy-4-methoxybenzophenone (2,2′OH–4MeO–BP or BP-8), compounds that are also used in cosmetic products as sunscreen agents[12–15] (see Fig. 1). Interestingly, there is evidence that 2,4OH–BP possesses an estrogenic activity higher than that of 2OH–4MeO–BP,[7–9,13,16] and it can also display antiandrogenic activity[8] *in vitro*. Additive estrogenic effects of 2,4OH–BP and 2OH–4MeO–BP, on pS2-gene transcription in MCF-7 cells, have been reported.[17] In addition, other BP derivatives, such as 2,2′,4,4′-tetrahydroxybenzophenone (2,2′,4,4′OH–BP or BP-2) and 4-hydroxybenzophenone (4OH–BP), have been shown to have estrogenic activities higher than that of 2OH–4MeO–BP.[7,9] Nevertheless, information regarding occurrence of BP derivatives in humans is scarce, except for 2OH–4MeO–BP.

2OH–4MeO–BP has been recently analyzed in water,[18–20] fish,[19] and human urine,[21–24] using liquid chromatograph (LC) - UV detection, gas chromatograph (GC) - mass spectrometer (MS) and LC - tandem mass spectrometer (MS/MS). Jeon *et al.*[25] detected 2OH–4MeO–BP, 2,4OH–BP, and 2,2′OH–4MeO–BP in water, soil, and sediment samples using GC–MS. Felix *et al.*[26] measured 2OH–4MeO–BP, 2,4OH–BP, and 2,2′OH–4MeO–BP in urine from a person using sunscreen product that contained 2OH–4MeO–BP; 2OH–4MeO–BP was detected in the urine, while 2,4OH–BP was not resolved adequately, and no peak was found for 2,2′OH–4MeO–BP. Thus, more selective and sensitive analytical methods are needed for the determination of BP derivatives in human specimens.[1]

In this study, we have developed a method for the determination of five BP derivatives, namely, 2OH–4MeO–BP; 2,4OH–BP; 2,2′OH–4MeO–BP; 2,2′,4,4′OH–BP; and 4OH–BP, in human urine, using LC-MS/MS. The chemical structures of the five BP derivatives are shown in Fig. 1. We believe that urine is a useful matrix for the biomonitoring of human exposure to BP derivatives, for the following reasons: polar metabolites are readily excreted in urine, concentrations of the polar compounds are generally higher in urine than in other biological matrices, and collection of urine is relatively easier than is the case for other matrices.

2. Experimental

2.1. Chemicals and reagents

2OH–4MeO–BP (purity: 98%), 2,4OH–BP (purity: 99%), 2,2′OH–4MeO–BP (purity: 98%), 2,2′,4,4′OH–BP (purity: 97%), 4OH–BP (purity: 98%), deuterated bisphenol-A (d_{16}-BPA), 4-methylumbelliferone, 4-methylumbelliferyl β-glucuronide, and β-glucuronidase/sulfatase (Type HP-2, from *Helix pomatia*, ≥100,000 units/ml glucuronidase and ≤7,500 units/ml sulfatase) were purchased from Sigma-Aldrich (St. Louis, MO). Analytical grade methanol (MeOH), methyl *tert*-butyl ether (MTBE), and ammonium acetate were obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ), and ethyl acetate was from Honeywell International Inc. (Morristown, NJ). Deionized (DI) water was generated with a NANOpure Diamond ultrapure water system (Barnstead International, Dubuque, IA), and had a resistance of 18.2 MΩ cm.

2.2. Standard solution

Stock solutions of BP derivatives, d_{16}-BPA, 4-methylumbelliferone, and 4-methylumbelliferyl β-glucuronide were prepared at 1 mg ml^{-1}, using MeOH, and were then stored at −20 °C in a dark room. The calibration standards, ranging in concentration from 0.05 to 100 ng ml^{-1}, were prepared from the stock solution through dilution with MeOH, whenever a new batch of samples was injected into the LC-MS/MS (*i.e.*, every 5 days). Solution at 10 ng ml^{-1} and 500 ng ml^{-1} of d_{16}-BPA, 50 ng ml^{-1} (283.82 nM) of 4-methylumbelliferone, and 10 μg ml^{-1} (28.385 μM) of 4-methylumbelliferyl β-glucuronide were made from each of the stock solutions, to check recoveries and deconjugation efficiencies. As described later, 100 ng (10 μl of 10 ppm solution) of 4-methylumbelliferyl β-glucuronide was spiked into each urine sample, and the final volume prior to LC-MS/MS analysis was set at 1 ml. The molarity of 4-methylumbelliferone deconjugated from 4-methylumbelliferyl β-glucuronide was calculated as 283.85 nM.

2.3. Sample collection

In this study, we analyzed 52 urine samples (50–200 ml) that were stored at −20 °C at the New York State Department of Health (NYSDOH); the samples had been collected during February-March 2005 from two cohorts: scientists, students, and office workers at Wadsworth Center of NYSDOH, located in Albany, New York, USA (male: $n = 14$ [24–63 years old], female: $n = 6$ [23–45 years old]); and a similar group of subjects at Center for Marine Environmental Studies (CMES), Ehime University, located in Matsuyama, Japan (male: $n = 19$ [22–55 years old], female: $n = 13$ [21–36 years old]). Information on use of sunscreen was not available for these 52 subjects. We also obtained urine samples (50 ml) from three NYSDOH scientists in September 2009: a sunscreen user (male; 47 years old) and two non-sunscreen users (male; 52 years old, female; 48 years old). Each sample was collected in a 50-ml polypropylene (PP) conical tube and stored at −20 °C until analysis. The samples were devoid of any personal identifiers. Institutional Review Board

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Table 1 MS/MS parameters optimized for analysis of five benzophenone-type UV filters

	2OH-4MeO-BP	24OH-BP	2,2′OH-4MeO-BP	2,2′,4,4′OH-BP	4OH–BP
Curtain Gas (CUR)	10	10	20	15	10
Collision Gas (CAD)	6	7	7	7	7
IonSpray Voltage (IS)	−4500	−4000	−4000	−4000	−4000
Temp (TEM)	400	400	400	400	400
Ion Source Gas 1 (GS1)	50	50	50	50	50
Ion Source Gas 2 (GS2)	60	60	60	60	60
Declustering Potential (DP)	−25	−30	−25	−25	−30
Focusing Potential (FP)	−400	−400	−300	−400	−400
Entrance Potential (EP)	−10	−10	−9	−9	−10
Collision Energy (CE)	−30	−40	−40	−40	−40
Collision Cell Exit Potential (CXP)	−7	−5	−5	−5	−5

approvals were obtained from NYSDOH for the analysis of urine samples.

2.4. Sample preparation

Urine samples were removed from the −20 °C freezer and thawed overnight at 4 °C. A 500-μl or 100-μl (for a sunscreen user) aliquot of urine was transferred into a 15-ml PP tube, using a tip ejector variable volume micropipetter. Then, 10 ng (20 μl of 500 ppb solution) of d_{16}-BPA and 100 ng (10 μl of 10 ppm solution) of 4-methylumbelliferyl β-glucuronide were spiked as internal and deconjugation standards, respectively. After gentle mixing, 300 μl of β-glucuronidase/sulfatase buffer, which contains 2 μl enzyme and 1 ml of 1.0 M ammonium acetate, was added. After vortex mixing, the sample was incubated overnight at 37 °C. Then, 3 ml of 50% MTBE/ethyl acetate was added, shaken for 30 min using a reciprocating shaker, and centrifuged at 3,000 *g* for 2 min. The organic phase, containing BP derivatives, was transferred into a new 15-ml PP tube, and the aqueous phase was extracted one more time with 3 ml of 50% MTBE/ethyl acetate. The organic phases were combined, evaporated to near-dryness using N_2, and redissolved with 1 ml of MeOH. After sonication for 10 s and centrifugation at 3,000 *g* for 2 min, the solution was transferred to a 1.5-ml amber vial for LC-MS/MS analysis.

2.5. LC-MS/MS conditions

An API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA) equipped with an Agilent 1100 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA) was used for the measurement of BP derivatives. The negative ion multiple reaction monitoring (MRM) mode was used, and the MRM transitions monitored were 227>211 (confirmation ion: 183) for 2OH–4MeO–BP, 213>91 (65) for 2,4OH–BP, 243>93 (108) for 2,2′OH–4MeO–BP, 245>91 (109) for 2,2′,4,4′OH–BP, and 197>92 (120) for 4OH–BP. Nitrogen was used as both curtain and collision gas. MS/MS parameters were optimized for each BP derivative, by infusion of 1 μg ml^{-1}-standard solution. The optimized MS/MS parameters for BP derivatives are summarized in Table 1. The MRM transitions monitored for d_{16}-BPA and 4-methylumbelliferone were 241>142 and 175>119, respectively.

Table 2 HPLC gradient parameters optimized for analysis of benzophenone derivatives

	Time/min				
	1.5	5	6	7	11.5
Mobile phase A (%)	15	80	80	90	90
Mobile phase B (%)	85	20	20	10	10

Mobile phase A: MeOH
Mobile phase B: deionized water
Injection volume: 10 μl
Flow rate: 300 μl min^{-1}

2.6. LC-MS/MS procedure

Ten microlitres of urine extract were injected onto a Thermo BETASIL C18 (100 mm length × 2.1 mm internal diameter, 5 μm particle diameter) chromatographic column serially connected with a guard column (20 × 2.1 mm, 5 μm; Thermo Electron Co., Bellefonte, PA), at a flow rate of 300 μl min^{-1}. The mobile phase was MeOH (solvent A) and deionized water (solvent B); the gradient parameters are shown in Table 2.

2.7. Data analysis

The analytes were quantified from an external calibration curve prepared at concentrations ranging from 0.05 to 100 ng ml^{-1}, when the retention times of BP derivatives in urine matched those of the standards within ±0.05 min. If

Table 3 Recoveries (n = 3) of benzophenone (BP) derivatives through the entire analytical procedure, after spiking of either of two concentrations (1.0 and 10 ppb) of BP derivatives into a urine sample

	Added amount/ng ml^{-1}	Mean value	(SD)	Recovery (%)	CV (%)
2,2′,4,4′OH-BP	0	<LOD			
	1	0.946	(0.02)	94.6	2.1
	10	9.16	(0.1)	91.6	1.1
2,2′OH-4MeO-BP	0	<LOD			
	1	0.852	(0.05)	85.2	5.9
	10	8.56	(0.24)	85.6	2.8
2,4OH-BP	0	<LOD			
	1	0.991	(0.01)	99.1	1.0
	10	9.84	(0.25)	98.4	2.5
2OH-4MeO-BP	0	<LOD			
	1	0.873	(0.02)	87.3	2.3
	10	8.78	(0.1)	87.8	1.1
4OH-BP	0	<LOD			
	1	0.996	(0.01)	99.6	1.0
	10	9.52	(0.05)	95.2	0.53

concentrations of BP derivatives in urine were above 100 ng ml^{-1}, the sample was re-analyzed using a smaller volume (100 μl). In this study, the urine sample from one sunscreen user had to be re-analyzed for 2OH–4MeO–BP and 2,4OH-BP due to high concentration (*i.e.*, further dilutions were needed). Data processing was performed with the Analyst 1.4.1 software package. Statistical analyses were conducted with Statistica V. 06J (StatSoft Inc., Tulsa, OK). The statistical significance of differences in concentrations of BP derivatives, between the 20 U.S. male and female samples collected in 2005, was evaluated by Mann-Whitney *U* test. Samples with concentrations less than the limit of detection (LOD) were treated as the LOD value, for the statistical analysis. A p value <0.05 was considered significant.

3. Results and discussion

3.1. Instrumental calibration and limit of detection

Calibration standards injected at eight different concentrations, ranging from 0.05 to 100 ng ml^{-1}, for each of the BP derivatives showed excellent linearity ($r > 0.99$). When 10 μl of 0.05 ng ml^{-1} (*i.e.*, 0.5 pg of 2,4OH–BP and 4OH–BP) or 10 μl of 0.1 ng ml^{-1} (*i.e.*, 1.0 pg of 2OH–4MeO–BP, 2,2′OH–4MeO–BP, and 2,2′,4,4′OH–BP) standard was injected, the signal to noise (S/N) ratio was 3.0 for 2,4OH–BP and 2,2′,4,4′OH–BP; 3.3 for 4OH–BP and 2OH–4MeO–BP; and 5.0 for 2,2′OH–4MeO–BP. Thus, the instrumental detection limits were in the range of 0.5–1.0 pg. The LOD and LOQ values for the analytical method were determined based on the standard deviations of six replicate analyses, using the lowest calibration standard, *i.e.*, 0.05 ng ml^{-1} for 2,4OH–BP and 4OH–BP, and 0.1 ng ml^{-1} for 2OH–4MeO–BP, 2,2′OH–4MeO–BP, and 2,2′,4,4′OH–BP. The LOD and LOQ values were calculated as $3S$ and $10S$, respectively, where S is the standard deviation. The calculated LOD and LOQ values were, respectively, 0.041 and 0.14 ng ml^{-1} for both 2,4OH–BP and 4OH–BP; 0.067 and 0.22 ng ml^{-1} for 2,2′OH–4MeO–BP; and 0.14 and 0.45 ng ml^{-1} for both 2OH–4MeO–BP and 2,2′,4,4′OH–BP. Because of the 2–fold dilution of the sample in the analytical procedure, the actual LOD and LOQ values for urine samples analyzed in this study were 0.082 and 0.28 ng ml^{-1} for both 2,4OH–BP and 4OH–BP; 0.13 and 0.44 ng ml^{-1} for 2,2′OH–4MeO–BP; and 0.28 and 0.90 ng ml^{-1} for both 2OH–4MeO–BP and 2,2′,4,4′OH–BP.

3.2. Analytical accuracy and precision

A recovery test was conducted, through spiking of two concentrations (1.0 and 10 ppb) of each of five BP derivatives into a urine sample that had been found not to contain any of the target compounds. The spiked urine sample was passed through the entire analytical procedure. The recoveries of five BP derivatives in urine were between 85.2 and 99.6%, and the coefficients of variation (CVs) of triplicate analyses were between 0.53 and 5.9% (Table 3). In addition, variations in the responses of BP derivatives within a day and between 5 days were checked by replicate analyses of a U.S. urine sample collected in 2005 that had been found to contain 2,4OH–BP, 2OH–4MeO–BP, and 4OH–BP. Respective CVs of five replicates within a day and between 5 days were 1.4 and 3.7% for 2,4OH–BP, 1.7 and 3.0% for 2OH–4MeO–BP, and 2.8 and 4.5% for 4OH–BP (Table 4). These results suggest that the analytical method developed for the analysis of five BP derivatives in urine provides adequate accuracy and precision.

When BP derivatives were analyzed in 55 urine samples from the U.S. and Japan, d_{16}-BPA was used as an internal standard, as reported in earlier studies on 2OH–4MeO–BP in urine; $^{13}C_{12}$-BPA has been used in those studies.[21–23] Recoveries of d_{16}-BPA spiked into urine samples, in our study, were between 78.5 and 96.0%. Deconjugation efficiencies, as estimated from the concentrations of 4-methylumbelliferone, were nearly 100%. No BP derivatives were detected in procedural blanks, which consisted of solvents and reagents passed through the entire analytical procedure.

3.3. Benzophenone-type UV filters in human urine

Typical chromatograms of BP derivatives detected in human urine and standard solutions are shown in Fig. 2. 2,2′,4,4′OH–BP and 2,2′OH–4MeO–BP were not detected in any of the urine samples analyzed. Detection frequencies of 2,4OH–BP, 2OH–4MeO–BP, and 4OH–BP in urine samples collected in February-March 2005 are shown in Fig. 3.

2,4OH–BP, 2OH–4MeO–BP, and 4OH–BP were respectively detected in 100%, 66.7%, and 33.3% of the urine samples from females, and in 42.6%, 21.4%, and 7.1% of the urine samples from males, in Albany, New York (Fig. 3). Concentrations of BP derivatives in urine samples from females were significantly higher than those in males ($p<0.05$). This suggests higher exposures in females than in males and can be attributed to higher

Table 4 Within-day and between-day variations in determinations of 2,4OH–BP, 2OH–4MeO–BP, and 4OH–BP, by replicate analyses of a urine sample

	Within-day precision ($n = 5$)			Between-day precision (1 run per day for 5 days)		
	2,4OH-BP	2OH-4MeO-BP	4OH-BP	2,4OH-BP	2OH-4MeO-BP	4OH–BP
1	4.9	7.1	0.82	4.6	7.3	0.76
2	4.9	7.2	0.79	4.9	6.8	0.77
3	4.9	7.0	0.78	4.5	6.8	0.76
4	4.8	7.0	0.82	4.6	7.0	0.84
5	4.9	7.2	0.78	4.4	6.8	0.76
Average	4.9	7.1	0.80	4.6	6.9	0.78
SD	0.067	0.12	0.023	0.17	0.21	0.035
CV (%)	1.4	1.7	2.8	3.7	3.0	4.5

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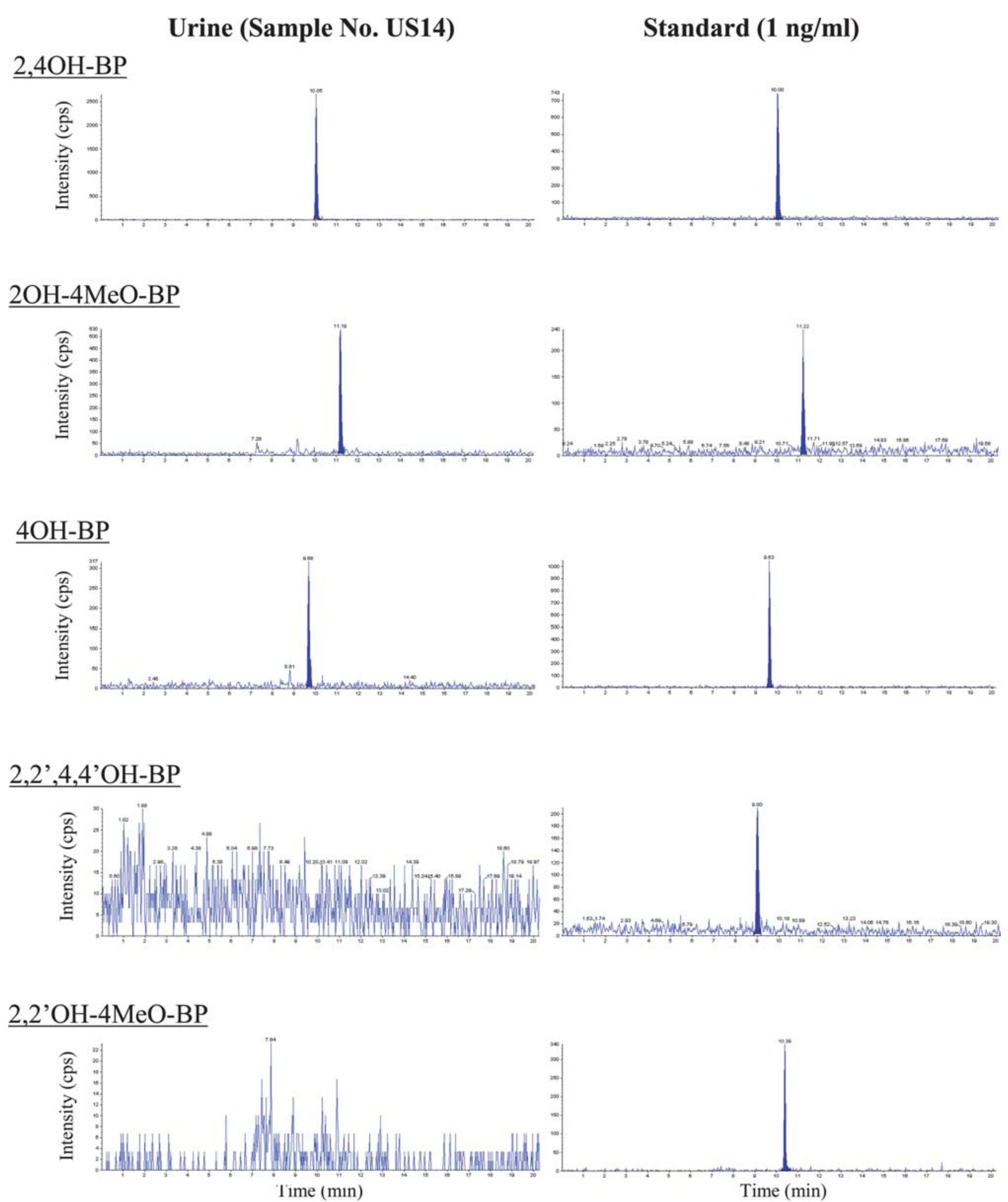

Fig. 2 Chromatograms of BP derivatives detected in human urine and in a standard solution.

usage of both sunscreen products and also other cosmetic products containing UV filters by females. Among 32 Japanese urine samples analyzed, only 2,4OH–BP was found in only two samples from females (15.4%); no samples from males had any detectable concentrations (Fig. 3). These results indicate that the U.S. females are exposed to relatively high concentrations of BP derivatives. Although published data for 2,4OH–BP and 4OH–BP are not available, geometric mean concentration of 2OH–4MeO–BP in 2517 urine samples collected from the U.S. general population ($\geq$ 6 years of age), as part of the 2003–2004 National Health and Nutrition Examination Survey, was 22.9 ng ml^{-1} (range: 0.4–21700 ng ml^{-1}).[23] Concentrations of this BP

derivative in females were significantly higher than in males in that study, indicating probable differences in the use of personal care products containing 2OH–4MeO–BP.[23] Concentrations of 2OH–4MeO–BP in all of the urine samples from Albany that were analyzed in the present study were lower than the U.S. mean value of 22.9 ng ml^{-1} reported earlier.[23] This difference could be ascribable in part to seasonal variations in personal exposure to BP derivatives. Wolff *et al.*[27] measured concentrations of 2OH–4MeO–BP in urine collected from 90 girls (6.4–9.2 years old) in New York City (NY), Cincinnati (OH), and northern California. 2OH–4MeO–BP concentrations ranged from <0.2 to 26700 ng ml^{-1} and were significantly higher in samples collected in summer than in other seasons.[27] We analyzed BP derivatives in urine collected from a male sunscreen user and two non-sunscreen users, in September 2009, and found notably high concentrations of 2OH–4MeO–BP and 2,4OH–BP in urine from the sunscreen user (Table 5), indicating that sunscreen products are one of the major exposure sources of BP derivatives in humans.

Concentrations of 2,4OH–BP in urine were comparable to those of 2OH–4MeO–BP (Fig. 3 and Table 5). This observation can be attributed to the formation of 2,4OH–BP from 2OH–4MeO–BP *via* metabolic activities of cytochrome P450 enzymes, although humans can also be exposed to 2,4OH–BP directly, through the use of personal care products; the latter BP derivative is also present in some U.S. cosmetics, although the frequency of use of this compound is much lower than that of 2OH–4MeO–BP.[28] In a skin absorption study,[29] sunscreen lotion containing 2OH–4MeO–BP was applied to the skin of three female volunteers; the major metabolite in the urine was found to be 2,4OH–BP. It has also been reported that 2OH–4MeO–BP is metabolized principally to 2,4OH–BP, in rats[12–14] and piglets.[15] There is evidence that 2,4OH–BP possesses higher estrogenic activity than does 2OH–4MeO–BP,[7–9,13,16] indicating that hydroxylated

Table 5 Concentrations (ng ml^{-1}) of BP derivatives in urine collected from a sunsceen user and two non-sunsceen users in September 2009 (Albany, NY)

	2OH-4MeO-BP	2,4OH-BP	4OH-BP	2,2′OH-4MeO-BP	2,2′,4,4′OH–BP
Sunscreen user					
Male (47)[a]	330	250	1.53	<LOD	<LOD
Non user					
Male (52)[a]	<LOD	0.39	<LOD	<LOD	<LOD
Female (48)[a]	<LOD	0.33	0.37	<LOD	<LOD

[a] Numbers in parentheses denote age (in years).

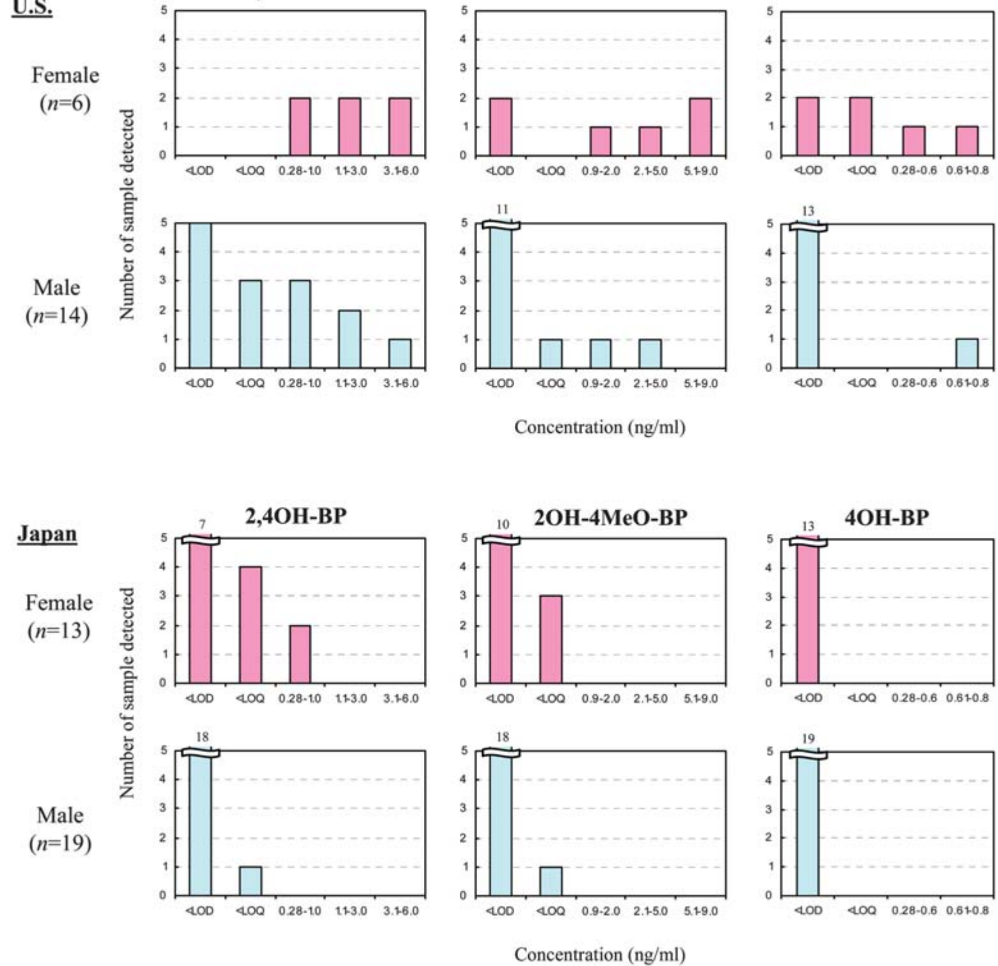

Fig. 3 Detection frequencies of BP derivatives in urine samples collected from subjects in Albany, New York and in Matsuyama, Japan, during February-March 2005.

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intermediates (such as 2,4OH–BP) can be potent xenoestrogens. Oral administration of 2OH–4MeO–BP to rats showed that the metabolite, 2,4OH–BP, decreased much more slowly in rat blood than did its parent compound, indicating that the metabolites can be retained in the body for a long time.[14] 4OH–BP detected in the present study could have been formed through oxidative metabolism of BP, but information on the use of 4OH–BP in personal care products, and evidence of its formation from 2OH–4MeO–BP and 2,4OH–BP are lacking. BP has been reported to be metabolized to 4OH–BP, following incubation with rat hepatocytes[30] and in an *in vivo* study.[14] The hydroxylated metabolite acted as a weak xenoestrogen on MCF-7 cells.[30]

4. Conclusions

We have developed a reliable LC-MS/MS method for the measurement of five BP derivatives, namely, 2OH–4MeO–BP, 2,4OH–BP, 2,2′OH–4MeO–BP, 2,2′,4,4′OH–BP, and 4OH–BP, in human urine. 2OH–4MeO–BP, 2,4OH–BP, and 4OH–BP were detected in the samples analyzed; concentrations of 2,4OH–BP were comparable to those of 2OH–4MeO–BP. Concentrations of the remaining two analytes, 2,2′OH–4MeO–BP and 2,2′,4,4′OH–BP, were below the limit of detection in all of the samples analyzed. Considering that elevated concentrations of 2OH–4MeO–BP and 2,4OH–BP found in the urine from a known sunscreen user sampled in late summer (September 2009) and considering that 2,4OH–BP possesses a higher estrogenic activity than does 2OH–4MeO–BP, comprehensive investigations on human exposures to not only 2OH–4MeO–BP but also its metabolite, 2,4OH–BP, are needed, especially for females and during the summer months.

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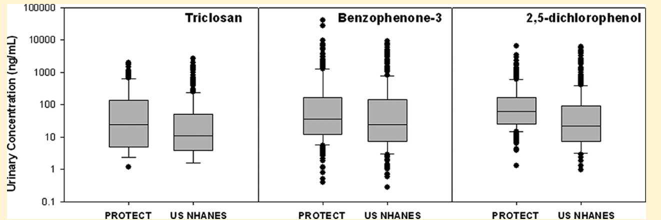
Distribution, Variability, and Predictors of Urinary Concentrations of Phenols and Parabens among Pregnant Women in Puerto Rico

John D. Meeker,*[,†] David E. Cantonwine,[†] Luis O. Rivera-González,[†] Kelly K. Ferguson,[†] Bhramar Mukherjee,[‡] Antonia M. Calafat,[§] Xiaoyun Ye,[§] Liza V. Anzalota Del Toro,[‖] Noé Crespo-Hernández,[‖] Braulio Jiménez-Vélez,[‖] Akram N. Alshawabkeh,[⊥] and José F. Cordero[‖]

[†]Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, United States
[‡]Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, Michigan, United States
[§]Centers for Disease and Control and Prevention, Atlanta, Georgia, United States
[‖]University of Puerto Rico Graduate School of Public Health, UPR Medical Sciences Campus, San Juan, Puerto Rico
[⊥]College of Engineering, Northeastern University, Boston, Massachusetts, United States

Supporting Information



ABSTRACT: Puerto Rico has higher rates of a range of endocrine-related diseases and disorders compared to the United States. However, little is known to date about human exposures to known or potential endocrine disrupting chemicals (EDCs) in Puerto Rico. We recruited 105 pregnant women in Northern Puerto Rico who provided urine samples and questionnaire data at three times (18 ± 2, 22 ± 2, and 26 ± 2 weeks) during gestation. We measured the urinary concentrations of five phenols and three parabens: 2,4-dichlorophenol (24-DCP), 2,5-dichlorophenol (25-DCP), benzophenone-3 (BP-3), bisphenol A (BPA), triclosan (TCS), butyl paraben (B-PB), methyl paraben (M-PB), and propyl paraben (P-PB). The frequent detection of these chemicals suggests that exposure is highly prevalent among these Puerto Rican pregnant women. Urinary concentrations of TCS, BP-3, and 25-DCP were higher than among women of reproductive age in the US general population, while concentrations of BPA, 24-DCP, and parabens were similar. Intraclass correlation coefficients (ICC) varied widely between biomarkers; BPA had the lowest ICC (0.24) and BP-3 had the highest (0.62), followed by 25-DCP (0.49) and TCS (0.47). We found positive associations between biomarker concentrations with self-reported use of liquid soap (TCS), sunscreen (BP-3), lotion (BP-3 and parabens), and cosmetics (parabens). Our results can inform future epidemiology studies and strategies to reduce exposure to these chemicals or their precursors.

■ INTRODUCTION

There is growing evidence that exposure to endocrine disrupting chemicals (EDCs) may contribute to various human diseases and disorders, such as adverse pregnancy outcomes, altered reproductive development or function, hindered brain development, obesity, and increased risk of metabolic syndrome and diabetes.[1,2] In the past two decades, Puerto Rico has experienced a steep increase in the rate of preterm birth, where rates have gone from being similar to the US average in the 1990s (12%) to now being the highest (18%) among all US states and territories.[3,4] As a nation, Puerto Rico would have the third highest preterm birth rate worldwide behind only Malawi and Congo.[5] Compared to the United States, Puerto Rico also has higher rates of childhood obesity and asthma[6−8] as well as of obesity, metabolic syndrome, and diabetes in adults.[9,10] There is some evidence for widespread endocrine disruption on the island, manifested in the form of elevated rates of developmental anomalies such as premature thelarche.[11,12] However, little is known to date about human exposures to EDCs in Puerto Rico.

Exposure to certain phenols (or their precursors) and parabens is widespread in the United States based on the detection of urinary biomarkers of exposure to these chemicals in virtually everyone tested in the National Health and Nutrition Examination Survey (NHANES), a large-scale study representative of the US population.[13] Use of consumer and personal care products are thought to contribute to exposure, but this remains unclear due to the lack of research to date. Among the environmental phenols, BPA represents the most studied. It is used in the manufacture of polycarbonate plastics and epoxy resins and may be found in a range of consumer products as well as in canned and other foods. The primary pathway of exposure for most people is likely through the diet, though other sources and pathways are possible.[14] BPA is weakly estrogenic, but may impact multiple endocrine-related

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pathways, and has been associated with a range health effects in animal and human studies.[15,16] Triclosan (TCS) is used as a preservative and antiseptic agent added to a range of products including soaps, toothpaste, mouthwash, and other personal care products.[17] This widespread use has resulted in contamination of the aquatic environment through residential wastewaters; TCS can also be further transformed into other toxic chemicals.[18,19] TCS has demonstrated effects on thyroid function and possibly reproduction in animal studies,[19] but there have been very few human studies. Benzophenone-3 (BP-3) is a UV filter and stabilizer used in sunscreens, lotions, conditioners, cosmetics, and plastics, which, like TCS, has led to its detection in surface and drinking waters.[13] It has been shown to be weakly estrogenic and antiandrogenic and/or impact thyroid function in experimental research, but human studies of BP-3 are also lacking.[20]

Dichlorophenols are also prevalent in human urine samples.[13] 2,4-Dichlorophenol (24-DCP) is a minor metabolite of the common herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and other environmental chemicals (including TCS[21]), while 25-DCP is a metabolite of 1,4-dichlorobenzene (paradichlorobenzene) that has been used in moth balls, as a room/toilet deodorizer and previously as an insecticide.[13] Urinary 25-DCP (but not 24-DCP) was associated with obesity in children[22] and with age at menarche in adolescent girls[23] in recent reports utilizing NHANES data. Inverse associations between 25-DCP and infant birth weight, particularly among male infants, have also been reported.[24,25] Finally, parabens are a class of chemicals widely used as preservatives in cosmetics and other personal care products and are also used as antimicrobials in various foods and pharmaceuticals.[26] They are suspected EDCs with demonstrated adverse impacts on endocrine and reproductive function in animal studies, but research on human health impacts has been extremely limited.[27,28]

With the exception of two recent studies of BPA[29,30] and a related study on parabens,[27] data on temporal variability and/or predictors of exposure to these chemicals related to product use in pregnant women are lacking. Given their ubiquity and potential to contribute to adverse human health, exposure characterization studies are needed to inform epidemiology studies, especially among susceptible populations such as pregnant women and children. Information is also needed on sources of exposure to inform potential interventions aimed at reducing exposures and associated health risks. The objective of this study was to determine distributions, variability, and predictors of urinary biomarkers of environmental phenols and parabens measured at multiple times during pregnancy among women living in Northern Puerto Rico.

■ METHODS

Study Participants. This study was conducted among pregnant women participating in the "Puerto Rico Testsite for Exploring Contamination Threats (PROTECT)" project, an ongoing prospective birth cohort in the Northern Karst Region of Puerto Rico, which is designed to evaluate the relationship between environmental toxicants and risk of preterm delivery. Study participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico during 2010−2012. Women were eligible if they were between the ages of 18 to 40 years, resided in a municipality within the Northern karst region, did not use oral contraceptives three months prior to pregnancy or *in vitro* fertilization as a method of assisted reproductive technology, and were free of known medical/obstetrics complications. Women provided spot urine samples at three separate study visits (18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks of gestation). Questionnaires to collect demographic information and data on self-reported product use in the 48 h preceding urine sample collection were also administered at each visit.

The present analysis reflects the first 105 women recruited into the study who had urinary biomarker data as of June 2012. The research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participating clinics, the University of Michigan School of Public Health, and Northeastern University. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research. The study was described in detail to all participants, and informed consent was obtained prior to study enrollment.

Measurement of Phenols and Parabens in Urine. Urine was collected in polypropylene containers, divided into aliquots, and frozen at −80 °C until shipped overnight to the CDC. All urine samples were analyzed at the National Center for Environmental Health of the CDC for five phenols (BPA, TCS, BP-3, 24-DCP, and 25-DCP) and three parabens (butyl paraben (B-PB), methyl paraben (M-PB), propyl paraben (P-PB)) by online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry.[31,32] The analytical method details are provided in the Supporting Information. To monitor for accuracy and precision, each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentrations. The limits of detection (LODs) varied slightly between analytes but were generally in the low ng/mL range. Concentrations below the LOD were assigned a value of LOD divided by the square root of 2. Specific gravity (SG) was measured at the University of Puerto Rico Medical Sciences Campus using a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan). For data analyses utilizing SG-corrected metabolite concentrations, the following formula was used: $P_c = P[(1.019-1)/(SG-1)]$ where P_c is the SG-adjusted urinary concentration (ng/mL), P is the measured urinary concentration, and SG is the specific gravity of the urine sample. An SG of 1.019 was the median SG value for this group of urine samples.

Statistical Analysis. Geometric means and selected percentiles were calculated to describe the distributions of urinary biomarkers of phenols and parabens among study participants and for comparison with other published reports. We compared concentrations measured in the present study with those measured in NHANES. We utilized publicly accessible urinary phenols and parabens concentration data from NHANES 2007−2008 and 2009−2010 among females between the ages of 18 and 40 years, along with appropriate sampling weights, to tabulate geometric means and selected percentiles.

Pearson and Spearman rank correlations were calculated to assess relationships between study visits and between the various biomarkers. Differences in geometric mean biomarker concentrations between study visits (i.e., time points in gestation) were tested using one-way ANOVA. To assess temporal variability in urinary biomarker concentrations intraclass correlation coefficients (ICCs) and their 95%

confidence intervals were calculated.[33] ICC is a measure of the reliability of repeated measures over time, defined as the ratio of between-subject variance to total (between-subject plus within-subject) variance. ICC ranges from zero to one, with values near zero indicating poor temporal reliability and values near one indicating high temporal reliability.[34]

Geometric means were compared between categories for maternal age, maternal education, marital status, household income, parity, prepregnancy body mass index (BMI), and time of day at urine collection. We examined the association between urinary concentrations of the biomarkers and demographic, sampling time, and 48-h recall of product use variables using linear mixed effects models with the compound symmetry covariance structure. Demographic factors were included as fixed time-invariant effects in our mixed models. Time of day of sample collection and product use variables were modeled as fixed time-dependent factors. Natural log-transformed unadjusted or SG-adjusted urinary concentrations of phenols or parabens were the dependent variable in mixed models, with separate models for each independent variable. Data were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

A total of 279 urine samples from 105 women were analyzed. Data on SG were missing for 2 samples. Statistical analysis was conducted for both unadjusted and SG-adjusted urinary concentrations, and results were highly consistent between the two approaches throughout. Demographic characteristics of our study sample are shown in Table 1. The mean age was 27.2 years; 82% of the women had an education above the high school level, and 73% were either married or in a domestic partnership. The majority of women reported a household income below $40,000 per year, and nearly all women did not smoke during pregnancy.

Table 1. Demographic Characteristics of n = 105 Pregnant Women from Puerto Rico (2010−2012)

variable	mean ± SD or n (%)
maternal age at enrollment (years)	27.1 ± 4.8
gravidity (# pregnancies)	1.9 ± 1.0
parity (# live births)	0.6 ± 0.7
years of maternal education	
<high school	12 (11.4)
high school/equivalent	7 (6.7)
college	86 (81.9)
household income (US$)	
missing	15 (14.3)
<$20,000	46 (43.8)
≥$20,000 to <$40,000	27 (25.7)
≥$40,000	17 (16.2)
marital status	
single	29 (27.6)
married or living together	76 (73.4)
prepregnancy BMI (kg m^{-2})	
≤25	60 (57.1)
>25 to ≤30	32 (30.5)
>30	13 (12.4)
smoked during pregnancy	
missing	2 (1.9)
yes	1 (1.0)
no	102 (97.1)
employment	
unemployed	42 (40.0)
employed	63 (60.0)

Distributions of urinary biomarker concentrations are presented in Table 2, along with distributions from 18 to 40 year old women from US NHANES 2007-08 and 2009-10. BPA, BP-3, both dichlorophenols, M-PB, and P-PB were detected in between 95% and 100% of samples. TCS was detected in 89% of samples, while B-PB was detected in 58%. When comparing distributions with NHANES women, women in our study had higher geometric mean concentrations of BP-3, TCS, and 25-DCP. Median concentrations of TCS and 25-DCP were 2- and 6-fold greater, respectively, among women in this study compared to NHANES 2009-10. For BP-3, median concentrations were similar, but the populations diverged greatly at the upper end of the distribution, which resulted in a higher geometric mean concentration among Puerto Rican women. Geometric mean and median concentrations of BPA, 24-DCP, and the three parabens were similar between the two populations. When looking across urinary biomarkers there was a strong correlation between 24-DCP and 25-DCP (Spearman r > 0.8) and between the three parabens, particularly between M-PB and P-PB (r = 0.8). There were also weak (r = 0.25 to 0.4) but statistically significant (p < 0.05) correlations between 24-DCP and TCS and between BP-3 and the parabens.

Box plot comparisons of the concentration distributions for each biomarker between study visits (approximately 18, 22, and 26 weeks gestation) are shown in Figure 1. There were no statistically significant differences between unadjusted or SG-adjusted geometric mean concentrations at the three visits for any of the biomarkers. ICCs, presented in Table 3, varied widely between biomarkers and ranged from weak to moderately strong. BPA had the lowest ICC (0.24) and BP-3 had the highest (0.62), followed by 25-DCP (0.49) and TCS (0.47).

Urinary biomarker concentrations in relation to sampling and demographic variable categories are presented in Table 4. Only urinary 24-DCP and 25-DCP concentrations were associated with time of day of urine sample collection, where concentrations in samples collected later in the day (between 3:00 and 8:00 p.m.) were significantly higher (p = 0.01 and p = 0.006, respectively) compared to the other time categories. There was a trend for increasing BP-3 concentrations with increasing age categories. The oldest age category (>30 years) was also associated with higher TCS and B-PB concentrations. BP-3 and P-PB concentrations were lower among women with <12 years of education. There were increasing trends between BP-3 concentrations and income status, B-PB concentrations and increased parity, and between BPA concentrations and prepregnancy BMI. Finally, dichlorophenols concentrations were lower and B-PB concentrations higher among women who were not currently employed.

Self-reported use of selected products in the 48 h preceding urine sample collection that were related to urinary biomarker concentrations are presented in Table 5. Use of hand or body lotion was associated with significantly higher (between 2- and 3-fold) geometric mean concentrations of BP-3, B-PB, M-PB, and P-PB. Self-reported use of colored cosmetics (makeup) was positively associated with similar changes in all paraben biomarker concentrations. Geometric mean BP-3 concentrations were 10-fold higher among women who reported recent use of sunscreen (503 ng/mL) than among other women (49

Table 2. Urinary Phenol and Paraben Concentrations (ng/mL) in n = 105 Pregnant Women from Puerto Rico[a] in 2010−2012 and Comparison with U.S. Population-Based Samples of Women Ages 18−40 from NHANES[b,c]

			percentiles				
	%>LOD	GM (95% CI)	25th	50th	75th	95th	max.
BPA							
PROTECT	97.9	2.6 (2.3, 2.9)	1.3	2.5	4.4	13.9	97.4
NHANES 07-08	96.2	2.5 (2.2, 2.9)	1.3	2.4	4.6	14.0	64.0
NHANES 09-10	92.1	2.0 (1.7, 2.2)	0.8	2.0	4.1	9.2	416
TCS							
PROTECT	88.9	29.9 (23.6, 37.9)	5.1	26.2	121	944	2000
NHANES 07-08	84.9	18.7 (14.7, 23.8)	4.4	14.0	67.7	520	2780
NHANES 09-10	79.0	16.9 (12.2, 23.4)	4.0	13.0	52.5	577	2690
BP-3							
PROTECT	100	52.2 (41.0, 66.4)	11.5	31.3	172	2150	39700
NHANES 07-08	97.8	38.6 (28.9, 51.6)	8.6	35.6	148	1256	21500
NHANES 09-10	99.3	36.3 (22.6, 58.4)	7.6	27.4	145	3340	8970
24-DCP							
PROTECT	97.9	1.5 (1.3, 1.8)	0.6	1.3	3.3	16.3	83.3
NHANES 07-08	90.1	0.9 (0.7, 1.1)	0.3	0.7	1.7	8.4	231
NHANES 09-10	85.1	0.7 (0.6, 0.9)	0.2	0.6	1.5	7.1	147
25-DCP							
PROTECT	100	26.0 (21.4, 31.7)	7.0	19.0	82.5	650	4110
NHANES 07-08	99.7	8.4 (6.4, 11.0)	2.6	6.1	20.1	333	11300
NHANES 09-10	97.6	5.1 (3.7, 7.1)	1.1	3.8	16.5	215	3820
B-PB							
PROTECT	58.4	1.0 (0.8, 1.3)	<0.2	0.4	5.5	36.4	148
NHANES 07-08	74.8	1.1 (0.9, 1.4)	<0.2	0.7	4.0	33.6	188
NHANES 09-10	59.0	0.7 (0.6, 0.8)	<0.2	0.4	2.2	22.1	127
M-PB							
PROTECT	100	140 (117, 167)	57.6	153	381	1590	6040
NHANES 07-08	99.7	132 (97.1, 179)	47.8	146	430	1444	7550
NHANES 09-10	99.5	111 (90.3, 138)	38.6	119	374	1269	4840
P-PB							
PROTECT	99.3	30.0 (24.1, 37.5)	10.1	36.7	130	493	1220
NHANES 07-08	98.1	28.0 (20.3, 38.6)	6.4	33.6	121	410	1400
NHANES 09-10	98.1	21.2 (16.0, 28.2)	4.6	24.8	110	434	3490

[a]Includes biomarker concentrations for up to 3 repeated samples per woman (n = 279 samples). [b]Females 18−40 years of age; n = 365 for metabolites measured in 2007−2008; n = 415 for 2009−2010. [c]NHANES, National Health and Nutrition Examination Survey; LOD, limit of detection; GM, geometric mean; NA, not applicable.

ng/mL). Use of mouthwash was associated with significant increases in BP-3 and BPA concentrations. Triclosan concentrations were also higher among women reporting use of liquid soap and hairspray compared to those who did not. The use of bar soap was negatively associated with BP-3 concentrations, and use of bar soap and pesticides were both negatively associated with M-PB concentrations. None of the questionnaire variables, including use of pesticides, were associated with increased dichlorophenol concentrations (not shown). When demographic and product use variables that were associated with concentrations of each biomarker were included simultaneously in multivariate models, results were similar for each product use variable though somewhat attenuated (not shown).

■ DISCUSSION

To our knowledge, this is the first study to report biomarkers of exposure to known or suspected endocrine disrupting environmental phenols and parabens in Puerto Rico and also the first to report temporal variability and/or predictors of most of these chemicals among pregnant women. We found that exposure to the chemicals measured is highly prevalent among pregnant women in Puerto Rico. We also found evidence that concentrations of TCS, BP-3, and 25-DCP were higher than among women of reproductive age in the US general population. On the other hand, concentrations of BPA, 24-DCP, and parabens were similar to those reported among US women.

The urinary biomarker concentrations measured in the present study can also be compared with other studies of pregnant women, though caution must be taken since there were potentially important differences between the studies (e.g., study design, year of sample collection) that may impact these comparisons. Urinary BPA concentrations in this study were somewhat higher than studies in Ohio,[29] New York,[25,35] Mexico City,[36] Germany,[37] and The Netherlands[38] that reported geometric mean and/or median concentrations between 1.0 and 2.0 ng/mL but similar to studies in Boston,[30] France,[24] and Spain[39] that reported median BPA concentrations of 2.6, 2.7, and 2.2 ng/mL, respectively. Studies of the other chemicals we measured have been much more limited in number. Median concentrations of TCS and BP-3 appear to be much higher in this Puerto Rico cohort (26 and 31 ng/mL, respectively) compared to studies of pregnant women in New

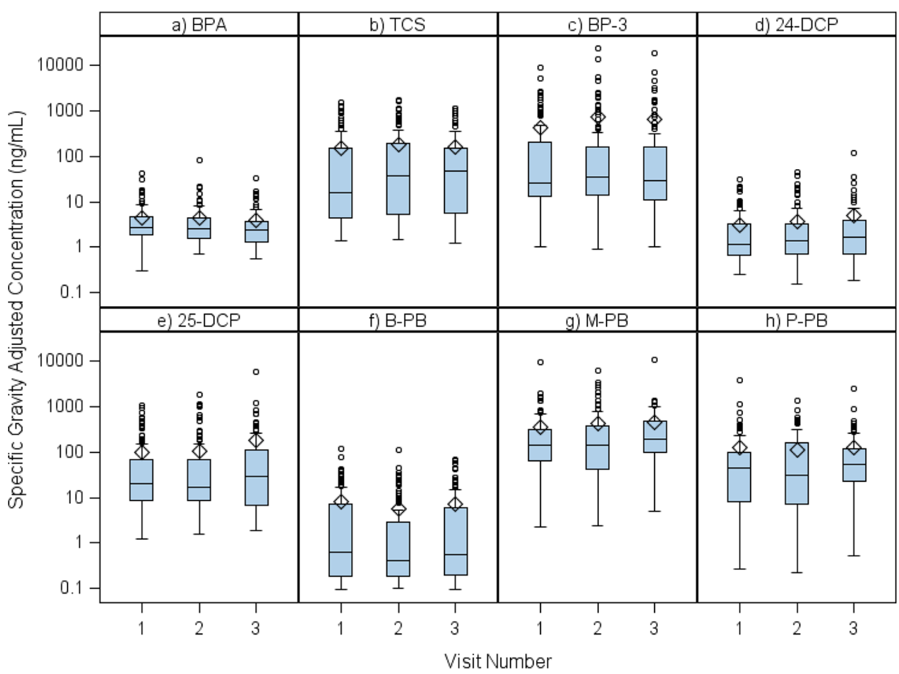

Figure 1. Boxplots comparing SG-adjusted concentrations of urinary biomarkers across study visits. Visit 1 (18 ± 2 weeks gestation), Visit 2 (22 ± 2 weeks), Visit 3 (26 ± 2 weeks).

Table 3. Intraclass Correlation Coefficients (ICCs) and 95% Confidence Intervals (95% CIs) for ln-Transformed Urinary Concentrations of Phenols and Parabens

	unadjusted[a]		SG-adjusted[b]	
urinary biomarker	ICC	95% CI	ICC	95% CI
BPA	0.27	0.15, 0.42	0.24	0.13, 0.40
TCS	0.42	0.30, 0.55	0.47	0.35, 0.59
BP-3	0.58	0.47, 0.68	0.62	0.51, 0.71
24-DCP	0.37	0.25, 0.50	0.38	0.27, 0.52
25-DCP	0.50	0.38, 0.62	0.49	0.38, 0.61
B-PB	0.45	0.33, 0.57	0.47	0.35, 0.60
M-PB	0.36	0.24, 0.50	0.39	0.27, 0.53
P-PB	0.31	0.19, 0.46	0.32	0.20, 0.47

[a] n = 279 samples from 105 participants. [b] n = 277 samples from 105 participants.

York (11 and 7.5 ng/mL)[25] and Spain (6.1 and 3.4 ng/mL).[39] Median concentrations of 25-DCP were similar in the Spanish study (17 ng/mL), but higher in the New York study (53 ng/mL), compared to this study (19 ng/mL). A recent pregnancy study in France reported a similar median TCS concentration (24 ng/mL) compared to our study, but BP-3 and 25-DCP concentrations were lower in that study (1.7 and 10.2 ng/mL, respectively).[24] For paraben concentrations in this study, B-PB was somewhat lower, P-PB was somewhat higher, and M-PB was similar compared to the Spanish and French studies.[24,39]

Characterizing temporal variability in exposure metrics, especially for biomarkers of nonpersistent compounds such as those measured in the present study, is a critical step in designing and interpreting an epidemiology study related to the potential for exposure measurement error. We found that temporal variability in urinary biomarker concentrations was not uniform across the chemicals measured. We found a weak ICC for BPA (ICC = 0.24), which is consistent with previous studies among pregnant women in Ohio (ICC = 0.10 to 0.28)[29] and Boston (ICC = 0.12 to 0.23).[30] This is likely attributable to the rapid metabolism of BPA in addition to intermittent exposure to BPA which occurs mostly through the diet. The ICCs we calculated for M-PB (ICC = 0.39) and P-PB (ICC = 0.32) were somewhat lower than those recently reported among pregnant women who had sought fertility treatment in Boston (ICC = 0.46 and 0.44, respectively).[27] The higher ICCs we reported for BP-3 (0.62), TCS (0.47), and 25-DCP (0.49) suggest more consistent exposure sources over time for these compounds. Only one other study, conducted among children in New York City, has assessed temporal variability of these chemicals.[40] The authors reported lower ICCs than we reported here (0.39, 0.35, and 0.37 for BP-3, TCS, and 25-DCP, respectively).

We found that only dichlorophenol concentrations were associated with time of day of urine sample collection, where samples collected later in the day had significantly higher concentrations of these biomarkers. The lack of association between urinary BPA and time of day in these women was inconsistent with time of day influences on urinary BPA reported by a previous study of pregnant women in Ohio[29] and an NHANES analysis.[41] On the other hand, the positive association between BPA and BMI in this study was consistent with previous studies reporting relationships between BPA exposure and obesity.[42,43] Our observation of increased TCS concentrations among women in their 30s is similar to an NHANES analysis.[17] Finally, our finding that dichlorophenol concentrations were lower among women not currently employed may suggest occupational sources of exposure to the parent chemicals, which may also be reflected in the time of day patterns we observed in these two biomarkers. However, additional detailed studies would be needed to support that conclusion.

Most of the associations we observed between self-reported product use and urinary biomarker concentrations are supported by what is known about the use of these chemicals. Liquid soap use in the 48 h preceding urine sample collection was positively associated with TCS concentrations in this study,

Table 4. Geometric Means of Specific Gravity Adjusted Urinary Concentrations of Phenols and Parabens According to Time of Urine Collection, Demographic, and Maternal Factors

	n (%)[a]	BPA	TCS	BP-3	24-DCP	25-DCP	B-PB	M-PB	P-PB
overall	277 (100)	2.8	31.8	55.8	1.7	28.0	1.1	148.4	31.8
time of day									
0600–0859	37 (13.4)	2.9	37.2	68.5	1.7	31.7	0.6	188.3	29.1
0900–1159	117 (42.2)	2.5	29.1	48.3	1.7	27.4	1.1	143.8	33.8
1200–1459	90 (32.5)	2.9	42.0	58.6	1.3	19.6	1.2	125.4	25.8
1500–2000	33 (11.9)	3.7	17.2	64.4	2.9	69.8	1.7	201.7	50.5
p-value[b]		0.12	0.31	0.73	0.01	0.006	0.17	0.30	0.37
maternal age (years)									
<25	96 (34.7)	2.8	33.8	32.1	1.5	26.5	0.9	142.6	28.4
25–30	95 (34.3)	2.8	19.9	47.3	1.6	31.6	0.8	147.0	33.1
>30	86 (31.0)	2.8	50.1	123.8	1.9	26.0	1.8	156.9	34.7
p-value[b]		0.95	0.01	0.0003	0.35	0.99	0.04	0.90	0.76
maternal education (years)									
<12	31 (11.2)	2.6	20.1	18.6	1.4	24.1	1.2	92.9	10.9
12	18 (6.5)	2.5	31.2	106.8	1.6	40.6	1.9	125.2	29.8
>12	228 (82.3)	2.8	34.0	61.5	1.7	27.7	1.0	160.4	37.0
p-value[b]		0.80	0.61	0.006	0.80	0.55	0.45	0.13	0.004
marital status									
married/civil union	202 (72.9)	2.7	33.4	57.1	1.7	28.4	1.1	159.3	32.8
unmarried	75 (27.1)	3.0	27.9	52.5	1.5	26.9	1.0	122.7	29.3
p-value[b]		0.43	0.70	0.77	0.79	0.91	0.33	0.12	0.55
income status (US $)									
<$20,000	120 (49.8)	2.7	31.0	38.3	1.4	25.0	1.2	159.2	27.0
≥$20,000 to < $40,000	74 (30.7)	2.9	34.0	65.6	1.9	31.6	1.2	136.9	37.5
≥$40,000	47 (19.5)	2.5	31.1	142.7	1.9	25.4	1.0	152.6	33.0
p-value[b]		0.66	0.94	0.003	0.39	0.89	0.88	0.68	0.67
parity									
0	126 (45.5)	2.8	34.3	63.3	1.7	32.4	0.8	144.4	32.4
1	93 (33.6)	3.0	30.5	54.9	1.6	23.3	1.0	165.1	38.1
>1	58 (20.9)	2.5	29.1	43.4	1.6	27.4	2.4	132.8	23.0
p-value[b]		0.47	0.99	0.48	0.99	0.50	0.005	0.67	0.32
prepregnancy BMI (kg m^{-2})									
≤25	158 (57.0)	2.8	31.4	50.7	1.7	29.5	1.1	173.4	36.1
>25 to ≤30	83 (30.0)	2.3	26.2	71.3	1.4	25.5	1.2	121.6	30.5
>30	36 (13.0)	4.3	52.7	48.4	1.8	27.5	0.8	118.9	20.3
p-value[b]		0.01	0.71	0.18	0.65	0.88	0.50	0.20	0.31
employment status									
unemployed	109 (39.4)	2.7	35.8	48.0	1.3	19.6	1.6	148.3	26.4
employed	168 (60.7)	2.8	29.5	61.4	1.9	35.2	0.8	148.7	36.0
p-value[b]		0.71	0.70	0.32	0.04	0.03	0.06	0.59	0.15

[a]*n* represents number of samples, not participants. [b]p-values from linear mixed effects models accounting for within-person correlations.

which is consistent with the high concentrations (>1,000 μg/g) of TCS recently measured in samples of "conventional" hand soap and liquid dish soap.[44] TCS concentrations were also higher among women reporting use of hairspray than among women who did not. However, we could not find evidence for the presence of TCS in hairspray.[17,44,45] The divergence of the exposure distribution we observed for BP-3 concentrations between women in this study compared to NHANES, but only at the high end of the distribution, may also represent a subpopulation of pregnant women in Puerto Rico with unique and direct exposure to a particular source. Self-reported use of sunscreen and hand/body lotion was positively associated with BP-3 concentrations. The greatest increase for any product use/biomarker combinations in our study was observed for sunscreen use and BP-3. BP-3, a UV filter, has been detected in both "conventional" and "alternative" sunscreens.[44] Many hand and body lotions advertise having a sun protection factor and thus may contain BP-3. We also found higher urinary concentrations of BP-3 and BPA in relation to self-reported mouthwash use. No information relating to the use of these chemicals in mouthwash could be located, and it may be possible that these associations were due to confounding or were chance findings due to multiple comparisons. Concentrations of parabens, which are commonly used in cosmetics and other personal care products,[45] were associated with self-reported use of cosmetics and lotion. Parabens were recently detected in a study that sampled various cosmetic and lotion products.[44]

Strengths of our study include the novel aspects described earlier, in addition to its focus on an understudied and potentially at-risk population, a fairly large sample size for assessing predictors of metabolite concentrations, its important and original contribution to informing future exposure assessment and epidemiology studies, and the collection of

Table 5. Frequencies of Product Use Reported in the 48-h Recall Questionnaire and Selected[a] SG-Adjusted Geometric Mean Concentrations of Phenols and Parabens (ng/mL) Associated with Self-Reported Use (Y) or Nonuse (N)[b,c]

	n[b] = 105	n[c] = 264	BPA	TCS	BP-3	B-PB	M-PB	P-PB
cleaning products								
laundry detergent	84	145						
fabric softener	74	132						
general cleaners	89	163						
creams and lotions								
hand/body lotion	95	217			Y: 62.7; N: 30.1 p = 0.05	Y: 1.1; N: 0.6 p = 0.05	Y: 180; N: 67.6 p = 0.0001	Y: 40.2; N: 11.8 p = 0.0001
shaving cream	13	15						
sunscreen	5	10			Y: 503; N: 49.8 p = 0.001			
toiletries and cosmetics								
perfume/cologne	93	221						
colored cosmetics	95	213				Y: 1.2; N: 0.6 p = 0.01	Y: 175; N: 82.3 p = 0.004	Y: 40.5; N: 12.6 p = 0.0002
bar soap	99	242			(negative) p = 0.07		(negative) p = 0.05	
liquid soap	103	223		Y: 36.7; N: 18.5 p = 0.06				
mouthwash	80	146	Y: 3.2; N: 2.5 p = 0.03		Y: 75.5; N: 37.2 p = 0.03			
hair and nail products								
hairspray	47	81		Y: 50.0; N: 27.5 p = 0.05				
conditioner	97	170						
shampoo	97	169						
nail polish	58	86						
chemical products								
pesticides	21	25					(negative) p = 0.09	
pet grooming products	7	8						

[a]Results shown for associations with p-value ≤ 0.1. [b]p-values from linear mixed effects models accounting for within-person correlations. [c](negative) = negative association; product use associated with lower urinary biomarker concentrations, contrary to hypothesis. n[b] = total number of participants who answered "yes" at least once. n[c] = total number of total responses that were "yes".

repeated data on urinary biomarkers and self-reported product use. The repeated data allowed for an especially powerful analysis for the time varying factors, as each participant can serve as their own reference in mixed effects models. A primary limitation of our study was the inability to ask more detailed questions on the product use form. However, the inclusion of increased detail on the product use form would result in an exponential increase in participant burden and may introduce additional recall errors. Added detail, such as specific product and brand names, would also result in a diffuse data set that may lack power to test associations. Another potential limitation may be in our ability to compare our results with other studies or generalize our findings to other populations due to differences in study (e.g., questionnaire) design and differences in product formulation, availability and use by country/region.

In conclusion, we found evidence that urinary concentrations of triclosan, BP-3, and 25-DCP were higher among a group of pregnant women in Puerto Rico compared to women of reproductive age in the US, while concentrations of BPA, 24-DCP, and parabens were similar. Although greatly limited, there is some evidence that exposure to these chemicals may be associated with adverse pregnancy outcomes and other health effects. Additional human epidemiology studies of these chemicals are greatly needed. We found positive associations between biomarker concentrations and self-reported use of liquid soap (TCS), sunscreen (BP-3), lotion (BP-3 and parabens), and cosmetics (parabens). This information, coupled with data from studies measuring these chemicals in specific products, may help inform pregnant women and others on how to reduce their exposure. Finally, the degree of temporal reliability observed for the urinary measures varied by analyte. Epidemiology studies utilizing these urinary biomarkers to estimate exposure during pregnancy should include as many repeated measurements at multiple times during gestation as feasible to reduce measurement error and to explore potential windows of susceptibility to adverse health outcomes. However, the collection and analysis of multiple urine samples must be reconciled with budget and logistic constraints of large-scale epidemiology studies given the high costs associated with the additional contact with participants and sensitive analytical chemistry methods required.

■ ASSOCIATED CONTENT

Supporting Information

Additional text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 734-764-7184. Fax: 734-936-7283. E-mail: meekerj@umich.edu. Corresponding author address: Department of Environmental Health Sciences, University of Michigan School of Public Health, Room M6017 SPH Tower, 1415 Washington Hts., Ann Arbor, MI 48109.

Notes

The authors declare no competing financial interest.

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Percutaneous absorption of benzophenone-3, a common component of topical sunscreens.

Gustavsson Gonzalez H1, Farbrot A, Larkö O.
Author information
1 Departments of Dermatology, and Clinical Chemistry, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden. helena.gonzales@vgregion.se

Abstract
Benzophenone-3 (BZ-3) is a commonly used, chemical UV-absorber. It has been used for many years to protect against UV-radiation. Previous studies have shown that BZ-3 penetrates the skin, and it can be found in urine, faeces, and blood. In this study we examined the percutaneous absorption of BZ-3. The amount of BZ-3 absorbed was measured in urine, as experimental studies in the rat have shown that urine is the major route of excretion. Eleven volunteers applied the recommended amount of a commercially available sunscreen and urine samples were collected during a 48-h period after application. The average total amount excreted was 11 mg, median 9.8 mg, which is approximately 0.4% of the applied amount of BZ-3. Some of the volunteers still excreted BZ-3 48 h after application. It is evident that BZ-3 undergoes conjugation in the body to make it water soluble. However, we do not know at what age the ability to conjugate is fully developed, and therefore for children physical filters such as titanium dioxide and/or zinc oxide might still be considered a more appropriate sunscreen component.

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Characteristic profiles of benzonphenone-3 and its derivatives in urine of children and adults from the United States and China.

Wang L1, Kannan K.
Author information
1 Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, United States.

Abstract
Widespread exposure of humans to benzophenone-3 (BP-3) is a concern due to this compound's potential to disrupt endocrine function. BP-3 can be metabolized by phase I and phase II reactions of the human cytochrome P450 system. Urinary measurements of BP-3 have been used as a biomarker of exposure. Nevertheless, metabolic transformation pathway and the transformation products of BP-3 in humans are still less known. In this study, 166 urine samples collected from children and adults in the U.S. and China were analyzed for free and total forms (free plus conjugated) of BP-3 as well as four of its metabolic derivatives, 4-OH-BP, 2,4-diOH-BP, 2,2',4,4'-tetraOH-BP, and 2,2'-diOH-4-MeO-BP, using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). BP-3 was found in almost all urine samples from the U.S. and China. Concentrations of BP-3 in urine from children (GM: 9.97 ng/mL) and adults (15.7 ng/mL) in the U.S. were significantly higher than those in children (0.622 ng/mL) and adults (0.977) from China. A significant positive relationship was found between the concentrations of urinary BP-3 and its derivatives. The profiles of BP derivatives in urine suggested that demethylation was a major route of BP-3 metabolism. The percentage of the free form of BP-3 in urine was used in the determination of efficacy of phase II metabolism among the different population groups studied. A significantly lower percentage of the free form of BP-3 was found in urine from the U.S. population than in the Chinese population.

Concentrations of the Sunscreen Agent Benzophenone-3 in Residents of the United States: National Health and Nutrition Examination Survey 2003–2004

Antonia M. Calafat, Lee-Yang Wong, Xiaoyun Ye, John A. Reidy, and Larry L. Needham

Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Background: The capability of benzophenone-3 (BP-3) to absorb and dissipate ultraviolet radiation facilitates its use as a sunscreen agent. BP-3 has other uses in many consumer products (e.g., as fragrance and flavor enhancer, photoinitiator, ultraviolet curing agent, polymerization inhibitor).

Objectives: Our goal was to assess exposure to BP-3 in a representative sample of the U.S. general population ≥ 6 years of age.

Methods: Using automated solid-phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometry, we analyzed 2,517 urine samples collected as part of the 2003–2004 National Health and Nutrition Examination Survey.

Results: We detected BP-3 in 96.8% of the samples. The geometric mean and 95th percentile concentrations were 22.9 μg/L (22.2 μg/g creatinine) and 1,040 μg/L (1,070 μg/g creatinine), respectively. Least-square geometric mean (LSGM) concentrations were significantly higher ($p \le 0.04$) for females than for males, regardless of age. LSGM concentrations were significantly higher for non-Hispanic whites than for non-Hispanic blacks ($p \le 0.01$), regardless of age. Females were more likely than males [adjusted odds ratio (OR) = 3.5; 95% confidence interval (95% CI), 1.9–6.5], and non-Hispanic whites were more likely than non-Hispanic blacks (adjusted OR = 6.8; 95% CI, 2.9–16.2) to have concentrations above the 95th percentile.

Conclusions: Exposure to BP-3 was prevalent in the general U.S. population during 2003–2004. Differences by sex and race/ethnicity probably reflect differences in use of personal care products containing BP-3.

Key words: benzophenone-3, biomonitoring, exposure, human, NHANES 2003–2004, sunscreen, urine. *Environ Health Perspect* 116:893–897 (2008). doi:10.1289/ehp.11269 available via *http://dx.doi.org/* [Online 21 March 2008]

Benzophenone-3 [2-hydroxy-4-methoxybenzophenone, oxybenzophenone (BP-3)], a commonly used sunscreen agent that absorbs and dissipates ultraviolet radiation, is used in a variety of cosmetic products (Gonzalez et al. 2006; National Library of Medicine 2007; Rastogi 2002). BP-3 also has been used as ultraviolet stabilizer in plastic surface coatings for food packaging to prevent polymer or food photodegradation (Suzuki et al. 2005) and is approved by the U.S. Food and Drug Administration as an indirect food additive.

Human exposure to BP-3 has not been associated with adverse health effects, and acute toxicity from BP-3 is low. However, results from animal studies—primarily dietary studies that affected body weight gain—showed alterations in liver, kidney, and reproductive organs in rats and mice administered BP-3 dermally and orally (National Toxicology Program 1992). Although the maximum dose that could be administered dermally was similar to the lowest orally administered dose, which produced little systemic toxicity, these results suggested that oral and dermal exposure routes might affect the animals similarly (National Toxicology Program 1992). BP-3 also shows estrogen-like activity *in vitro* and *in vivo* (Schlumpf et al. 2001, 2003, 2004a, 2004b; Suzuki et al. 2005), although in one study BP-3's estrogenic activity was observed only in the presence of a rat liver preparation, suggesting metabolic activation of BP-3 (Morohoshi et al. 2005). BP-3 can also display antiandrogenic activity *in vitro* (Ma et al. 2003; Schreurs et al. 2005). Thus, BP-3 might exhibit endocrine-disrupting action via both mechanisms in animals. Therefore, *in vivo* effects due to these combined activities should be further investigated.

The focus of pharmaceuticals and ingredients in personal care products, including organic sunscreen agents, as environmental pollutants is increasing because these compounds may enter the aquatic environment not primarily as a result of manufacturing practices but from their steady and widespread use in human and veterinary daily activities. Furthermore, little is known about the potential hazards associated with recurring human or ecologic exposures to these synthetic substances, many of which are bioactive (Daughton 2002; Daughton and Ternes 1999). BP-3, one of these substances, has been detected in surface waters (Balmer et al. 2005; Cuderman and Heath 2007), drinking water (Loraine and Pettigrove 2006; Stackelberg et al. 2004), and wastewater [Balmer et al. 2005; Centers for Disease Control and Prevention (CDC) 2003; Loraine and Pettigrove 2006] in North America and in Europe.

The widespread inclusion of sunscreen agents in personal care and consumer products (Gonzalez et al. 2006; National Library of Medicine 2007; Rastogi 2002) increases the potential for human exposure to BP-3. Data support the absorption of BP-3 through human skin (Gonzalez et al. 2006; Hayden et al. 2005; Janjua et al. 2004; Jiang et al. 1999; Sarveiya et al. 2004). Application of some of these products to large areas of the body and frequent reapplication increase the daily systemic absorption of BP-3. In some cases, as much as 10% of the applied dose can be absorbed (Jiang et al. 1999).

Like many xenobiotics, BP-3 undergoes phase I and phase II biotransformations. In rats, after oral and dermal administrations of 100 mg BP-3/kg body weight (Kadry et al. 1995; Okereke et al. 1993, 1994, 1995), the parent compound and three oxidative metabolites (2,4-dihydroxylbenzophenone, 2,2′-dihydroxy-4-methoxybenzophenone, and 2,3,4-trihydroxybenzophenone) were detected in plasma, tissues, and urine. Urine was the major route of excretion; BP-3 and its metabolites were excreted mainly as glucuronide conjugates (Kadry et al. 1995; Okereke et al. 1993). Similarly, BP-3 and 2,4-dihydroxylbenzophenone were detected in human urine collected after a volunteer applied a commercially available sunscreen (Felix et al. 1998). These data suggest that the conjugated species of BP-3 and its metabolites in urine can be used as biomarkers of exposure. Oxidative metabolites of BP-3 can themselves be used as sunscreen agents. Although BP-3 can be biotransformed to several metabolites, exposure to BP-3 can be assessed by measuring the total (free plus conjugated) concentrations of BP-3 in urine.

Address correspondence to A.M. Calafat, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy., NE, Mailstop F53, Atlanta, GA 30341 USA. Telephone: (770) 488-7891. Fax: (770) 488-4371. E-mail: Acalafat@cdc.gov

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The detection of BP-3 in the aquatic environment and the widespread use of products containing BP-3 have raised interest about assessing human exposure to this compound for risk assessment. We report here the first nationally representative data on the urinary concentrations of BP-3 in the U.S. general population ≥ 6 years of age, stratified by age group, sex, and race/ethnicity.

Materials and Methods

The National Health and Nutrition Examination Survey (NHANES), conducted continuously since 1999 by the CDC, assesses the health and nutritional status of the civilian noninstitutionalized U.S. population ≥ 2 months of age (CDC 2003). The survey includes household interviews, medical histories, standardized physical examinations, and collection of biologic specimens, some of which can be used to assess exposure to environmental chemicals. NHANES 2003–2004 included examinations of 9,282 people (CDC 2006a). We measured BP-3 by analyzing a random one-third subset of urine samples (*n* = 2,517) collected from NHANES participants ≥ 6 years of age. Because this subset was randomly selected from the entire set, it maintained the representativeness of the survey. Participants provided informed written consent; parents provided informed written consent for their children.

Urine specimens were shipped on dry ice to the CDC's National Center for Environmental Health and stored frozen at or below –20°C until analyzed. We measured total (free plus conjugated species) concentrations of BP-3 in urine by online solid-phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometry described in detail elsewhere (Ye et al. 2005a). Briefly, conjugated BP-3 in 100 µL of urine was hydrolyzed using β-glucuronidase/sulfatase (*Helix pomatia*; Sigma Chemical Co., St. Louis, MO). After hydrolysis, samples were acidified with 0.1 M formic acid; BP-3 was preconcentrated by online solid-phase extraction, separated by reversed-phase high-performance liquid chromatography, and detected by atmospheric pressure chemical ionization–tandem mass spectrometry. Because a stable isotope-labeled BP-3 was not available, we used $^{13}C_{12}$-bisphenol A as internal standard (Ye et al. 2005a). The limit of detection (LOD), calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero (Taylor 1987), was 0.34 µg/L, and the precision ranged from 17.6% (at 18.5 µg/L) to 16.2% (at 46 µg/L). Low-concentration (~ 20 µg/L) and high-concentration (~ 45 µg/L) quality control materials, prepared from pooled human urine, were analyzed with standard, reagent blank, and NHANES samples (Ye et al. 2005a).

We analyzed the data using Statistical Analysis System (version 9.1.3; SAS Institute, Inc., Cary, NC) and SUDAAN (version 9.0.1; Research Triangle Institute, Research Triangle Park, NC). SUDAAN calculates variance estimates after incorporating the sample population weights, nonresponse rates, and sample design effects. We calculated the percentage of detection and the geometric mean and distribution percentiles for both the volume-based (in micrograms per liter urine) and creatinine-corrected (in micrograms per gram creatinine) concentrations. For concentrations below the LOD, as recommended for the analysis of NHANES data (CDC 2006b), we used a value equal to the LOD divided by the square root of 2 (Hornung and Reed 1990).

A composite racial/ethnic variable based on self-reported data defined three major racial/ethnic groups: non-Hispanic black, non-Hispanic white, and Mexican American. We included participants not defined by these racial/ethnic groups only in the total population estimate. Age, reported in years at the last birthday, was stratified in groups (6–11, 12–19, 20–59, and ≥ 60 years of age) for calculation of the geometric mean and the various percentiles.

We used analysis of covariance to examine the influence of several variables, selected on the basis of statistical, demographic, and biologic considerations, on the concentrations of BP-3. For the multiple regression models, we used the variables described below and all possible two-way interactions to calculate the adjusted least square geometric mean (LSGM) concentrations. LSGM concentrations provide geometric mean estimates (in micrograms per liter) after adjustment for all covariates in the model. Because the distributions of BP-3 and creatinine concentrations were skewed, these variables were log transformed. We analyzed two separate models: one for adults (≥ 20 years of age) and one for children and teenagers (≤ 19 years of age). We considered age (continuous), age squared, sex, race/ethnicity, and log-transformed creatinine concentration for both models. When the model included both age and age squared, we centered age by subtracting 50 from each participant's age, thus avoiding multicollinearity and obtaining the least weighted correlation between these two variables

Table 1. Geometric mean and selected percentiles of BP-3 concentrations in urine for the U.S. population ≥ 6 years of age: data from NHANES 2003–2004.[a]

Variable[b]	Geometric mean	Selected percentile 10th	25th	50th	75th	90th	95th	Sample (*n*)
Total	22.9 (18.1–28.9)	2.20 (1.50–2.60)	5.80 (4.70–7.10)	18.0 (15.3–23.1)	94.0 (67.5–123)	364 (225–570)	1,040 (698–1,390)	2,517
	22.2 (17.6–28.0)	2.28 (1.73–2.89)	5.24 (4.27–6.21)	16.2 (12.7–21.6)	82.0 (58.7–108)	409 (283–577)	1,070 (686–1,600)	2,514
Age group (years)								
6–11	21.2 (16.4–27.3)	3.60 (2.40–4.50)	6.70 (5.20–9.50)	17.2 (14.9–25.9)	63.6 (38.7–102)	154 (106–246)	227 (154–618)	314
	25.8 (19.5–34.1)	4.30 (2.86–5.19)	8.25 (5.98–10.5)	22.4 (14.4–33.7)	83.6 (41.0–131)	171 (132–365)	427 (171–710)	314
12–19	22.9 (18.0–29.3)	3.30 (2.30–4.10)	7.80 (5.60–9.60)	20.0 (16.1–25.1)	66.5 (45.2–93.8)	170 (137–240)	407 (183–717)	715
	17.2 (13.7–21.5)	3.17 (2.24–4.03)	5.86 (4.81–6.93)	12.9 (10.3–16.5)	42.9 (29.5–57.7)	136 (91.7–239)	350 (173–646)	713
≥ 20	23.1 (18.0–29.6)	1.80 (1.20–2.40)	5.50 (4.50–6.70)	18.1 (14.7–23.3)	108 (72.1–140)	450 (315–733)	1,200 (769–1,750)	1,488
	22.8 (17.8–29.1)	1.98 (1.48–2.59)	4.89 (3.71–6.12)	16.2 (12.7–21.9)	93.2 (66.0–130)	486 (361–700)	1,330 (880–1,880)	1,487
Sex								
Female	30.7 (23.7–39.8)	2.50 (1.80–3.40)	7.30 (5.40–9.10)	26.0 (20.2–34.1)	137 (105–172)	596 (403–769)	1,340 (776–1,790)	1,288
	35.5 (27.1–46.4)	3.16 (2.28–4.13)	7.42 (5.83–9.39)	28.2 (20.2–37.0)	144 (101–224)	686 (491–1,130)	1,850 (1,220–2,580)	1,286
Male	16.8 (13.2–21.3)	1.80 (1.30–2.20)	5.00 (4.30–5.90)	13.6 (11.4–16.8)	54.4 (33.2–86.5)	178 (134–324)	567 (238–1,350)	1,229
	13.6 (10.8–17.1)	1.82 (1.55–2.16)	3.81 (3.33–4.87)	10.2 (8.36–12.9)	40.0 (24.9–62.5)	169 (93.3–316)	378 (229–685)	1,228
Race/ethnicity								
Non-Hispanic white	27.7 (20.3–37.8)	2.30 (1.50–3.00)	6.80 (5.10–8.60)	23.5 (16.8–32.0)	120 (83.6–162)	501 (316–769)	1,250 (733–2,070)	1,092
	28.3 (20.6–38.8)	2.55 (1.80–3.62)	6.07 (4.88–8.33)	21.9 (14.6–32.7)	116 (73.5–175)	510 (380–760)	1,330 (852–2,410)	1,091
Mexican American	16.5 (10.9–25.1)	2.30 (1.70–3.70)	5.00 (3.70–6.60)	11.9 (8.30–18.3)	45.5 (25.9–78.2)	176 (68.7–346)	412 (178–2,180)	613
	15.1 (9.44–24.0)	2.39 (1.68–3.26)	4.10 (2.95–6.71)	11.0 (6.95–16.0)	40.7 (18.3–85.8)	158 (87.4–362)	595 (118–1,860)	612
Non-Hispanic black	12.8 (9.38–17.4)	2.10 (1.30–2.70)	4.60 (3.20–6.20)	10.2 (7.40–14.3)	34.2 (22.8–50.6)	127 (90.8–176)	209 (143–499)	652
	8.78 (6.49–11.9)	1.50 (1.05–2.35)	3.18 (2.42–4.14)	6.80 (5.27–9.00)	19.6 (13.5–33.4)	78.1 (46.8–139)	185 (79.8–536)	651

[a]Concentrations are given as micrograms per liter (unshaded) and micrograms per gram creatinine (shaded), with 95% CIs in parentheses. [b]Participants not defined by the three racial/ethnic groups shown were included only in the total population estimate.

(Bradley and Srivastava 1979). Additionally, to further evaluate the relation between the log-transformed BP-3 concentration and age, we used age group (20–29, 30–39, 40–49, and ≥ 50 years of age) as a categorical variable in the model and generated a bar chart of LSGM concentrations by age group.

To reach the final reduced model, we used backward elimination with a threshold of $p < 0.05$ for retaining the variable in the model, using Satterwaite-adjusted F statistics. We evaluated for potential confounding by adding each of the excluded variables back into the final model one by one and examining changes in the β coefficients of the statistically significant main effects or interactions. If addition of one of these excluded variables caused a change in a β coefficient by ≥ 10%, we re-added the variable to the model.

We also conducted weighted univariate and multiple logistic regressions to examine the association of BP-3 concentrations above the 95th percentile with sex, age group, and race/ethnicity for all ages.

Results

We detected BP-3 in 96.8% of the 2,517 samples at concentrations ranging from 0.4 to 21,700 µg/L; the geometric mean and 95th percentile concentrations were 22.9 µg/L (22.2 µg/g creatinine) and 1,040 µg/L (1,070 µg/g creatinine), respectively (Table 1).

The final model for adults included sex, race/ethnicity, age, age squared ($p = 0.038$), creatinine concentration (log scale), and the interaction terms creatinine*sex ($p < 0.001$) and age*race/ethnicity ($p = 0.04$) (Table 2). Females had significantly higher BP-3 concentrations ($p \leq 0.04$) than did males, regardless of creatinine level [see Supplemental Tables S1 and S2 (http://www.ehponline.org/members/2008/11269/suppl.pdf). Although BP-3 concentrations increased linearly as log creatinine increased for both sexes ($p < 0.001$), the increase was more pronounced for males than for females (β for males, 1.12; for females, 0.65). Also, as age increased, BP-3 LSGM concentrations showed a significant quadratic trend for Mexican Americans ($p = 0.016$) and a significant linear positive trend for non-Hispanic blacks ($p = 0.022$) but no significant linear or quadratic trend for non-Hispanic whites (Figure 1). LSGM concentrations of BP-3 for non-Hispanic whites were significantly higher than for non-Hispanic blacks, regardless of age ($p \leq 0.01$), and significantly higher than for Mexican Americans only for 20- to 29-year-olds ($p = 0.01$). LSGM concentrations of BP-3 were significantly higher for Mexican Americans than for non-Hispanic blacks only for 30- to 39-year-olds ($p = 0.01$) [see Supplemental Tables S1 and S2 (http://www.ehponline.org/members/2008/11269/suppl.pdf)].

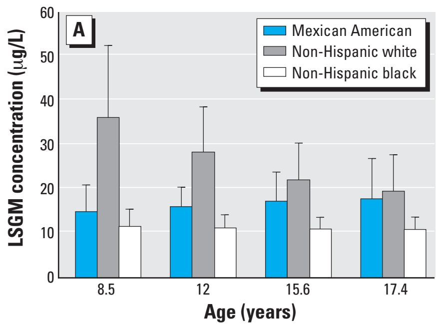
The final model for children and adolescents included sex ($p < 0.001$), race/ethnicity, age, creatinine concentration (log scale) ($p < 0.001$), and a race/ethnicity*age ($p = 0.01$) interaction term (Table 2). LSGM concentrations of BP-3 increased as log creatinine increased (β = 0.77, $p < 0.001$). LSGM BP-3 concentrations for girls [30.2 µg/L; 95% confidence interval (CI), 21.4–42.6 µg/L] were significantly higher ($p < 0.001$) than for boys (16.1 µg/L; 95% CI, 13.2–19.8 µg/L). BP-3 concentrations also decreased linearly as age increased ($p = 0.0005$) for non-Hispanic whites but not for Mexican Americans and non-Hispanic blacks [Figure 1; see also Supplemental Table S3 (http://www.ehponline.org/members/2008/11269/suppl.pdf)]. LSGM concentrations of BP-3 for non-Hispanic whites were significantly higher than LSGM concentrations for non-Hispanic blacks, regardless of age, and for Mexican Americans only at younger ages [$p < 0.001$ at 8.5 years, $p < 0.01$ at 12 years; Supplemental Table S4 (http://www.ehponline.org/members/2008/11269/suppl.pdf)]. LSGM BP-3 concentrations were significantly higher for Mexican Americans than for non-Hispanic blacks only for older children ($p = 0.01$, at 12 and at 15.6 years, $p = 0.03$ at 17.4 years) [Supplemental Table S4 (http://www.ehponline.org/members/2008/11269/suppl.pdf)].

For participants with urinary concentrations above the 95th percentile of BP-3, sex ($p < 0.001$) and race/ethnicity ($p = 0.03$), but not age, were significantly associated univariately. In the final multiple logistics regression, sex ($p < 0.001$) and race/ethnicity ($p = 0.03$) were significant [Supplemental Table S5 (http://www.ehponline.org/members/2008/11269/suppl.pdf)]. Females were 3.5 times more likely than males to be above the 95th percentile [adjusted odds ratio (OR) = 3.5; 95% CI, 1.9–6.5]. Non-Hispanic whites were 6.8 times more likely to have BP-3 concentrations above the 95th percentile (adjusted OR = 6.8; 95% CI, 2.9–16.2) than were non-Hispanic blacks, and Mexican Americans were four times more likely to be above the 95th percentile (adjusted OR = 4.04; 95% CI, 1.1–15.5) than were non-Hispanic blacks. We found no significant difference between non-Hispanic whites and Mexican Americans.

Table 2. Coefficients for the significant variables from the multiple regression models of the BP-3 urinary concentration (log-transformed) by age group [β coefficient (p-value)].

Variable	Children and adolescents (6–19 years of age)	Adults (≥ 20 years of age)
Intercept	–0.33985 (0.14913)	–0.08999 (0.73675)
Sex		
Male	–0.27143 (0.00079)	–1.39213 (0.00079)
Female	Reference	
Race/ethnicity		
Mexican American	0.01857 (0.91283)	0.24104 (0.15686)
Non-Hispanic white	0.73888 (0.00035)	0.48352 (0.00001)
Non-Hispanic black	Reference	
Age	–0.00243 (0.73485)	0.00155 (0.50696)[a]
Creatinine concentration (log transformed)	0.76653 (<0.001)	0.64519 (0.00008)
Age squared (centered)		–0.00018 (0.03848)
Race/ethnicity*age		
Mexican American	0.01139 (0.4116)	–0.00365 (0.2125)[a]
Non-Hispanic white	–0.02787 (0.0249)	–0.00784 (0.02026)[a]
Non-Hispanic black	Reference	
Sex*log creatinine		
Male		0.47423 (0.00811)
Female		Reference

[a]Age centered at 50 years.



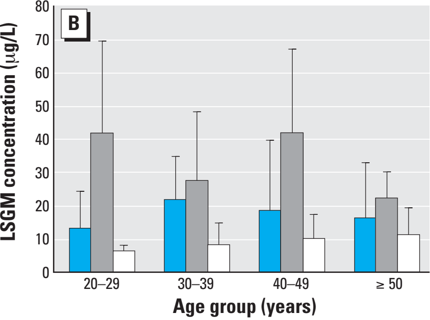


Figure 1. LSGM concentrations of BP-3 (in micrograms per liter) by age and race/ethnicity: (*A*) children and adolescents and (*B*) adults. Error bars indicate 95% CIs.

Discussion

The detection of BP-3 in almost all samples suggests that exposure to BP-3 was widespread in the U.S. general population during 2003–2004. This high level of detection most likely resulted from routine use of consumer products that contain BP-3, such as sunscreen, skin care lotion, lipstick, and hair spray (National Library of Medicine 2007). The wide range of urinary concentrations—10% of participants had BP-3 concentrations < 2.3 µg/g creatinine and 5% had concentrations > 1,070 µg/g creatinine (Table 1)—may be related to lifestyle differences that result in exposure differences and to individual variations in bioavailability, distribution kinetics, or metabolism of BP-3.

The frequent detection of BP-3 and the magnitude and range of urinary concentrations in NHANES 2003–2004 are comparable with data from two smaller studies in the United States. In 30 anonymous adult volunteers with no documented BP-3 exposure, we detected BP-3 in 90% of samples, and total urinary concentration (free plus conjugates) of BP-3 ranged from the LOD (0.5 µg/L) to 3,000 µg/L (Ye et al. 2005b). In a pilot study of 90 prepubertal girls from New York City, New York; Cincinnati, Ohio; and Northern California, we detected BP-3 in 86% of samples (Wolff et al. 2007). The creatinine-adjusted geometric mean concentration of BP-3 (30.8 µg/g) for these girls was similar to that for NHANES 2003–2004 children 6–11 years of age (25.8 µg/g creatinine).

The relation between age and LSGM BP-3 concentrations differed by race/ethnicity (Figure 1). These differences most likely result from increased use of sunscreen or other personal-care products containing BP-3 by people with light skin pigmentation. For instance, sunscreen use among non-Hispanic whites is reportedly higher than for non-Hispanic blacks and other race/ethnic groups of outdoor workers and the general population (Briley et al. 2007; Pichon et al. 2005). Likewise, differences by age might reflect differences in use of personal-care products that contain BP-3. Non-Hispanic white parents may apply sunscreen regularly to protect their young children from sunburn, whereas teenagers might not apply sunscreen as often (Jones and Saraiya 2006; Livingston et al. 2007). Non-Hispanic white adults in their twenties and forties might be more preoccupied about their skin appearance than non-Hispanic whites in their thirties (who may devote more time to work and family responsibilities than to themselves) or people in their fifties (who may see little benefit in applying sunscreen at older ages).

We found differences by sex in the adjusted LSGM concentrations of BP-3. Compared with males, females tend to use more sunscreen (Eide and Weinstock 2006; Hall et al. 1997; Jones and Saraiya 2006) and other personal-care products that may contain BP-3. Therefore, higher concentrations of BP-3 for females than for males most likely result from their higher exposure to BP-3.

Females and non-Hispanic whites not only had significantly higher LSGM concentrations than did males and non-Hispanic blacks, respectively, but also were more likely to exhibit concentrations of BP-3 above the 95th percentile. In particular, females were 3.5 times more likely than males, and non-Hispanic whites were 6.8 times more likely than non-Hispanic blacks to have BP-3 concentrations above the 95th percentile. Mexican Americans were about four times more likely than non-Hispanic blacks to present BP-3 concentrations above the 95th percentile. Although young children had LSGM concentrations of BP-3 comparable with those of adults in their twenties and forties, age was not significantly associated with having concentrations above the 95th percentile. Our data suggest that females and non-Hispanic whites represent two segments of the general population with higher exposures to BP-3 compared with other demographic groups.

Protection against sunburn and squamous cell carcinoma by application of sunscreens is important, even though the use of sunscreen may not protect against melanoma, the deadliest form of skin cancer (Lin and Fisher 2007). Sun protection is critical for outdoor workers, who are at higher risk for squamous cell carcinoma than other population groups (Pichon et al. 2005), and in situations where sun exposure, even during peak times, is unavoidable. In other situations, although behavioral measures, such as wearing a hat, sunglasses, and sun protective clothes and avoiding the sun during peak exposure times, can reduce the risk for skin damage, sunscreens may be the primary means of sun protection, especially in societies that value outdoor activities (Lautenschlager et al. 2007). Toxicologic and epidemiologic data on BP-3, one of these sunscreens, are lacking. Nevertheless, the NHANES 2003–2004 data demonstrating Americans' exposure to BP-3 can be used to establish a nationally representative baseline assessment of exposure to this sunscreen agent and may promote the use of biomonitoring to complement the questionnaire or survey information in studies designed to evaluate sun-safety practices. These NHANES 2003–2004 data could also be of benefit in a risk assessment for BP-3 if indicated by toxicologic or epidemiologic studies.

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Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood

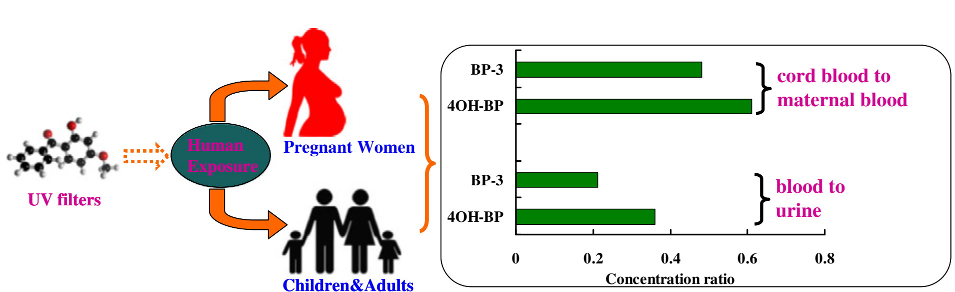
Tao Zhang [a], Hongwen Sun [a], Xiaolei Qin [a], Qian Wu [b,c], Yanfeng Zhang [a], Jing Ma [d], Kurunthachalam Kannan [b,c,e,*]

[a] College of Environmental Sciences and Engineering, Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin 300071, China
[b] Wadsworth Center, New York State Department of Health, Albany, NY 12201, USA
[c] Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, NY 12201, USA
[d] Applied Radiation Institute, School of Environmental and Chemical Engineering, Shanghai University, 99 Shangda Road, P.O. Box 144, Shanghai 200444, China
[e] International Joint Research Center for Persistent Toxic Substances, State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

HIGHLIGHTS

- Benzophenone (BP) concentrations are determined in paired blood and urine for the first time.
- BP-3 and 4OH-BP partition preferentially into urine.
- Cord to maternal blood ratios of BP-3 and 4OH-BP were 0.48 and 0.61, respectively.
- Exposure to BPs was significantly associated with sex.

GRAPHICAL ABSTRACT



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ABSTRACT

Limited information exists on the exposure of benzophenone (BP)-type UV filters (i.e., sunscreen compounds) in children, adults, and pregnant women in China. In this study, we determined the concentrations of five BP derivatives, BP-1, BP-2, BP-3, BP-8, and 4OH-BP in urine ($n = 101$) as well as paired specimens of blood and urine ($n = 24$ pairs) collected from adults; in matched maternal and fetal cord blood ($n = 20$ pairs) collected from pregnant women; and in blood collected from children ($n = 10$). 4OH-BP, BP-1, and BP-3 were found in 61%, 57%, and 25%, respectively, of the urine samples analyzed. 4OH-BP was found in all blood samples; BP-3 was found more frequently in the blood of adults (83%), followed, in decreasing order, by pregnant women (35%) and children (30%). Among all adults, urinary BP-3 concentrations were significantly ($p < 0.001$) positively correlated with urinary BP-1 concentrations. Nevertheless, no significant correlations were found between urinary concentrations of BP-3 (or BP-1) and 4OH-BP. Our results suggest that human exposure to BP-3 and BP-1 is related, whereas 4OH-BP originates from a discrete source. Females had higher urinary concentrations of BP-3, BP-1 and 4OH-BP than males. The distribution profiles of BP-1 and its parent compound (i.e., BP-3) in urine decreased with increasing age of donors ($p < 0.05$). The ratio of concentrations of BP-3 between blood and urine was 0.21 in adults, which was significantly lower than that for 4OH-BP (0.36). The concentration ratio of BPs between cord blood and maternal blood was higher for 4OH-BP (0.61) than that for BP-3 (0.48), which suggested

* Corresponding author at: Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509, USA. Tel.: +1 518 474 0015; fax: +1 518 473 2895.
E-mail address: kkannan@wadsworth.org (K. Kannan).

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greater trans-placental transfer potential of 4OH-BP. This is the first study to document the occurrence of BPs in paired urine and blood, and in matched maternal and fetal cord blood.
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1. Introduction

The toxic potential of UV (ultra violet light) filters, used in a variety of sunscreen and personal care products to attenuate the negative effects of harmful UV radiation on skin and hair, has been a concern. In the U.S., 2-hydroxy-4-methoxybenzophenone (2OH-4MeO-BP or BP-3) is a commonly used sunscreen agent (Environmental Working Group, EWG, 2012). In China, the use of BP-3 in cosmetics has been rapidly increasing over the past decade (Gao et al., 2011).

Because of the extensive use of BP-3 in personal care products, human exposure to this compound is widespread. BP-3 was found in >95% of urine samples collected from the U.S. general population (Calafat et al., 2008; Ye et al., 2005a, 2005b; Wolff et al., 2007), at concentrations ranging from 0.4 to 21700 ng/mL. The application of some personal care products that contain UV filters on the skin and frequent reapplication can increase the systemic absorption (Janjua et al., 2008; Jiang et al., 1999; León et al., 2010). In some cases, as much as 10% of the applied dermal dose was absorbed into the systemic circulation (Jiang et al., 1999). BP-3 was shown to be weakly estrogenic in in vitro and in vivo (Fent et al., 2008; Schlumpf et al., 2001) and anti-androgenic in in vitro studies (Schreurs et al., 2005). Thus, BP-3 is a potential endocrine-disrupting compound. A recent study also reported a significant association between exposure to BP-3 and an estrogen-mediated disease, endometriosis in women (Kunisue et al., 2012).

In addition to BP-3, the family of benzophenone (BP)-type UV filters comprises several structurally related compounds that are used in cosmetic products as sunscreen agents. These include analogs of 2,4-dihydroxybenzophenone (2,4OH-BP or BP-1) to 2-hydroxy-4-*n*-octoxybenzophenone (BP-12) and other, less-known compounds, such as 4-hydroxybenzophenone (4OH-BP). It has been reported that, in humans (Felix et al., 1998) and animals (Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1994), BP-3 is metabolized to BP-1 and 2,2′-dihydroxy-4-methoxybenzophenone (2,2′OH-4MeO-BP or BP-8). It is noteworthy that the metabolite, BP-1, possesses greater estrogenic activity than does BP-3 (Kawamura et al., 2003, 2005; Nakagawa and Suzuki, 2002; Suzuki et al., 2005; Takatori et al., 2003). In addition, other BPs, such as 2,2′,4,4′-tetrahydroxybenzophenone (2,2′,4,4′OH-BP or BP-2) and 4OH-BP, possess estrogenic activities higher than those of BP-3 (Kawamura et al., 2003, 2005). Significant estrogenic effects of BP-2 in fish were previously reported (Weisbrod et al., 2007). Nevertheless, information on the occurrence of BPs, except for BP-3, in humans is scarce. To our knowledge, exposure of the Chinese population to BPs has never been documented previously.

Although some studies have shown that BP-3 is found widely in various human bodily fluids, such as urine (Calafat et al., 2008; Kunisue et al., 2010, 2012; Wolff et al., 2007; Ye et al., 2005a, 2005b), blood (Ye et al., 2008b), breast milk (Ye et al., 2008a), and semen (León et al., 2010), occurrence and partitioning of BP-3 and other BPs in paired samples of blood and urine have not been examined. Measurement of BPs in paired samples of blood and urine would enable the understanding of the partitioning of these compounds between these two matrices. Further, little is known on prenatal exposure to BPs in the developing fetus. Because the developing fetus is more sensitive to the effects of environmental chemicals than are adults, an understanding of BP exposures during pregnancy and the mechanisms underlying trans-placental transfer of BPs is essential.

In this study, we determined total urinary and blood concentrations (free plus conjugated) of five BPs (i.e., BP-1, BP-2, BP-3, BP-8 and 4OH-BP) (chemical structures are shown in Fig. 1) in children, adults, and pregnant women from China. Based on the measured blood and urinary BP concentrations, gender and age-related accumulation, partition between blood and urine and trans-placental transfer were examined.

2. Materials and methods

Details of the chemicals and reagents, sample extraction, and instrumental analysis are provided in the Supplementary material. Briefly, urine and whole blood samples were extracted by liquid–liquid extraction (Kunisue et al., 2010, 2012) and ion-pair extraction (Zhang et al., 2013), respectively, after enzymatic deconjugation, as described previously. Total BP concentrations (free plus conjugated) were determined by high-performance liquid chromatography–isotope dilution tandem mass spectrometry (HPLC–ID/MS/MS). Creatinine levels in urine were analyzed by HPLC–MS/MS in an electrospray positive ionization mode, and the MRM transitions monitored were 114>44 for creatinine, and 117>47 for d_3-creatinine (Park et al., 2008). Isotopically-labeled internal standards of ^{13}C-BP-3 and $^{13}C_{12}$-bisphenol A ($^{13}C_{12}$-BPA) as well as d_3-creatinine were spiked into samples prior to extraction.

2.1. Study participants and sample collection

From February 2010 to May 2010, a total of 101 urine samples were collected from the general adult population in Tianjin ($n = 50$), Shanghai ($n = 26$) and Qiqihar ($n = 25$), China; paired blood and urine samples were collected from 24 of 50 participants in Tianjin. Further, 20 matched maternal blood and fetal cord blood samples were collected from women who were delivering in Tianjin; whole blood samples were collected from 10 children (1–5 yrs) in Nanchang, China. The sampling locations

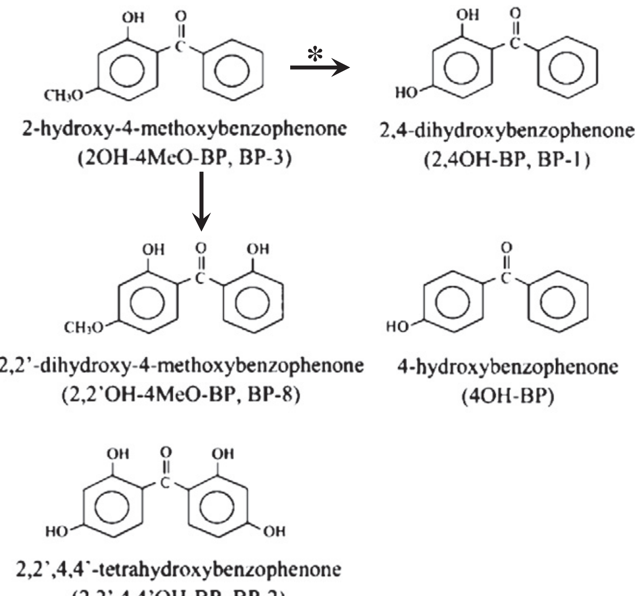

Fig. 1. Chemical structures of the benzophenone-type UV filters analyzed in this study. Arrows indicate that the metabolic pathway of BP-3 was suggested from some experimental studies (Felix et al., 1998; Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1994), * represents that BP-1 is mainly formed from BP-3.

are shown in Fig. S1. The maternal blood samples were collected in the preoperative holding area within 1 h prior to delivery, and fetal cord blood samples were collected at delivery. Urine samples from Tianjin and Qiqihar, and paired urine and blood samples from Tianjin, were first morning voids (fasting time: ~8 h). Whole blood samples from children were randomly collected. Urine samples were collected in 50-mL polypropylene tubes; blood samples were collected in heparinized vacutainers. All whole blood and urine specimens were frozen at −20 °C until analysis.

All participants were healthy, and none reported work-related exposure to BPs. The mean (range) ages are 2.83 (1–5), 39.0 (19–63), and 30.0 (21–36) yrs for children, adults, and pregnant women, respectively. Overall, 52 of 101 general adults were males; 70% of pregnant women were primiparities, and the rest (30%) gave birth to their second child. Detailed demographic information of the donors is provided in Table S1. The sample collection and analysis were approved by the Institutional Review Boards of Nankai University, China, and Wadsworth Center, New York State Department of Health, Albany.

2.2. Quality assurance and quality control

For every batch of 20 samples analyzed, a procedural blank, an instrumental blank, and a matrix-spiked sample were analyzed. Procedural blanks were prepared by substitution of 0.5 mL of Milli-Q water for blood ($n = 3$) or urine ($n = 3$), followed by passage through the entire analytical procedure; pure solvent (methanol) was injected at the beginning and after every 10 samples as a check for instrument contamination that would arise from carryover. BPs were not detected in procedural or instrumental blanks. Ten nanograms each of native BP (100 μL, 0.1 ng/μL in methanol) was spiked into sample matrices ($n = 3$ for each matrix type) and passed through the entire analytical procedure. The recoveries of BPs ranged from 73 ± 18% to 128 ± 16% for urine and from 83 ± 16% to 101 ± 16% for blood. A few exceptions, with low recoveries, were noted: BP-8 and BP-2 showed recoveries <50% in blood; however, these BPs were rarely found in humans in this study or in previous studies (Kunisue et al., 2010, 2012; Ye et al., 2008a), and the poor recoveries for these two compounds, therefore, did not affect the interpretation of our results. The relative standard deviation (RSD) of replicate analysis of selected samples was <20%.

A calibration curve was prepared every day at the beginning and at the end of every batch of 20 samples analyzed. The regression coefficient of calibration standards, injected at concentrations ranging from 0.05 to 50 ng/mL, was >0.999. As a check for instrumental drift in response factors, a midpoint calibration standard was injected after every 10 samples. The limit of quantitation (LOQ) was determined based on the linear range of the calibration curve; concentrations in samples that were at least three fold greater than the lowest acceptable standard concentration were considered valid. The LOQ for the target analytes was (ng/mL): 0.17 ± 0.06 (BP-3), 0.07 ± 0.01 (4OH-BP), 0.08 ± 0.01 (BP-1), 0.09 ± 0.02 (BP-8), and 0.21 ± 0.03 (BP-2) for urine; and 0.47 ± 0.06 (BP-3), 0.06 ± 0.01 (4OH-BP), 0.09 ± 0.03 (BP-1), 0.41 ± 0.31 (BP-8), and 0.67 ± 0.46 (BP-2) for blood. Dilution factors and the mass of samples taken for analysis were included in the calculation of LOQ. Quantification was performed by isotope-dilution; $^{13}C_{12}$-BP-3 was used as an internal standard for BP-3, BP-1, and BP-8, and $^{13}C_{12}$-BPA was used as an internal standard for 4OH-BP and BP-2. BP concentrations in urine can fluctuate, depending on the volume of urine output at each sampling event. To enable the comparisons of concentrations between samples through reducing variability that would arise from volume of urine output, we also corrected the data for creatinine levels. Creatinine adjustment has been suggested to improve the comparability of chemical measurements across individuals, although this is still a subject of debate.

2.3. Statistical analysis

Data analysis was performed with SPSS, Version 17.0. Concentrations below the LOQ were substituted with a value equal to LOQ divided by 2 or the square root of 2 for the calculation of the arithmetic mean and geometric mean (GM), respectively. Differences between groups were compared by one-way ANOVA. Data were tested for normality using the Kolmogorov–Smirnov test. Pearson correlation coefficients were used for the analysis of relationship between two sets of data with normal distribution (or log-normal distribution); otherwise, the Spearman's rank correlation coefficient was used. A value of $p < 0.05$ denoted significance.

3. Results and discussion

Total concentrations (GM, median, and range) of BPs (free plus conjugated) in urine from adults, and in whole blood from children, adults, and pregnant women and their fetuses (i.e., cord blood), from China are shown in Table 1.

3.1. Urinary BP concentrations

4OH-BP, BP-1, and BP-3 were found in 61%, 57%, and 25%, respectively, of 101 urine samples analyzed; BP-8 and BP-2 were detected in only 3% and 5% of urine samples; therefore, these two BPs were excluded from further discussions. Elevated concentrations of BP-3 [475 ng/mL or 197 μg/g creatinine (Cr)] and BP-1 (338 ng/mL or 140 μg/g Cr) were found in a female adult; based on our investigation, this donor reported frequent use of sunscreen that contains BP-3, for skin protection. These two outlier values also were excluded from further discussions.

We compared urinary BP concentrations between frequent users of personal care products (including sunscreen) ($n = 7$) and non-users of personal care products ($n = 13$) and found notably high concentrations of BP-3 and BP-1 in urine from frequent users of personal care products (Table 1). Our results indicate that sunscreen or other personal care products are important exposure sources of BP-3 and BP-1, as has been reported earlier (Kunisue et al., 2012).

The GM concentration of BP-3 (0.26 ng/mL or 0.21 μg/g Cr) measured in all urine samples was similar to that of BP-1 (0.28 ng/mL or 0.18 μg/g Cr); the levels of 4OH-BP were low (GM: 0.19 ng/mL or 0.12 μg/g Cr). A significant positive correlation was found between BP-3 and BP-1 concentrations in urine ($r^2 = 0.671$; $p < 0.001$) (Fig. 2), across all samples. No significant correlation was found between BP-3 and 4OH-BP or between BP-1 and 4OH-BP concentrations (data not shown). These results suggest that the sources of human exposure to BP-3 and BP-1 are common or related, whereas 4OH-BP originates from a discrete source. In a dermal absorption study (Felix et al., 1998), a sunscreen product that contains BP-3 was applied on the skin of volunteers; the major metabolite found in urine was BP-1. It has been reported that BP-3 is metabolized principally to BP-1 in rats (Jeon et al., 2008; Nakagawa and Suzuki, 2002; Okereke et al., 1994), zebrafish (Blüthgen et al., 2012) and piglets (Kasichayanula et al., 2005). Further, humans can be exposed to BP-1 directly via the use of personal care products, but the use of this compound in personal care products is much lower than that of BP-3 (Environmental Working Group, EWG, 2012). Information on the use of 4OH-BP in personal care products and the evidence of its formation from other BPs is lacking. However, high detection frequency of 4OH-BP in urine (61%) indicates that this compound is extensively used in China.

To our knowledge, this is the first study on urinary (and blood) BP concentrations in Chinese populations. The urinary concentrations of BP-3 (median: <0.22 ng/mL) and BP-1 (median: 0.29 ng/mL) found in China were much lower than those found in the U.S. (median: 6.1–82.3 ng/mL for BP-3; 6.1 ng/mL for BP-1) (Calafat et al., 2008; Kunisue et al., 2010, 2012; Wolff et al., 2007; Ye et al., 2005a, 2005b) but

Table 1
Benzophenone-type UV filter[a] concentrations in whole blood (ng/mL) from children, adults, pregnant women and fetuses (cord blood); and in urine (ng/mL; μg/g Cr[b]) from the adults in China.

Variables	n (M/F[c])	BP-3				4OH-BP				BP-1			
		DR[d]/%	GM	Median	Range	DR/%	GM	Median	Range	DR/%	GM	Median	Range
BPs in blood													
Children	10 (5/5)	30	0.43	<0.52	<0.52–2.20	100	0.31	0.32	0.23–0.40	10	0.03	<0.06	<0.06–0.09
Fetuses[e]	22 (11/11)	55	0.52	0.59	<0.41–2.55	100	0.41	0.41	0.26–0.51	0	< 0.12	<0.12	<0.12
Pregnant women	20 (0/20)	35	0.38	<0.41	<0.41–2.30	100	0.67	0.58	0.32–1.78	0	< 0.12	<0.12	<0.12
Adults	23 (12/11)	83	1.51	2.09	<0.52–3.38	100	0.43	0.35	0.26–1.29	4	0.03	<0.06	<0.06–0.15
BPs in paired blood and urine													
Paired blood	23 (12/11)	83	1.51	2.09	<0.52–3.38	100	0.43	0.35	0.26–1.29	4	0.03	<0.06	<0.06–0.15
Paired urine	23 (12/11)	65	1.63	5.06	<0.11–45.2	48	0.16	<0.06	<0.06–3.70	83	1.32	2.01	<0.07–14.3
			0.73	**1.48**	**<0.11–46.1**		**0.12**	**<0.06**	**<0.06–2.32**		**0.48**	**0.55**	**<0.07–14.6**
BPs in urine from adults													
Total	100 (52/48)	25	0.26	<0.22	<0.11–45.2	61	0.19	0.29	<0.06–8.11	57	0.28	0.23	<0.07–20.3
			0.21	**<0.22**	**<0.11–46.1**		**0.12**	**0.14**	**<0.06–5.14**		**0.18**	**0.14**	**<0.07–14.6**
BPs in urine by gender and age													
Males	52 (52/0)	17	0.18	<0.22	<0.11–24.3	54	0.15	0.25	<0.06–2.82	50	0.21	0.11	<0.07–20.3
			0.14	**<0.22**	**<0.11–6.91**		**0.10**	**0.10**	**<0.06–1.79**		**0.12**	**0.07**	**<0.07–5.62**
Females	48 (0/48)	33	0.39	<0.22	<0.11–45.2	69	0.23	0.33	<0.06–8.11	65	0.38	0.33	<0.07–14.3
			0.31	**<0.22**	**<0.11–46.1**		**0.16**	**0.17**	**<0.06–5.14**		**0.28**	**0.29**	**<0.07–14.6**
18–40 yrs	48 (27/21)	26	0.31	<0.22	<0.11–24.0	74	0.25	0.39	<0.06–8.11	57	0.28	0.20	<0.07–12.4
			0.23	**<0.22**	**<0.11–13.4**		**0.14**	**0.15**	**<0.06–3.29**		**0.19**	**0.14**	**<0.07–8.85**
41–50 yrs	23 (6/17)	30	0.33	<0.22	<0.11–45.2	52	0.16	0.23	<0.06–4.14	79	0.54	0.71	<0.07–14.3
			0.26	**<0.22**	**<0.11–46.1**		**0.15**	**0.23**	**<0.06–5.14**		**0.31**	**0.23**	**<0.07–14.6**
51–65 yrs	29 (19/10)	17	0.17	<0.11	<0.11–24.3	48	0.12	<0.06	<0.06–3.70	42	0.16	<0.09	<0.07–20.3
			0.14	**<0.11**	**<0.11–6.72**		**0.09**	**<0.06**	**<0.06–2.32**		**0.11**	**<0.09**	**<0.07–5.62**
BPs in urine by sampling location													
Tianjin	49 (27/22)	35	0.34	<0.11	<0.11–45.2	29	0.08	<0.06	<0.06–3.70	61	0.43	0.74	<0.07–14.3
			0.22	**<0.11**	**<0.11–46.1**		**0.07**	**<0.06**	***<0.06–2.32***		**0.20**	**0.21**	**<0.07–14.6**
Shanghai	26 (15/11)	8	0.15	<0.22	<0.22–13.1	88	0.31	0.37	<0.08–8.11	38	0.12	<0.09	<0.09–11.3
			0.15	**<0.22**	**<0.22–5.10**		**0.13**	**0.14**	**<0.08–3.14**		**0.12**	**<0.09**	**<0.09–8.85**
Qiqihar	25 (10/15)	20	0.27	<0.22	<0.22–24.3	96	0.51	0.45	<0.08–4.14	68	0.31	0.25	<0.09–20.3
			0.25	**<0.22**	**<0.22–13.4**		**0.37**	**0.3**	**<0.08–5.14**		**0.23**	**0.18**	**<0.09–5.62**
BPs in urine by lifestyle													
PCPs users[f]	7 (2/5)	86	2.68	5.55	<0.11–45.2	43	0.12	<0.06	<0.06–2.08	86	2.35	3.08	<0.07–14.3
			1.17	**2.14**	**<0.11–46.1**		**0.10**	**<0.06**	***<0.06–1.35***		**0.75**	**1.64**	**<0.07–14.6**
Non-PCPs users	13 (8/6)	31	0.28	<0.11	<0.11–7.90	15	0.06	<0.06	<0.06–1.20	77	0.87	1.95	<0.07–2.66
			0.15	**<0.11**	**<0.11–1.48**		**0.04**	**<0.06**	**<0.06–0.15**		**0.18**	**0.25**	**<0.07–0.47**

[a] BP-8 and BP-2 were not detected in blood samples, and were only detected in <5% of urine samples, the data were not shown; one urine sample containing outlier value of 475 ng/mL for BP-3 and 338 ng/mL for BP-1, and those outlier values were excluded for calculation.
[b] Bold italic: creatinine-adjusted concentration (μg/g Cr).
[c] M/F = male/female.
[d] DR = detection rate.
[e] The concentration of benzophenone-type UV filters in cord blood was considered to be blood BP levels for fetuses at the time of delivery.
[f] PCPs users = personal care products (include sunscreen) users.

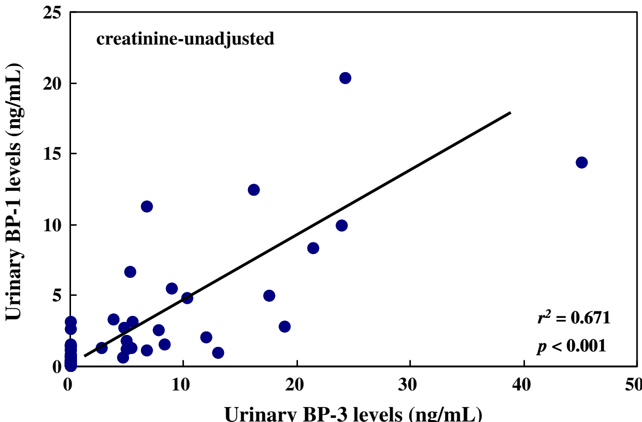

Fig. 2. Correlation between (creatinine-unadjusted) urinary concentrations ($n = 100$) of BP-3 and BP-1.

comparable to those from Japan (median: <0.45–0.52 ng/mL for BP-3; <0.14 ng/mL for BP-1) (Kawaguchi et al., 2009; Kunisue et al., 2010; Wolff et al., 2008). However, the concentrations of 4OH-BP in urine from China (median: 0.29 ng/mL) were similar to those reported for the U.S. (median: 0.36 ng/mL) (Kunisue et al., 2010, 2012) but higher than those reported for Japan (<LOQ for all samples) (Kunisue et al., 2010). These results suggest that the Chinese population is exposed to lower concentrations of BP-3 and BP-1 than is the general U.S. population. The differences are likely the result of the widespread use of sunscreens or other personal care products that contain BP-3 (or BP-1) in the U.S. (Environmental Working Group, EWG, 2012), whereas the use of sunscreen products in China and Japan is uncommon. Another possible reason for lower concentrations of BP-3 found in China in this study than those reported in the U. S. might be due to the use of first morning voids, because the urine collection time is simply out of the exposure window. Moreover, sampling season has an important influence on the levels of BPs; people are exposed to high doses of BPs during the summer months (Kunisue et al., 2010, 2012; Wolff et al., 2007, 2010). In this study, all urine samples from China were collected during winter or spring; the differences in urinary concentrations of BP-3 and BP-1

between China and the U.S. could be ascribable, in part, to the sampling season.

3.2. Blood BP concentrations

BP-3 was found in the blood of 83% of investigated adults, followed, in decreasing order, by pregnant women (35%) and children (30%) (Table 1). The GM concentration of BP-3 in the blood of adults (1.51 ng/mL) was at least three times higher than that determined for children (0.43 ng/mL) and pregnant women (0.38 ng/mL). This suggests higher BP-3 exposures in adults than in children or pregnant women. 4OH-BP was found in all blood samples (100%) analyzed. This finding reiterates the widespread exposure to the Chinese population of 4OH-BP. BP-8 and BP-2 were not detected in any blood samples. BP-1 was found in 2 of 75 blood samples, although it was found more frequently (83%) in urine samples analyzed; BP-1 measured in urine was expected to arise mainly from the metabolic transformation of BP-3 (Felix et al., 1998; Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1994). The low detection frequency of BP-1 in blood suggests the lack of direct exposure to this compound.

An earlier study (Ye et al., 2008b) conducted by the Centers for Disease Control and Prevention (CDC) analyzed BP-3 in 15 serum samples collected in the U.S. between 1998 and 2003; this compound was not detected in any samples [limit of detection (LOD) = 0.50 ng/mL]. Lower blood BP-3 concentrations found in the U.S. than those from China were unexpected.

3.3. Gender, age, and regional differences in urinary BP concentrations

Although the differences were not statistically significant ($p > 0.05$), females had higher urinary BP-3 and BP-1 concentrations (both creatinine-adjusted and -unadjusted concentrations) than did males (Table 1 and Table S2), except for similar urinary concentrations of BP-3 between males and females from Qiqihar. Overall, females had marginally significantly higher ($p = 0.062$ for creatinine-adjusted, $p = 0.055$ for creatinine-unadjusted) concentrations of 4OH-BP than did males, and the difference was pronounced in samples from Tianjin ($p < 0.05$) but not in samples from Shanghai and Qiqihar (Table S2). For all participants with urinary concentrations above the 90th percentile value for BP-3 (12.1 ng/mL), BP-1 (4.93 ng/mL), and 4OH-BP (1.61 ng/mL), sex was significantly associated univariately. Females were 4.0, 9.0, and 2.5 times more likely than males to have concentrations above the 90th percentile for BP-3, BP-1, and 4OH-BP, respectively. A similar trend was previously reported for the general population in the U.S. (Calafat et al., 2008; Kunisue et al., 2010). Our data suggest that females are exposed to higher concentrations of BPs than are males. The greater use of cosmetics and skin care products by females than males can explain this finding.

We categorized the urine samples from adults into three groups on the basis of donor's age: 18–40, 41–50, and 51–65 yrs; the wide age range for the group of 18–40 yrs was due to the small sample size of n = 5 available for ages of 31–40 yrs. This categorization was performed individually for each city (Table S2) and for the entire sample set (Table 1). The lowest urinary concentrations of BP-3, BP-1, and 4OH-BP were found for the age group 51–65 yrs, although the differences were not significant among the three age groups. Further, urinary concentrations of BP-3 ($p < 0.05$) and BP-1 ($p < 0.05$) were significantly negatively correlated with age for an adult population from Tianjin (Fig. S2 and Fig. S3) when the two outlier values were excluded. However, this age-related trend was not observed for the Shanghai and Qiqihar populations, due to the small sample size. Our data suggest higher exposures to BP-3 and BP-1 by young adults than the elderly in Tianjin. In the U.S. (Calafat et al., 2008; Wolff et al., 2007), no age-related exposures were found, although non-Hispanic whites who were in their twenties and forties had significantly higher urinary BP-3 concentrations than did those who were in their thirties and fifties (Calafat et al., 2008).

The urine samples collected from Qiqihar had significantly higher 4OH-BP concentrations than those from Shanghai ($p < 0.05$) and Tianjin ($p < 0.05$). However, the urinary BP-3 and BP-1 concentrations in samples from Tianjin were comparable to those from Qiqihar but were marginally higher than those from Shanghai ($p = 0.0508$ for creatinine-unadjusted levels). It is worth noting that urine samples from Tianjin and Qiqihar were first morning voids, whereas urine samples from Shanghai were spot samples; therefore, our results should be interpreted with caution. Further studies with large sample sizes are needed to explore the influence of demographic factors on BP exposures in China.

3.4. BP-1 versus BP-3 in urine

BP-3 can be metabolized principally to BP-1 in humans (Felix et al., 1998) and animals (Blüthgen et al., 2012; Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1994). To assess the differences in metabolic transformation, a coefficient, *f*, to elucidate the distribution of metabolites of BP-3 (e.g., BP-1) in relation to sum concentrations, was calculated using Eq. (1):

$$f = \frac{C_2}{C_1 + C_2} \quad (1)$$

where C_1 and C_2 (ng/mL) are the individual urinary concentration of BP-3 and BP-1, respectively, for each adult. The *f* values (range: 0.06–0.62) varied among 25 participants with measurable concentrations of both BP-1 and BP-3 in urine (>LOQ). The GM values of *f* were 0.28, 0.21, and 0.22 for participants from Tianjin, Shanghai, and Qiqihar, respectively. Our results suggest considerable differences in the proportions of BP-1 and BP-3 in humans. This information is important for the assessment of risks associated with BP-3 exposures because the metabolism of BP-3 yields more estrogenic BP-1 (Kawamura et al., 2003, 2005; Nakagawa and Suzuki, 2002; Suzuki et al., 2005; Takatori et al., 2003).

We examined gender and age-related differences in *f* values for Tianjin's population. No significant gender-related differences in *f* values (GM: 0.27 for males, 0.24 for females) were found. Nevertheless, a significant inverse relationship was found between ln (*f*) and ln (age) ($p < 0.05$) (Fig. 3) as well as between the values of *f* and age ($r^2 = -0.242$; $p < 0.05$) (Fig. S4). The age group of 18–40 yrs had significantly higher ($p < 0.01$) *f* values (GM: 0.34) than did those of the age group of 41–62 yrs (GM: 0.19). Although the number of samples analyzed is small, the age-related differences in *f* values

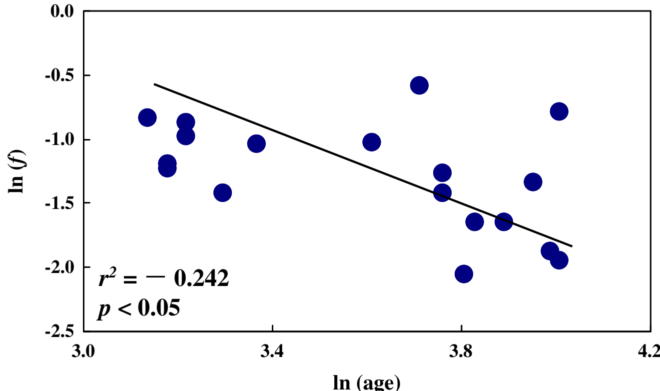

Fig. 3. Correlation between ln (*f*) [ratio between urinary concentrations of BP-1 and sum of urinary concentrations of BP-3 and BP-1] and ln (age) of donors ($n = 18$) with measurable concentrations of both BP-1 and BP-3 in urine (>LOQ).

suggest faster metabolism of BP-3 by younger age groups than that by elderly people.

3.5. Partition of BPs between blood and urine

Among the 23 individuals from Tianjin who donated matched blood and urine, BP-3 was found in 19 blood and 15 urine samples. Concentrations of BP-3 ranged from <LOQ to 4.41 ng/mL in blood and from <LOQ to 45.2 ng/mL in urine (Table 1). Higher concentrations of BP-3 observed in urine than in blood suggests that this compound excretes readily in urine. 4OH-BP was detected in all blood samples, at concentrations ranging from 0.26 to 1.29 ng/mL and, in 11 of 23 urine samples, at concentrations ranging from 0.23 to 3.70 ng/mL.

The ratios of concentrations of BPs between blood and urine (PB/PU ratio) were calculated by dividing blood BP concentrations by creatinine-unadjusted urinary BP concentrations. The GM (range) PB/PU ratio for BP-3 was 0.21 (0.07–0.92) in the 15 blood-urine pairs that contained measurable concentrations of BP-3; the PB/PU ratio for 4OH-BP was significantly higher ($p < 0.01$) than that of BP-3, with a GM value of 0.36 (range: 0.12–1.42) for the 12 blood-urine pairs with detectable concentrations of 4OH-BP (Table 2). This finding suggests that 4OH-BP has a slower clearance rate than that of BP-3 from human bodies. In general, hydrophilic chemicals excrete into urine relatively more quickly than do hydrophobic chemicals. Although some BPs are hydrophobic (or lipophilic) chemicals, 4OH-BP is more hydrophilic (log Kow = 3.07) than is BP-3 (log Kow = 3.52) (Zhang et al., 2011). Therefore, the PB/PU ratio of 4OH-BP was expected to be lower than that of BP-3. Previous studies have shown that BPs excrete as conjugated forms (e.g., glucuronidated or sulfated) in urine (Gonzalez et al., 2006, 2008; Ye et al., 2005b). The physicochemical properties (e.g., hydrophilicity/hydrophobicity) are expected to be different between conjugated and free-form of BPs. Needham and Wang (2002) found that conjugated chemicals are more hydrophobic than are the corresponding free forms. Therefore, the differences in hydrophilicity between conjugated and free forms of 4OH-BP and BP-3 may be an important factor that drives the differential partitioning of these two compounds between blood and urine. However, no information is available on the log Kow of conjugated BPs. Further studies are needed to clarify the factors that influence partitioning of BPs between blood and urine.

To our knowledge, this is the first study to report BP concentrations in paired blood and urine samples from the general population. No significant relationship existed between concentrations of BP-3 or 4OH-BP (creatinine-adjusted and creatinine-unadjusted) in blood and urine. No gender/age-related differences in PB/PU ratios were found for BP-3 and 4OH-BP (Table 2).

Table 2
Concentration ratios of BP-3 and 4OH-BP between blood and urine from an adult population and between cord blood and maternal blood.

	Partition ratios between blood and urine[a]					Trans-placental transfer ratios[b]		
	All	<40 yrs	≥40 yrs	Males	Females	All	Males[c]	Females[c]
BP-3								
GM	0.21	0.25	0.19	0.30	0.17	0.48	0.64	0.32
Median	0.24	0.27	0.20	0.30	0.15	0.51	0.55	0.33
Range	0.07–0.92	0.10–0.92	0.07–0.66	0.11–0.92	0.07–0.66	0.24–0.92	0.51–0.92	0.24–0.43
4OH-BP								
GM	0.36	0.28	0.41	0.34	0.37	0.61	0.53	0.67
Median	0.36	0.29	0.37	0.38	0.36	0.68	0.56	0.75
Range	0.12–1.42	0.14–0.67	0.12–1.42	0.15–0.67	0.12–1.42	0.21–1.61	0.21–1.61	0.23–1.29

[a] Partition ratios were calculated as blood BP-3 and 4OH-BP concentrations divided by creatinine-unadjusted urinary concentrations.
[b] Trans-placental transfer ratios were calculated as fetal cord blood BP concentrations divided by maternal blood BP concentrations.
[c] Gender of fetus.

3.6. Placental transfer of BPs

4OH-BP was found in all matched fetal cord blood and maternal blood samples, at concentrations ranging from 0.26 to 0.51 ng/mL in cord blood, and from 0.32 to 1.78 ng/mL in maternal blood. 4OH-BP concentrations in maternal blood were higher than those found in cord blood. The ratio of concentrations of 4OH-BP between cord blood and maternal blood (CB/MB ratio) varied among the 22 mother-fetus pairs analyzed. The GM (range) value for the CB/MB ratio of 4OH-BP was 0.61 (0.21–1.61) (Table 2). BP-3 was found in 12 cord blood (range: 0.55–2.55 ng/mL) and 7 maternal blood samples (0.74–2.30 ng/mL). The CB/MB ratios of BP-3 were lower than those of 4OH-BP, with a GM value of 0.48 (range: 0.24–0.92) (Table 2). Vela-Soria et al. (2011a, 2011b) determined BP-3, 4OH-BP, and other BPs in 66 placental tissues collected from Spain and reported frequent detection of 4OH-BP, while BP-3 was not found in placenta samples. These results indicated that BP-3 did not efficiently cross the placental barrier. The lower CB/MB ratios found for BP-3 than those for 4OH-BP could, in part, be due to greater lipophilicity of the former than the latter (Zhang et al., 2011).

4. Conclusions

This study provides the first report on the occurrence of and human exposure to five BPs by children, adults, and pregnant women in China. Body burdens of BP-3 and BP-1 in Chinese were lower than those reported for the U.S. populations. As previously reported and confirmed in the present study, females were considerably more exposed to BP-3 than males. Our comparison of benzophenone concentrations between paired blood and urine as well as between fetal and maternal blood provided novel information. BP-3 and 4OH-BP in blood were readily partitioned into urine. BP-3 and 4OH-BP can be transferred by trans-placental routes from mother to fetus.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.04.074.

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Determination of benzophenone-UV filters in human milk samples using ultrasound-assisted extraction and clean-up with dispersive sorbents followed by UHPLC-MS/MS analysis.

Rodríguez-Gómez R1, Zafra-Gómez A2, Dorival-García N1, Ballesteros O1, Navalón A1.
Author information

Abstract
A new sample preparation method for the determination of five benzophenone UV-filters in human breast milk has been developed. The procedure involves the lyophilization of the sample, and its subsequent extraction by ultrasound sonication using acetonitrile. In order to reduce matrix effects produced by milk components that are coextracted, mainly proteins, sugars and lipids, a further clean-up step with a mixture of dispersive-SPE sorbents, C18 and PSA, was applied. Extraction parameters were optimized using experimental design, and the compounds were detected and quantified by ultrahigh performance liquid-chromatography tandem mass spectrometry (UHPLC-MS/MS) in positive ESI mode. Analytes were separated in 10 min. BP-d10 was used as internal standard. The limits of detection (LODs) were between 0.1 and 0.2 ng mL(-1), and the limits of quantification (LOQs) were between 0.3 and 0.6 ng mL(-1) for the target analytes. The inter- and intra-day variability was <12%. The method was validated using matrix-matched calibration and recovery assays with spiked samples. Recovery rates were between 90.9 and 109.5%. The method was successfully applied for the determination of these compounds in human milk samples collected from volunteers lactating mothers with no known occupational exposure to these compounds who live in the province of Granada (Spain). The analytical method developed here may be useful for the development of more in-depth studies on the prenatal exposure and biomonitoring of these commonly used UV-filters.

Oxybenzone HEL Monograph - 4 of 7

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Joe DiNardo <jmjdinardo@aol.com>

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Cc:cadowns@haereticus-lab.org <cadowns@haereticus-lab.org>;

9 attachments (9 MB)

21 Hines 2015 Concentration of environmental phenols and parabens in milk.pdf; 22 Gago-Ferrero ABC-Review biota UV F_publicado.pdf; 23 Molins-Delgado Articulo publicado_BZTs medio urbano_2017.pdf; 24 Alonso Toxic Heritage.pdf; 25 Molins-Delgado UVFs in Eggs_EST 2017 publicado.pdf; 26 Popkin Effects of pH on weak and positive control mutagens in the Ames Salmonella plate assay.docx; 27 Zeiger Salmonella mutagenicity tests- III. Results from the testing of 255 chemicals.docx; 28 Knowland Sunlight-induced mutagenicity of a common sunscreen ingredient.docx; 29 NTP Report on Benzophenone.zip;

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,
Craig Downs – Executive Director – Haereticus Environmental Laboratory
Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:
- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.
- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.
- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:

1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone
2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

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Concentrations of Environmental Phenols and Parabens in Milk, Urine and Serum of Lactating North Carolina Women

Erin P. Hines[1], **Pauline Mendola**[2], **Ondine S. vonEhrenstein**[3], **Xiaoyun Ye**[4], **Antonia M. Calafat**[4], and **Suzanne E. Fenton**[5]

Pauline Mendola: pauline.mendola@nih.gov; Ondine S. vonEhrenstein: ovehren@ucla.edu; Xiaoyun Ye: xay5@cdc.gov; Antonia M. Calafat: aic7@cdc.gov; Suzanne E. Fenton: fentonse@niehs.nih.gov

[1]Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency, RTP, NC

[2]Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD

[3]Department of Community Health Sciences, Fielding School of Public Health, University of California Los Angeles, Los Angeles, CA

[4]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA

[5]Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, RTP, NC

Abstract

Phenols and parabens show some evidence for endocrine disruption in laboratory animals. The goal of the Methods Advancement for Milk Analysis (MAMA) Study was to develop or adapt methods to measure parabens (methyl, ethyl, butyl, propyl) and phenols (bisphenol A (BPA), 2,4- and 2,5-dichlorophenol, benzophenone-3, triclosan) in urine, milk and serum twice during lactation, to compare concentrations across matrices and with endogenous biomarkers among 34 North Carolina women. These non-persistent chemicals were detected in most urine samples (53-100%) and less frequently in milk or serum; concentrations differed by matrix. Although urinary parabens, triclosan and dichlorophenols concentrations correlated significantly at two time points, those of BPA and benzophenone-3 did not, suggesting considerable variability in those exposures. These pilot data suggest that nursing mothers are exposed to phenols and parabens; urine is the best measurement matrix; and correlations between chemical and endogenous immune-related biomarkers merit further investigation.

Correspondence: Erin P. Hines, Ph.D. U.S. EPA, ORD, NCEA, EMAG, B243-01, Research Triangle Park, NC 27711, Tel: (919) 541-4204, hines.erin@epa.gov.

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Keywords

Biomonitoring; BPA; breast milk; lactation; MAMA Study; parabens phenols; serum; urine

Introduction

Humans, house pets, and parts of our food chain are exposed to a mixture of man-made chemicals through industrial pollution, pesticide use, consumer and personal care products, house dust, drinking water, and food packaging. The National Health and Nutrition Examination Survey (NHANES), conducted by the Centers for Disease Control and Prevention (CDC), has demonstrated widespread exposure to some of these chemicals, such as phenols (e.g., bisphenol A [BPA], triclosan) and parabens, among the U.S. general population [1]. As these particular chemicals are commonly found in cosmetics, UV filters, anti-microbial soaps, lotions and plastics used in toys and food storage, at-risk populations (i.e., pregnant women, infants, children, and the elderly) may have more potential for exposure due to enhanced use.

Some persistent environmental chemicals, such as brominated flame retardants (BFRs) and perfluoroalkyl substances (PFAS) can be measured at higher serum concentrations in children than adults [2, 3]. It is not known whether this is due to different metabolic rates, varied exposure patterns, or smaller blood volumes in children compared to adults. Of note, PFASs and BFRs can also be found in breast milk and can be transferred to the infant [4, 5]. However, few studies have examined the extent to which many non-persistent chemicals are found in breast-feeding women and their milk [6-8]. Characterization of chemical exposure in breastfeeding women and the potential for transfer of those chemicals or their metabolites to breast milk would aid in exposure assessment in infants/children and is of interest to risk assessors [9].

Certain phenols and parabens have endocrine disrupting effects in cell lines and animal models [10-12]. In laboratory animals, exposures to some phenols and parabens have been linked to pathologies or disorders such as obesity, thyroid dysfunction, and breast cell hyper-proliferation [13-17]. NHANES and other studies have reported the concentrations of certain phenols and parabens in the serum or urine of adults [18-21], but information on the transfer to milk, and the ratios of the chemical concentrations in the various matrices of at-risk populations, such as lactating women [6-8], especially women from the USA, is limited.

Because early life is a critical and influential period for potential health effects of endocrine disrupting factors [22], our goals were to develop or adapt methods to collect biological matrices (i.e., milk, serum, urine) from lactating women and measure the total concentrations of phenols and parabens in these different biological specimens (total and free in serum) at two time points (i.e., visits). These methods are integral for evaluating the effects of environmental exposures in longitudinal health studies, such as the National Children's Study [23, 24] or large developmental cohort studies conducted in other countries [25-27]. Those types of studies also have interests in major health afflictions of children, such as puberty timing, obesity, diabetes, allergy and asthma. We had previously validated assays that may serve as health biomarkers and were endogenous components of the

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matrices we collected [28]. Therefore, we also assessed correlations between phenol and/or paraben concentrations and endogenous components of milk [glucose, triglycerides, secretory immunoglobulin A (sIgA), prolactin, estradiol, interleukin-6, and tumor necrosis factor-alpha (TNF-α)] and serum (including the aforementioned milk biologics with the addition of IgE, IgM, IgG, and IgA instead of sIgA) for the individuals in our study. We did this hypothesizing that there may be significant correlations between these chemical exposures and endogenous components that would mirror correlations reported in animal model studies, especially those indicating estrogen agonist activity (i.e., BPA). We also evaluated correlations between measured concentrations of these chemicals and potential exposure routes, using information gathered from an extensive questionnaire administered at the first of two visits [28].

Materials and Methods

MAMA Study Details

Healthy (no acute illness at the time of sample collection), lactating, English-speaking women between the age of 18 and 38 were recruited for the Methods Advancement in Milk Analysis (MAMA) study by the US Environmental Protection Agency (EPA) contractor, Westat (Chapel Hill, NC). The women visited the US EPA Human Studies Facility clinic in Chapel Hill, NC, between December 2004 and July 2005. Participants (n=34) were asked to fast before sample collection and to avoid the use of breast creams. The method of recruitment and demographic information on the participants has been previously reported [28] with study design including the use of a convenience sampling of women with limited ethnic diversity, e.g., majority Caucasian. The research with human subjects was approved by the Institutional Review Boards (IRBs) of the University of North Carolina-Chapel Hill Medical School under IRB number 03-EPA-207 and the CDC under IRB number 3961. Study volunteers were briefed on the study goals, risks and inclusion and exclusion criteria and provided informed consent (verbal and written) prior to donation and answering an extensive questionnaire.

Milk, urine and serum were collected at 2-7 weeks and 3-4 months postpartum into polypropylene containers using a previously described protocol [29]. Breasts were cleaned with water and a cloth towel before milk collection. Women provided all of the milk (including hind milk) available at the time of collection (volume was to equal/exceed 3 ounces). A log was kept to record details of the sample collection, including date and time of day. The samples from multiple matrices were collected within an hour of each other. All samples, including freshly collected, mixed milk samples were aliquoted into multiple tubes at collection and stored at or below -20 °C until analysis. Aliquots of each sample were available for endogenous biomarker analyses and analytical chemical analyses. A questionnaire was administered to the women at the first visit and it was aimed at understanding the sources of their potential chemical exposures, including age, race/ethnicity, education, years at current address, personal care product use (i.e., nail polish, hair styling products, hair color, foundation makeup), number of prior children and number breastfed, pregnancy complications (diabetes, preeclampsia, excess weight gain), information on current breastfeeding, source and amount of water consumed daily, and body

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mass index. Many answers were categorical (i.e., none, seldom, moderate, often) and others were continuous.

Analytical Chemical Measurements

Details of the analytical procedures used to measure the total (free plus conjugated) or free concentrations of the environmental chemicals can be found in the Supplementary Data. Specifically, we measured the total (free plus conjugated) concentrations in urine, milk, or serum, but only the free concentrations in serum. Briefly, the target analytes in urine, milk, or serum were pre-concentrated by online solid phase extraction, separated from other matrix components by reverse-phase high performance liquid chromatography, and detected by atmospheric pressure chemical ionization or atmospheric pressure photoionization–isotope dilution–tandem mass spectrometry with peak focusing as described before [30, 31]. Because certain compounds measured in this analysis are ubiquitous in the environment, quality control procedures, including the use of blanks, were used at all steps to monitor for BPA contamination from the procedures for sample collection, handling, and analysis [32-34].

The number of samples available for chemical analysis varied due to the method development nature of this study. Two urine, serum and milk ($Milk_A$= stored at -20 °C; $Milk_B$= stored at -80 °C to compare stability of these chemicals in milk) aliquots per participant were collected for analyses in this study. One of the two aliquots of serum was previously analyzed for other chemicals (i.e., PFASs) prior to BPA and benzophenone-3 (2-hydroxy-4-methoxybenzophenone) measurements [35]. We measured concentrations of parabens (methyl-, ethyl-, butyl- and propyl), BPA, benzophenone-3, 2,5- and 2,4-dichlorophenol, and triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) in urine, serum and milk. Measurements of phenols and parabens were made on 1st visit (V1) milk (n=1), 2nd visit (V2) milk (n=9), V1 serum (n=34), V2 serum (n=30), V1 urine (n=33), and V2 urine (n=30) samples. Only 10 milk samples (representative of 9 women) were analyzed for phenols and 8 milk samples for parabens because the initial collection protocol added a preservative (potassium dichromate) to the milk at collection that adversely affected the performance of the method used for analysis of parabens and phenols. This problem was identified after sample collection had begun and the methodology was altered to not include the preservative in the remaining samples.

Measurements of Endogenous Immune-Related Biomarkers

Concentrations of serum IgG, IgM, IgA, IgE, glucose, triglycerides, estradiol, prolactin, IL-6, and TNF-α and milk sIgA, IL-6, leptin, prolactin, TNF-α, triglycerides, glucose and estradiol were measured for each MAMA study participant by LabCorp Inc. (Burlington, NC) as defined in the detailed protocols previously reported [28]. Serum was assayed on the same day as or within 24 hours of their co-paired milk samples. Unlike the analytical methods described above, the preservative did not interfere in these assays, therefore the n=31 for V1 milk and n=21 for V2 milk end points. The n for serum samples is identical to those reported above (34 and 30, respectively, for V1 and V2). The assay coefficients of variation and limits of detection have been previously reported [28].

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Statistics

We report milk, serum and urine concentrations of benzophenone-3, BPA, methyl paraben and propyl paraben for each woman. The minimum, maximum and 20th-80th percentile concentrations of the endogenous immune-related biomarkers have been previously reported (Table 4 in [28]). Concentration distributions are described for each compound, but comparisons across matrices and across visits were done only for compounds detected in > 50% of samples [36]. Spearman correlations were calculated to evaluate the relationships within and between phenol and paraben concentrations to compare V1 and V2 for the same compound and to examine interrelationships among parabens and phenols. For concentrations below the limit of detection (LOD; reported in Table 1 by analyte and matrix), we assigned a value equal to the LOD divided by the square root of 2 [63]. Comparisons across visits were not performed for milk concentration because only one milk sample was available at V1 and power was limited (total n=10).

Questionnaires were completed at V1 to capture usual behavior and demographic characteristics. We examined questionnaire data to determine associations between 1) demographic, behavioral and dietary characteristics and 2) phenol and paraben concentrations. Spearman correlations and analysis of variance were used to assess relationships between questionnaire variables and the concentrations of the target analytes. Adjustments were not made for multiple comparisons given the exploratory nature of the study and our intent to identify signals that could be useful to suggest future avenues of investigation. In addition, we note that substantial variability was observed and with our limited power, we only comment in the text on questionnaire data that were associated with a compound at both study visits. However, all correlations are reported in supplemental tables (see Supplemental Data Tables S1-S3). We conducted all analyses using SAS Enterprise Guide 4.1 (SAS Inst., Cary, NC). Significance was denoted at p 0.05.

Results

Parabens and phenolic compounds detected in MAMA samples

Table 1 lists the detection frequency of the parabens and phenols in specific matrices (milk, serum or urine) by visit. Even though the LODs were comparable across all matrices (Table 1), urine yielded the highest number of detectable concentrations, and for all compounds evaluated, the majority of individual urinary concentrations were detectable. As a chemical class, the parabens were the most frequently detected compounds across all matrices. With the exception of methyl paraben, detected in nearly 100% of all samples in all matrices at all visits, other parabens were more often detectable in urine than in serum. Parabens were detected in the majority of milk samples, but no milk samples contained detectable concentrations of butyl paraben.

For the phenols, BPA was the most frequently detected in milk and urine (80-90%) but was seldom detected in serum. 2,4- and 2,5-dichlorophenol were rarely detected in milk (and were thus not measured in serum), but were detected in the majority of urine samples. Benzophenone-3 was detectable in about half of milk samples, in the majority of urine samples and in less than 30% of serum samples. Triclosan was detected in one third of milk

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samples, in nearly 90% of urine samples, and in only one serum sample. Although not shown in Table 1, the chlorinated compound triclocarban was measured but not detected in milk.

Comparison of concentrations of parabens and phenols in various matrices

To better understand the disposition of these chemicals in the individual participants, and how that may vary across the participants, we compared the concentrations of chemicals across matrices. First, we evaluated chemical concentrations in serum and urine of all women with 2 collections (n=30). In Table 2, we show the urine:serum ratio of methyl and propyl paraben, at visit 1 and 2, as they were the only chemicals detected in the serum of at least 50% of the samples. There was wide variation across participants, and for the majority of participants there was a change in concentrations between visits, with both parabens changing in the same direction over time.

To better understand the potential transfer of the parabens and phenols into breast milk, we also evaluated the ratios of chemicals in all the participants that donated milk in which we could make measurements. In Tables 3–6, we present the concentrations of the parabens or phenols (benzophenone-3, BPA, methyl paraben and propyl paraben) that were detected in greater than 50% of the samples. Corresponding individual urine and serum (total and free) concentrations are also reported in these tables.

For benzophenone-3 (Table 3), the milk:urine concentration ratio was heavily skewed toward urine, with ratio values ranging from 1:57 to 1:738. The milk to urine BPA ratios ranged from 1:1 to 1:80 (Table 4), with 3 of 5 individuals (for which a M:U concentration ratio could be calculated) suggesting a <1:10 relationship. Although methyl paraben (Table 5) was detected in all milk samples, the milk concentrations were 21-764 times lower than the urinary concentrations from the same women. Milk and total serum methyl paraben concentrations were not as varied as the difference in milk and urine concentrations, but concentrations in serum were consistently higher (11-30 fold) than in milk. Comparing across matrices, propyl paraben (Table 6) was detected at the highest concentrations in urine (ranging from 0.5 to 279 μg/L), and for individual participants, the milk and total serum propyl paraben concentrations were comparable, while the milk to urine ratio was heavily skewed toward urine (5 to nearly 700 fold higher).

Correlations of paraben and phenol concentrations across visit

Because of the relatively short biological half-lives of the compounds measured and the likely episodic nature of the exposures, we hypothesized that there would be a large variability between the concentrations of an individual compound between visits (collections). Table 7 shows median, minimum, maximum, and selected percentiles of the nine parabens and phenols measured in urine (total samples: n=33, V1; n=30, V2), separated by visit. The Spearman correlations demonstrate the relationship/ranking of each woman's set of measures, reflecting variation by individual (not with respect to absolute values). These correlations indicate that the relative rank of a woman is significantly related from one visit to the other. Surprisingly, there was a significant correlation between V1 and V2 measurements for 7 of 9 phenols and parabens measured when both collections and all

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participants are considered. Benzophenone-3 median concentrations were higher in V2 than in V1 and were not significantly correlated between visits. These findings suggest varied or changing exposures to benzophenone-3 over time, but they may also reflect the variation in timing of sample collection at one or both visits in relation to an individual's potential exposure events. BPA concentrations in urine were also not correlated across time, and our data could not determine if it was due to exposures changing over time or due to the timing of sample collection after a potential exposure.

We made similar comparisons for the concentrations in serum, but were limited in our analysis because from the nine compounds analyzed in serum, only methyl paraben and propyl paraben were detected in greater than 50% of samples. Median, minimum, maximum, and selected percentiles of paraben and phenol concentrations in serum, separated by visit are shown in Table 8. As was the case in urine, there was a significant correlation for both serum methyl paraben and propyl paraben between visits, with the serum range being nearly identical over time for both individual parabens. Butyl paraben was detected in 9% of V1 samples and 17% of V2 samples with a maximum value of 0.7 μg/L. Ethyl paraben was detected in 38% of V1 samples and 35% of V2 samples with a maximum value of 2.4 μg/L. Triclosan was detected in serum samples from two women with a maximum value of 1.5 μg/L, very close to the LOD of 1.1 μg/L.

Only methyl paraben and propyl paraben had detectable concentrations in greater than 50% of both urine and serum samples (as shown in Table 2). Expanding upon this further, methyl paraben urine to serum concentration correlations were significant for V1 (rho=0.40, p=0.02) but not V2 (rho=0.15, p=0.42). Propyl paraben concentrations were not significantly correlated between urine and serum at the same study visit (V1 rho=0.32, p=0.07; V2 rho=0.23, p=0.22). These findings suggest a consistent exposure pattern for the parabens when measured within a matrix over time, but predictions should not be made across matrices based on single collections.

Correlations of parabens and phenols with questionnaire data

Questionnaire data (collected at visit 1) provided information on living, working, and dietary habits, overall health, water source, education, breast feeding practices, how long the participant lived in her locale, and some information on how much time she spent in her car/home/yard, near a computer, and how often she used makeup, nail polish, hair styling products, etc. Those data were analyzed for associations with phenols and parabens concentrations from matrices in which the compounds were detected in more than 50% of the samples (no milk analysis for V1). Detailed questionnaire data results are reported in the supplementary data section of this publication (Supplementary Data Tables S1-S3). Spearman correlation analysis of questionnaire data yielded significant correlations at V1 and V2 for urine methyl paraben and nail polish use (Table S1); urine and serum propyl paraben and nail polish use (Tables S1 and S2); serum methyl paraben and hair styling product use (Table S2); and an inverse correlation between urine concentrations of BPA and education and hair styling product use (Table S3). Other significant outcomes lacked a consistent pattern, with certain correlations appearing at V1 or V2, but not both visits. The

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spreadsheets denoting both positive and negative correlations are included in Supplementary Data Tables S1-S3.

Correlation of parabens and phenols concentrations with endogenous immune-related biomarkers

Correlations between the phenol and paraben concentrations and endogenous immune-related biomarkers (cytokines, immunoglobulins, hormones, glucose or triglycerides), which were only measured at visit 1, were explored to try to get a better understanding of potential health related indices associated with these exposures. Data for seven different compounds are reported in Supplementary Data Tables S4-S10. These immune-related biomarkers were measured in all three of the matrices described and significant correlations are denoted based on matrix (see subscripts). Milk IL-6 had significant positive correlations with most of the phenols and parabens measured (all Rho > 0.5): BPA_{U1}, 2,4-dichlorophenol$_{U2}$, 2,5-dichlorophenol$_{U2}$, ethyl paraben$_{S}$1, ethyl paraben$_{U2}$, methyl paraben$_{S2}$, propyl paraben$_{S}$1 and propyl paraben$_{U2}$. Milk sIgA also had significant correlations, some highly correlated (i.e., Rho values of 0.74 and 0.75 with parabens), with many phenols and parabens: BPA_{U1}, 2,4-dichlorophenol$_{U2}$, 2,5-dichlorophenol$_{U2}$, ethyl paraben$_{U2}$, and methyl paraben$_{S}$1. Serum IgA had significant correlation with benzophenone-3$_{U1}$ and ethyl paraben$_{S}$1. Serum and milk IgM had significant correlation with methyl paraben$_{U2}$ and propyl paraben$_{U2}$, respectively. Serum TNF-α had a significant correlation with propyl paraben$_{U1}$. Glucose and triglycerides had negative and positive significant correlations with BPA_{U1} and benzophenone-3$_{U1}$, respectively. None of the parabens or phenolic compounds had significant correlations with circulating or milk-derived hormone concentrations (estradiol or prolactin).

Discussion

We report concentrations in multiple matrices of parabens and phenols measured at 2 different periods of lactation from individual women. One of our goals was to determine which matrices provide useful data (easily collected, analysis successful in that matrix, and over 50% detects for these chemicals). Urine provided the most useful data, with all parabens and phenols yielding detectable measurements in greater than 2/3 of the individual samples at both visits. Milk concentrations, albeit limited due to the methods development nature of this study, were detectable in >50% of samples for ethyl paraben, methyl paraben, propyl paraben, benzophenone-3 and BPA, suggesting potential usefulness of larger milk biomonitoring efforts on these endocrine active compounds. Measurements in serum were not worthwhile for many of these compounds, as only methyl and propyl paraben were detectable in >50% of samples.

As a methods development study on novel endpoints, we conducted exploratory analysis without adjustment for multiple comparisons. Because we provided data on only a small number of lactating women, our results should be confirmed in future studies. However, we learned several things that should advance this field: 1) Relatively high detection incidence (56-100%) in breast milk of ethyl, methyl and propyl parabens, BPA and benzophenone-3, so this matrix could be used for exposure analysis in future studies; 2) Relatively low

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milk:urine concentration ratio for BPA, so although this is a short-lived compound in the body, it may transfer to milk; 3) Statistically significant between-visit correlations for 7 of 9 of these compounds, suggesting consistent and/or recurrent exposure to these compounds over time; and 4) Consistently positive correlations of some of these compounds with immune end points in the milk of the study participants (see Supplementary data), suggesting further studies into the relationships of phenols and parabens with immune response may be fruitful.

Many of the chemicals measured in these lactating women are hormonally active in laboratory animals. BPA is employed in the manufacture of polycarbonate plastics and epoxy resins used in dental sealants, and as coatings lining food and soda cans [20], among other applications. In this study, individual urinary BPA concentrations were inversely related with the maternal education level and her reported use of hair styling products (Table S3). We did not evaluate the correlation between education and hair styling product use during pregnancy/lactation, but theorize that they are related. In rodent studies, BPA is associated with multiple adverse health outcomes following early life exposures [37]. Our data add to the limited reports of BPA concentrations in individual breast milk samples from US women analyzed mainly for method development studies [7, 30, 38-40]. Other researchers measured BPA in colostrum of Japanese women (n=110) by ELISA [41]. However, ELISA lacks adequate analytical selectivity and specificity, and matrix effects may induce performance anomalies for BPA quantification in human samples [37]. Studies in rats directly exposed to BPA report very low transfer of the compound to milk [42, 43], but the limited number of samples in our study show BPA is present in >50% of milk samples. However, because BPA is a ubiquitous environmental contaminant, we cannot rule out completely the potential for external contamination with BPA during collection, storage, or analysis [33]. We rarely detected total BPA in the serum, but we detected BPA in most urine samples. These data confirm previous reports [44] [45] that serum is not an adequate matrix for biomonitoring of BPA in adults or children, or to estimate dose in most rodent studies.

Benzophenone-3, often used as an ingredient in sunscreen, ultraviolet light stabilizer in plastics, and to inhibit photodegradation [12], has also been shown to be estrogenic [10, 18]. Our report of a mean milk concentration of 3.7 μg/L for the 7/10 samples (from 9 participants) with detectable concentrations adds to the limited data on benzophenone-3 concentrations in milk from US women [30, 39]. Previous studies have suggested that exposure to benzophenone-3 may vary by season [46] and race/ethnicity [19]. The majority of the samples in the current study were collected within the winter and spring season and most women (85%) were white. Benzophenone-3 had been previously reported in milk from Swiss women (n=34) with a median concentration of 19.8 ng/g lipid [6] and detection frequency of ∼18% (LOD = 2 ng/g). The other phenols examined show similar interquartile ranges and medians in urine to data reported in a recent study on pregnant Spanish women and their children [47].

Chlorophenols are found in biocides including pesticides, fungicides, and insecticides [31], and are used in dye synthesis intermediates, moth repellants, room deodorizers, and in treated wood. 2,5-dichlorophenol, the primary metabolite of p-dichlorobenzene, is common

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in US populations [1]. Exposure to high doses of 2,4-chlorophenol in laboratory animals causes immunological and liver related effects, in addition to smaller litters and offspring with decreased birth weight [48]. There are a couple of technical manuscripts devoted to analytical method development that report rarely detecting 2,4- and 2,5-dichlorophenol in milk [39, 49]. By contrast, these dichlorophenols are detected in the urine of the majority of the US general population [1], in residents of a California agricultural community [50], and in pooled serum samples of US children [45]. In the present study, we detected 2,4-dichlorophenol in 11% of the milk samples, whereas 2,5-dichlorophenol was undetectable; both compounds were detected in >82% of the urine samples, consistent with previous results.

Triclosan is added to some detergents, toothpastes, cosmetics, clothing and plastics to prevent microbial growth. Triclosan has been shown to depress serum testosterone at high doses in male rats without effects on puberty (pre-putial separation) or reproductive organ weight [17]. In female mice, triclosan is an exogenous estrogen enhancer in the weanling uterotrophic assay [11]. The first report of triclosan in human milk was a Swedish study in which five randomly collected samples were analyzed. In 3 of 5 samples triclosan was detected, although the method of sample collection was unknown [51]. Another Swedish study carefully collected samples from 34 women who either used or did not use triclosan containing products [52]. Triclosan was present in the serum of all women tested (even the “controls”) and in the milk of nearly half of the controls and all of the exposed women. When 62 U.S. milk bank samples were tested for triclosan [53], it was present in 51 of them above the LOD of 150 ng/kg and concentrations were highly variable, as was seen in our study. In another study, triclosan was detected (LOD = 1 ng/mL) in two of the four breast milk samples analyzed [30]. In these studies, the milk collection method was unknown. Triclosan has been measured in serum of breast-feeding women [51, 52], in the urine of young American girls [54], and in the general US population [1, 19]. The urinary concentrations of triclosan in these MAMA participants are similar to those reported in NHANES [1] and by Wolff *et al* [54] although these are all vastly different populations (lifestage, sex, age). Also, women from earlier studies had lower milk triclosan concentrations than they did serum concentrations [52]; our data did not allow for these comparisons because we detected triclosan in only one serum sample and in one third of milk samples. In the present study, we assured a fastidious collection procedure with no triclosan-containing product coming in contact with the biological sample or the skin. In our study we detected triclosan in nearly 90% of urine samples, which likely reflects the accurate exposure of the woman to this compound.

Parabens are antimicrobial agents found in personal care products including lotions, cosmetics, medicines, and soaps [55] and certain parabens are approved for food use in the USA [37]. Parabens have weak estrogenic activity [56] and can induce proliferation of breast cancer cells [13]. In male rodents, butyl or propyl paraben exposure decreased sperm production, fetal testosterone, and/or epididymal weight [57, 58], and caused epigenetic changes in sperm [59].

Parabens have been measured in 100% of urine samples from pregnant women and children previously [47], which is identical to the data on lactating women in the present study. There

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are currently only two publications that have measured parabens in human milk samples. In the first one, a method development paper, the authors analyzed four samples and detected (LODs = 0.1 ng/mL) methyl paraben in all of them, but propyl paraben in only one [30]. In the second study, methyl, ethyl and propyl parabens were detected in 54 carefully collected breast milk samples [6]. Neither dataset reported detectable concentrations of butyl paraben in milk, identical to our findings. Previous data show methyl paraben and propyl paraben had the highest concentrations in urine with mean values of 43.9 and 9.05 μg/L, respectively [60]. Earlier studies on measures of urine parabens in women and men reported substantial temporal variability [61, 62], and we observed significant correlations within paraben concentration over time, but greater than two collections would give increased clarity to inter-individual variability over time. Interestingly, propyl and methyl paraben had significant correlations with nail product use (Table S1) and methyl paraben concentration was associated with hair product use (Table S2) in our study. Furthermore, the parabens were the class of chemicals with the most correlations with endogenous biomarkers. Consistencies across parabens were seen in correlations with milk IL-6 and milk Ig (sIgA and IgM), and for ethyl, methyl and propyl paraben, these correlations were with urine and serum concentrations of the chemicals. These findings deserve further investigation.

In summary, there are strengths of this study that set it apart from others. These strengths include the first report of collection of multiple matrices (urine, serum and breast milk) obtained at two separate visits, from each individual, and analytical measurements by a highly experienced laboratory using validated methods, allowing for an initial assessment of disposition and variability of exposure over time and matrix. There are also limitations. They include the relatively small “n”, with multiple comparisons, and exploratory nature of the exposures and outcomes (e.g., immunological biomarkers). Because this was a pilot study, the milk data are limited but still provide interesting insights for future investigation. Given those limitations, these data suggest that serum is not an appropriate medium for detection of these non-persistent compounds. Urine, and, to a lesser extent milk, represent better matrices for detecting select phenols and parabens. These data provide support to risk assessors working on these non-persistent organic compounds and insight for future studies that may look at the partitioning of phenols and parabens in breast feeding women. Finally, these data suggest that trace levels of phenols and parabens can be present in breast milk, but should not preclude women from breastfeeding. Detection of the total concentrations of these phenols and parabens in breast milk is not proof that a nursing infant will actually absorb the target chemical. The target chemical in breast milk may be inactive, particularly if it is a metabolite. The chemical may be bound to other compounds in milk and have low bioavailability, or it may not be absorbed from the infant GI tract in an active form (e.g., a form capable of binding to target receptors). Breast milk is proven to be an extremely beneficial start to a child's life.

Supplementary Material

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The research described in this article has been reviewed by the U.S. EPA, the NICHD, the NIEHS, and the CDC and approved for publication. Approval does not signify that the contents necessarily reflect the official views and policies of the agencies or institute, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors.

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Abbreviations

BPA	Bisphenol A
CDC	Centers for Disease Control and Prevention
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin 6
LOD	Limit of Detection
MAMA	Methods Advancement in Milk Analysis
NHANES	National Health and Nutrition Examination Survey
PFAS	Perfluoroalkyl substance
s1	Serum from visit 1
s2	Serum from visit 2
sIgA	Secretory Immunoglobulin A
TNF-α	Tumor Necrosis Factor Alpha
u1	Urine from visit 1
u2	Urine from visit 2
US EPA or EPA	United States Environmental Protection Agency
V1	Visit 1
V2	Visit 2

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Highlights

- Parabens and phenols were detected in urine of 2/3 of lactating moms in MAMA study
- Ethyl, methyl, or propyl paraben, benzophenone-3 and BPA were detected in breast milk
- BPA and benzophenone-3 exposures could not be predicted by single daily collections
- Correlations between chemical and endogenous immune-related biomarkers are reported

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Table 1

Percent of Samples with Detectable Total Concentrations of Phenols and Parabens

Compound	Milk		Urine			Serum		
	LOD (µg/L)	V1+V2 %	LOD (µg/L)	Visit 1 %	Visit 2 %	LOD (µg/L)	Visit 1 %	Visit 2 %
Butyl Paraben	0.1	0	0.2	81	83	0.2	9	17
Ethyl Paraben	0.1	50	1.0	73	53	0.1	38	37
Methyl Paraben	0.1	100	1.0	100	100	0.1	100	97
Propyl Paraben	0.1	100	0.2	97	100	0.2	56	80
2,4-Dichlorophenol	0.16	11	0.17	85	83	NM	NM	NM
2,5-Dichlorophenol	0.42	0	0.12	85	93	NM	NM	NM
Benzophenone-3	0.51	56	0.34	67	80	0.5	17	27
Bisphenol A	0.28	89	0.36	91	80	0.3	3	10
Triclosan	1.5	38	2.27	85	87	1.1	3	3

‘Total’ concentrations refer to the sum of the free plus conjugated species. LOD = limit of detection; NM=not measured (due to very low detects in milk). Milk: n=9 women; 10 samples.

Visits denoted by visit 1 (V1) and visit 2 (V2). Urine V1: n=33, V2: n=30. Serum V1: n=34, V2: n=30.

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Table 2

Urine:Serum (U:S) total concentration ratios for chemicals present in the serum of 50% of participants

Participant	Methyl Paraben		Propyl Paraben	
	Visit 1 U:S Ratio	Visit 2 U:S Ratio	Visit 3 U:S Ratio	Visit 4 U:S Ratio
1	17	62	22	18
2	22	22	54	20
4	231	43	580	355
5	49	130	<1	<1
6	54	128	100	135
7	429	27	178	35
8	473	22	414	14
9	82	92	31	<1
10	2	4	3	6
11	16	5	23	8
13	28	141	97	25
14	24	68	19	96
15	39	28	11	16
17	38	13	98	3
18	131	21	681	29
19	23	40	7	2
20	265	970	652	242
21	49	13	226	33
22	9	3	15	<1
24	40	2	57	2
25	193	203	418	279
26	104	92	304	246
27	217	135	265	129
28	286	50	470	20
29	312	6081	406	85
30	64	25	18	4
31	39	88	117	347

Participant	Methyl Paraben		Propyl Paraben	
	Visit 1 U:S Ratio	Visit 2 U:S Ratio	Visit 3 U:S Ratio	Visit 4 U:S Ratio
32	67	67	3	84
33	24	68	25	116
34	11	4	1	4

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Table 3

Individual Benzophenone-3 concentrations (μg/L) by matrices

Individual	Visit	$Milk_A$ 'Total'	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	1.9	1.9	4.6	1.4	108	1:2:57
1	2	<LOD	<LOD	0.8	<LOD	26.1	N/A
2	2	4.9	4.1	6.9	3.2	2710	1:1:661
3	2	<LOD	<LOD	<LOD	<LOD	61.7	N/A
4	2	<LOD	<LOD	<LOD	<LOD	<LOD	N/A
5	2	10.4	10.2	59.2	47.7	1880	1:6:184
6	2	0.7	<LOD	1.4	<LOD	125	N/A
7	2	<LOD	<LOD	<LOD	<LOD	20.8	N/A
8	2	1	1	2.6	1	738	1:3:738
9	2	<LOD	0.5	1.5	<LOD	138	1:3:276

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; A and B subscripts denote two aliquots from the same sample ($Milk_A$ and $Milk_B$). A portion of $Milk_A$ was used in previous analyses for other environmental chemicals (vonEhrenstein et al 2010). M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values.

N/A=not applicable; limit of detection (LOD).

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Table 4

Individual Bisphenol A concentrations (μg/L) by matrices

Individual	Visit	$Milk_A$ 'Total'	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'"Total' M:S:U Ratio
1	1	1.1	<LOD	<LOD	<LOD	0.6	N/A
1	2	0.6	0.6	0.80	0.8	0.7	1:1:1
2	2	0.7	0.5	<LOD	<LOD	4.1	1:<1:8
3	2	<LOD	<LOD	0.3	<LOD	5.0	N/A
4	2	0.7	<LOD	<LOD	<LOD	1.0	N/A
5	2	<LOD	0.3	<LOD	<LOD	1.1	1:<1:4
6	2	0.7	0.5	<LOD	<LOD	7.5	1:<1:15
7	2	0.3	<LOD	0.3	0.3	1.9	N/A
8	2	0.5	0.4	<LOD	<LOD	31.9	1:<1:80
9	2	0.4	<LOD	0.7	<LOD	3.0	N/A

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with 603 milk samples available; A and B subscripts denote two aliquots from the same sample ($Milk_A$ and $Milk_B$). A portion of $Milk_A$ was used in previous analyses for other environmental chemicals (vonEhrenstein et al 2010). M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values.

N/A=not applicable; limit of detection (LOD).

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Table 5

Individual methyl paraben concentrations (μg/L) by matrices

Individual	Visit	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	2.3	40.1	7.1	75.3	1:17:33
1	2	0.8	8.8	4.4	17.1	1:11:21
2	2	1.1	13.8	0.3	291	1:13:265
3	2	1.3	24.1	4.9	993	1:19:764
4	2	1.1	24.0	2.4	324	1:22:295
5	2	0.5	5.4	1.8	90.3	1:11:181
6	2	NM	17.2	<LOD	430	N/A
7	2	1.4	42.1	11	968	1:30:691
8	2	NM	11.8	3.5	234	N/A
9	2	0.8	18.5	4	270	1:23:338

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values. N/A=not applicable; limit of detection (LOD); NM=not measured

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Table 6

Individual propyl paraben concentrations (μg/L) by matrices

Individual	Visit	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	0.2	<LOD	<LOD	<LOD	N/A
1	2	0.1	<LOD	<LOD	0.5	1:<1:5
2	2	0.3	0.4	<LOD	34.3	1:1:114
3	2	0.6	5.4	1.0	279	1:9:465
4	2	0.3	3.5	0.6	77.5	1:12:258
5	2	0.2	1.5	0.7	13.8	1:8:69
6	2	NM	4.8	2.2	187	N/A
7	2	0.3	2.9	0.6	208	1:10:693
8	2	NM	0.9	0.5	41.8	N/A
9	2	0.4	5.2	1.3	151	1:13:377

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values. N/A=not applicable; limit of detection (LOD); NM=not measured.

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Table 7

Urine paraben and phenol total concentration (μg/L) quintiles and between visit correlations

Compound	Visit (n)	Min	5th	25th	Median	75th	95th	Max	Significance
Butyl paraben	1 (34)	<LOD	<LOD	0.4	1.4	4.6	16.7	65.5	*
	2 (33)	<LOD	<LOD	0.5	2.9	6.8	69.6	110	
Ethyl paraben	1(34)	<LOD	<LOD	<LOD	5.3	19.4	84	135	***
	2(33)	<LOD	<LOD	<LOD	1.0	29	63.6	497	
Methyl paraben	1(34)	<LOD	11.6	75.3	143	266	1610	1710	**
	2(33)	6.7	17.1	45.8	125	248	968	993	
Propyl paraben	1(34)	<LOD	1	13.8	28.6	69	207	243	**
	2(33)	0.4	0.5	7.5	41.3	93.9	208	279	
Triclosan	1(34)	<LOD	<LOD	8.2	18.4	54.7	405	567	**
	2(33)	<LOD	<LOD	6.3	17.9	71.5	289	295	
Benzophenone-3	1(34)	<LOD	<LOD	<LOD	4.7	22.3	1440	3200	No
	2(33)	<LOD	<LOD	0.7	36.7	158	1880	2710	
Bisphenol A	1(34)	<LOD	0.6	0.6	1.1	4.1	16.9	20	No
	2(33)	<LOD	<LOD	0.5	1.0	3	28.3	31.9	
2,4-Dichlorophenol	1(34)	<LOD	<LOD	0.3	0.5	0.9	3.1	3.2	***
	2(33)	<LOD	<LOD	0.2	0.7	1.1	3.8	19.5	
2,5-Dichlorophenol	1(34)	<LOD	<LOD	0.6	1.9	6.2	53.5	65.3	***
	2(33)	<LOD	<LOD	0.6	3.1	6.9	84.1	778	

'Total' concentrations refer to the sum of the free plus conjugated species. Urine paraben/phenol significant between visit correlation, *p*-value

* <0.05,

** <0.01,

*** <0.001.

Concentrations below the limit of detection (LOD) were assigned a value equal to LOD divided by the square root of 2, for all calculations.

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Table 8

Serum paraben total concentration (µg/L) quintiles and between visit correlations

Compound	Visit(n)	Min	5th	25th	Median	75th	95th	Max	%>LOD	Significance
Methyl paraben	1(34)	0.2	0.3	0.8	2.1	5.1	20.9	34	100	**
	2(33)	0.1	0.2	1.7	3.35	4.9	22.2	34.7	97	
Propyl paraben	1(34)	<LOD	<LOD	<LOD	0.3	0.8	8.9	74	56	*
	2(33)	<LOD	<LOD	0.4	0.9	4.9	33.5	80.1	80	

Paraben correlation between visits 1 and 2, *p*-value

* <0.05,

** <0.01.

LOD=limit of detection.

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REVIEW

An overview of UV-absorbing compounds (organic UV filters) in aquatic biota

Pablo Gago-Ferrero · M. Silvia Díaz-Cruz · Damià Barceló

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Abstract The purpose of this article is to summarize biological monitoring information on UV-absorbing compounds, commonly referred as organic UV filters or sunscreen agents, in aquatic ecosystems. To date a limited range of species (macroinvertebrates, fish, and birds), habitats (lakes, rivers, and sea), and compounds (benzophenones and camphors) have been investigated. As a consequence there is not enough data enabling reliable understanding of the global distribution and effect of UV filters on ecosystems. Both liquid chromatography and gas chromatography coupled with mass spectrometry-based methods have been developed and applied to the trace analysis of these pollutants in biota, enabling the required selectivity and sensitivity. As expected, the most lipophilic compounds occur most frequently with concentrations up to 7112 ng g^{-1} lipids in mussels and 3100 ng g^{-1} lipids (homosalate) in fish. High concentrations have also been reported for 4-methylbenzilidenecamphor (up to 1800 ng g^{-1} lipids) and octocrylene (2400 ng g^{-1} lipids). Many fewer studies have evaluated the potential bioaccumulation and biomagnification of these compounds in both fresh and marine water and terrestrial food webs. Estimated biomagnification factors suggest biomagnification in predator–prey pairs, for example bird–fish and fish–invertebrates. Ecotoxicological data and preliminary environmental assessment of the risk of UV filters are also included and discussed.

Keywords UV filters · Biota · Chromatography · Mass spectrometry · Bioaccumulation · Toxicity

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P. Gago-Ferrero · M. S. Díaz-Cruz (✉) · D. Barceló
Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council of Scientific Research (CSIC),
Jordi Girona 18-26,
08034 Barcelona, Spain
e-mail: sdcqam@cid.csic.es

D. Barceló
Catalan Institute for Water Research (ICRA),
Parc Científic i Tecnològic de la Universitat de Girona,
C/ Emili Grahit, 101 Edifici H2O,
17003 Girona, Spain

Introduction

UV filters, including both inorganic and organic sunscreen agents, constitute a group of emerging environmental pollutants, potentially hazardous compounds that have been receiving steadily growing attention over the last decade as society has become aware of the dangerous effects of UV solar radiation. These chemicals can be found not only in cosmetics but also in other personal care products, food packaging, pharmaceuticals, plastics, textiles, and vehicle-maintenance products to prevent photodegradation of polymers and pigments [1, 2].

Incomprehensibly, there are scarce data about, and limited understanding of, the environmental occurrence, fate, distribution and effects of many UV filters and their metabolites and other transformation products, despite their extensive use. According to market studies, sunscreen product sales were higher than half a billion US dollars in 2005, and it is estimated that 10,000 tons of UV filters are produced annually for the global market [3].

It is likely that usage of sunscreen agents is going to increase in the future, because of the recommendations of health authorities on the prevention of skin cancer. One of the main reasons for the scarcity of data was the lack of suitable analytical methods capable of detecting emerging

pollutants at trace levels in, usually, complex environmental matrices. However, as a consequence of increasing concern about the potential effects of sunscreens on ecosystems and humans, in last five years environmental analytical scientists have developed sensitive and selective analytical methods.

Eco-toxicological data on both UV filters and their degradation products is also missing. Despite the small amount of information available about their toxicity, the low environmental concentrations reported so far suggest a low potential risk. However, the long-term risk associated with the pseudo-persistence of these chemicals in the environment is largely unknown.

The purpose of this review is to summarize scarce and scattered information about the profiles of UV filters in aquatic organisms, analytical methods, bioaccumulation/biomagnification, ecotoxicity, and environmental analysis and risk assessment (ERA). Finally, the article identifies current gaps in our knowledge and potential future research needs in ERA.

Physicochemical properties

UV filters are substances with almost null absorption of visible radiation but important light absorption in the UVA (315–400 nm) and UVB (280–315 nm) ranges [4]. Sunscreens can be classified into organic (chemical) absorbers and inorganic (physical) blockers on the basis of their mechanism of action. Organic UV filters absorb UV radiation with excitation to a higher energy state. Excess energy is dissipated by emission of higher wavelengths or relaxation by photochemical processes, for example isomerization and heat release. They include camphors, benzophenones, cinnamates, triazines, among others. Inorganic sunscreens, i.e. titanium dioxide and zinc oxide, protect the skin by reflecting and scattering UV radiation.

The focus of this review is on organic UV filters. A feature common to all of these is the presence of an aromatic moiety with a side-chain with different degrees of unsaturation. Their structures and other physicochemical properties are listed in Table 1. Some, for example 4-methylbenzylidene camphor (4MBC), ethylhexylmethoxy cinnamate (EHMC), and octocrylene (OC), are chiral compounds. Although the enantiomers of these compounds are not expected to have different physicochemical properties, isomers and enantiomers may differ in biological behavior. Commercial formulations contain mainly geometrical (*E*) isomers, although some UV filters (e.g., methoxycinnamates) contain both the (*E*) and the (*Z*) isomers. Because of the high lipophilicity and poor biodegradability of many UV filters (mostly with log K_{ow} 4–8) they end up in sewage sludge during wastewater treatment [5–8], and accumulate in river sediments [9–12] and biota [13–20].

Analytical methodology

Sampling and sample preparation

Sampling procedures for analysis of residues of UV filters in aquatic biota mainly involve traditional fishing, either by native fishers or by electric fishing, for which special permission is often needed. Unlike other matrices, there is the added difficulty of the availability of samples of the desired species, which often depends on external factors which are difficult to control. Moreover, the variability between individuals of the same species (size and living cycle) hinders comparison of results. Most studies have focused on fish, a representative matrix of the aquatic environment assumed to be able to retain and bioaccumulate UV filters because of the lipophilicity of the compounds. The most usual sample analyzed is muscle, probably because of its low lipid content in comparison with other tissues and because it is part of the human diet. Studies have also been conducted on macrozoobenthos, mussels, and birds. Selected tissues are homogenized by blending and often freeze-dried before extraction.

Extraction and clean up

Extraction of UV filters from tissues has been achieved by conventional Soxhlet extraction (which has become less attractive because of the time and solvent consumed) [16, 18], pressurized-liquid extraction (PLE) [14], solid–liquid extraction [13–15, 17, 19], and microwave-assisted extraction (MAE) [20]. These techniques lead to coextraction of a lipid fraction that must be removed before determination of the UV filters. Clean-up of biota sample extracts is usually a two-stage process. The sample extracts can first be subjected to gel-permeation chromatography (GPC), primarily to remove lipids, followed by adsorption chromatography on silica or Florisil columns. Quite often RP-HPLC has also been used for extraction and purification.

In the first work published on UV filter levels in biota [18], benzophenone-3 (BP3), 4MBC, homosalate (HMS), EHMC, ethylhexyl dimethyl PABA (OD-PABA), isopropyl dibenzoyl methane (IDM), and butyl methoxy dibenzoyl methane (BM-DBM) were extracted from fish tissue by Soxhlet extraction. The tissue was first homogenized and dried with sodium sulfate, then extracted with petroleum ether–ethyl acetate 2:1 (*v*/*v*). Lipids and other potential matrix interferences were removed by GPC (Bio Beads SX-3) with cyclohexane–acetone 3:1 (*v*/*v*) as mobile phase. For analysis of IDM and BM-DBM, CH_3I/NaH was added to half of the extract to form their derivatives for further GC–MS analysis. This half of the extract was then purified on a silica column (elution with hexane–ethyl acetate 7:3 (*v*/*v*)). The other half was also loaded on to a silica column and

Table 1 Physicochemical properties of the organic UV filters addressed in this review. In parentheses, the key system adopted herein

Name (INCI nomenclature)[a]	CAS no.	Structure	Molecular weight (g mol^{-1})	Log K_{ow}	Solubility (g L^{-1})[b]
Benzophenones					
Benzophenone-1 (BP1)	131-56-6		214.22	3.15[c]	0.39[c]
Benzophenone-2 (BP2)	131-55-5		246.22	2.78[d]	0.98[c]
Benzophenone-3 (BP3)	131-57-7		228.24	3.79[d]	0.10[c]
Benzophenone-4 (BP4)	4065-45-6		308.31	0.993[c]	11[c]
4,4′-Dihydroxybenzophenone (4DHB)	611-99-4		214.22	2.19[d]	0.6[c]
p-Aminobenzoic acid derivatives					
Ethylhexyldimethyl PABA (OD-PABA)	21245-02-3		277.4	5.412[c]	4.7×10^{-3}[c]
Ethyl-PABA (Et-PABA)	94-09-7		165.19	1.86[d]	1.31[d]
Salicylates					
Homosalate (HMS)	118-56-9		262.35	5.947[c]	0.021[c]
Cinnamates					
Ethylhexyl methoxycinnamate (EHMC)	5466-77-3		290.4	5.8	6.4×10^{-3}[c]
Camphor derivatives					
4-Methylbenzylidene camphor (4MBC)	36861-47-9		254.37	4.95	0.017
3-Benzylidene camphor (3BC)	15087-24-8		240.34	2.84	0.034
Dibenzoylmethane derivatives					
Butylmethoxydibenzoylmethane (BM-DBM)	70356-09-1		310.39	4.191	4.3×10^{-3}[c]
Isopropyldibenzoylmethane (IDM)	63250-25-9		266.33	4.382	0.027
Crylenes					
Octocrylene (OC)	6197-30-4		361.49	6.88	3.6×10^{-4}[d]

[a] INCI (International Nomenclature for Cosmetic Ingredient) established by CTFA and COLIPA

[b] In water at 25 °C

[c] Calculated by use of Advanced Chemistry Development (ACD/Labs) Software V11.02 (1999–2011 ACD/Labs)

[d] Experimental values from database of physicochemical properties; Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm

the same solvent mixture in a different proportion (91:9 *v*/*v*) was used for elution.

A similar method was developed by Meinerling and Daniels [16] for analysis of 4MBC, BP3, EHMC, and OC in the muscle of rainbow trout. In this case Soxhlet extraction with *n*-hexane–acetone 9:1 (*v*/*v*) was followed by GPC (Bio Beads SX-3) with cyclohexane–ethyl acetate 1:1 (*v*/*v*) as eluent. In a further clean-up step, a Florisil column was used to remove polar compounds.

In the procedure followed by Balmer et al. [14] for analysis of 4MBC, BP3, EHMC, and OC, fish samples were homogenized with sodium sulfate and column extracted or PLE extracted with dichloromethane (DCM)–cyclohexane 1:1 (*v*/*v*). The extracts were then cleaned by GPC on a Biobeads S-X3 column with DCM–cyclohexane 35:65 (*v*/*v*) as eluent, followed by silica purification. Buser et al. [15] extracted 4MBC and OC by successive extraction with potassium oxalate (2 mL, 35 %), ethanol (100 mL), diethyl ether (50 mL), and *n*-pentane (70 mL). After extraction, matrix components were removed by GPC and silica purification.

The methods described above are only suitable for extracting UV filters with similar physicochemical properties. A method for simultaneous determination of nine UV filters, from polar to lipophilic, in fish has been reported by Zenker et al. [17]. Mid-polar and lipophilic UV filters were extracted from homogenized tissue by solvent extraction with ethyl acetate–*n*-heptane–water 1:1:1 (*v*/*v*) and further purified by reversed-phase RP-HPLC. The fraction containing mid-polarity UV filters was analyzed by HPLC–MS whereas the fraction containing the lipophilic ones was determined by GC–MS. Polar and medium-polarity UV filters were extracted with a mixture of methanol (MeOH) and acetonitrile (ACN), followed by HPLC–MS analysis. This is the procedure requiring the smallest amount of sample (4 g); good limits of detection are achieved for most compounds. The same method proved to be suitable for analysis of macrozoobenthos and bird samples also.

Bachelot et al. [20] developed a method for determination of EHMC, OC, and OD-PABA in marine mussels. MAE was performed with 25 mL acetone–heptane 1:1 (*v*/*v*). After extraction, the liner was rinsed with the same solvent mixture. The extracts were percolated through anhydrous sodium sulfate. Further purification was performed by RP-HPLC on a RP Spherisorb ODS2 column (4.6 mm×150 mm, 5.0 μm) following a procedure adapted from Zenker et al. [17].

GPC or column purification with silica or Florisil is useful whenever compounds with similar physicochemical properties must be separated from interfering matrix substances, for example lipids, present in the sample. When these methods are used for a mixture of compounds with different physicochemical properties they are less effective. RP-HPLC is a suitable alternative when UV filters with a large range of physicochemical properties must be analyzed.

Instrumental analysis

LC is the technique of choice for the analysis of UV filters in cosmetic products. In contrast, GC is preferred for their environmental analysis. Nevertheless, both techniques have been applied to the analysis of biological samples. The low concentration of the target analytes in biota samples requires high sensitivity and selectivity. Therefore, mass spectrometric (MS) detection is the most suitable technique for determination of these compounds in such complex matrices. Relevant data on analytical methods are summarized in Table 2.

GC–MS

UV filters are, with very few exceptions (e.g., octyl triazone (OT) and BM-DBM), amenable to GC. Matrix effects are not critical for the ionization modes, e.g. electron impact (EI) or chemical ionization (CI), typically used in GC–MS. As a consequence, method detection limits (MDL) are usually quite low [21]. On the other hand, this technique can only be successfully applied to a limited number of non-polar and volatile compounds. For more polar or thermally unstable compounds an additional derivatization step is required; here differences in matrix components may result in quite different derivatization efficiencies which may affect both precision and accuracy of the analysis.

Analysis has always been performed in electron-impact mode (GC–EI-MS). Quantification is achieved by operating in selected ion monitoring mode (SIM). The fragment ions usually selected for the quantification and confirmation of the analytes are listed in Table 2.

BM-DBM, IDM, 4MBC, OD-PABA, HMS, EHMC, and BP3 have been analyzed by GC–MS on a SE-54-CB column (50 m×55 mm, 0.25-μm film), working in SIM mode [18]. Balmer et al. [14] analyzed 4MBC, BP3, EHMC, and OC in fish by GC–EI-MS using two different columns a BGB-5 (30 m×0.25 mm; 0.25 μm) and an SE54 (25 m×0.32 mm; 0.25 μm). In that study $^{13}C_{12}$-PCB 77 was used as surrogate standard. Under the same GC–EI-MS conditions Buser et al. [15] analyzed 4MBC and OC in fish tissue, using $^{15}N_3$-musk xylene as internal standard. Zenker et al. and Fent et al. [17, 19] analyzed nine UV filters with a large range of physicochemical properties (log K_{ow} from 0.9 to 5.7) in fish, macrozoobenthos, and bird tissue. Four out of the nine UV filters investigated, BP3, 3BC, 4MBC, and EHMC (the most lipophilic) were detected by GC–EI-MS on an Optima-5-MS (50 m×0.2 mm; 0.35 μm) column. In this study benzophenone-d_{10} was used as surrogate standard.

Mottaleb et al. [13] analyzed 4MBC and OC with 10 other personal care products in fish tissue by both GC–EI-MS and GC–EI-IT (with an ion trap mass spectrometer). The GC–EI-MS analysis was carried out with a XTI-5 capillary column (30 m×0.25 mm; 0.25 μm) operating in

Table 2 Analytical methodology and occurrence data for the UV filters addressed in this review

Matrix	Specie	Tissue	UV filter	Sample amount	Extraction	Purification	Technique
Fish	Bluegill (*Lepomis macrochirus*)	Muscle	4MBC, OC	1 g	Rotatory extraction with acetone	Silica	GC-EI-MS
	Sonora sucker (*Catostomus insignis*)	Muscle, belly flap and skin	4MBC, OC	1 g	Sonication with acetone	GPC Silica	GC-EI-IT
Fish	White fish (*Coregonus sp.*)	Muscle	4MBC, BP3, EHMC, OC	5 g	ASE extraction: Homogenized with diatomaceous earth 3 cycles DCM/ cyclohexane (1:1, v/v) at room temperature	GPC (EnviroSep-ABC or Biobeads S-X3) Silica	GC-EI-MS
	Roach (*Rutilus rutilus*)			20 g	Homogenized with sodium sulphate		
	Perch (*Perca fluviatilis*)				Column extracted with DCM/cyclohexane (1:1, v/v))		
Fish	Brown trout (*S.Trutta fario*)	Muscle plus adipose tissue under the skin	4MBC, OC	10–25 g	Homogenized in 100 ml water with hand blender Solvent Extraction using potassium oxalate (2 mL, 35 %), ethanol (100 mL), diethyl ether (50 mL) and n-pentane (70 mL)	GPC (EnviroSep-ABC or Biobeads S-X3) Silica	GC-EI-MS
Fish	Rainbow trout (*Oncorhynchus mykiss*)	Muscle	4MBC, BP3, EHMC, OC	10 g	Homogenized with sodium sulphate Soxhlet extracted with n-hexane/acetone (9/1, v/v)	GPC (Biobeads S-X3) Florisil	LC-ESI-MS/MS
Fish	Barb (*Barbus barbus*) and Chub (*Leucisus cephalus*)	Muscle plus adipose tissue under the skin	4MBC, 3BC, BP1, BP2, 4DHB, BP3, BP4, EHMC, Et-PABA	4 g	Solvent extraction using ethyl acetate, n-heptane and HPLC water (1:1:1, v/v/v) or Solvent extraction with MeOH:ACN (1:1, v/v)	RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 μm)	LC-ESI-MS/MS and GC-EI-MS
	Barb (*Barbus barbus*) and Chub (*Leucisus cephalus*)	Muscle plus adipose tissue under the skin	BP4, 4DHB, BP1, BP2, Et-PABA	1 g	Solvent extraction with MeOH/ACN (1:1, v/v)	Syringe filtration	LC-ESI-MS/MS
Fish	Roach (*Rutilus rutilus*)	Muscle, offal, rest and whole fish	IDM, BM-DBM, 4MBC, OD-PABA, HMS, EHMC, BP3		Homogenized with sodium sulphate	GPC (Biobeads S-X3)	GC-EI-MS
	Perch (*Perca fluviatilis*)				Soxhlet extracted with petroleum ether:Etyl acetate (1:1, v/v)		

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Table 2 (continued)

Matrix	Specie	Tissue	UV filter	Sample amount	Extraction	Purification	Technique
macrozoobenthos	Mussels (*Dreissena polymorpha*)	Whole macroinvertebrate	BP1, BP2, BP3, BP4, 4DHB, Et-PABA, EHMC, 4MBC, 3BC	4 g (fraction 1) and 1 g (fraction 2)	Fraction 1: Solvent extraction using ethyl acetate, n-heptane and HPLC water (1:1:1, v.v:v) or Solvent extraction with MeOH: ACN (1:1, v/v)	Fraction 1: RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 μm) Fraction 2: Syringe filtration	LC-ESI-MS/MS and GC-EI-MS
	Gammarus sp				Fraction 2: Solvent extraction with MeOH: ACN (1:1, v/v)		
Fish	Chub (*Leuciscus cephalus*) Brown trout (*Salmo trutta*) Barb (*Barbus barbus*) Eel (*Anguilla anguilla*)	Muscle plus adipose tissue under the skin					
Bird	Cormorants (*Phalacrocorax sp*)	Muscle					
Mussel[a]	*Mytilus edulis* and *Mytilus galloprovincialis*	Soft tissue	EHMC OC OD-PABA	3 g	MAE extraction with acetone:heptane (1:1, v/v)	Filtered (0.2 μm)) through 10 g anhydrous sodium sulphate RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 μm)	GC-EI-IT

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
Fish	XTI-5 capillary column (30 m × 0.25 mm; 0.25 μm)	4MBC: 115, 211, 254 OC: 177, 249, 361	98–99	5.3–17 ng/g	nd	13
	VF-5 MS capillary column (30 m × 0.25 mm; 0.25 μm)	4MBC: 211>169,155 OC: 250>248, 221	57–79	36–120 ng/g	nd	
Fish	BGB-5 (30 m × 0.25 mm; 0.25 μm) or SE54 (25 m × 0.32 mm; 0.25 μm)	4MBC: 254, 239 BP3: 228, 229	93–115	7–380 ng/g lipid	72 (OC)	14
		EHMC: 178, 290				
		OC: 249, 361		3–37 ng/g lipid	44–94 (4MBC), 66–118 (BP3), 64 (EHMC)	
				10–56 ng/g lipid	166 (4MBC), 123 (BP3), 25(OC)	
Fish	BGB-5 (30 m × 0.25 mm; 0.25 μm) or SE54 (25 m × 0.32 mm; 0.25 μm)	4MBC: 254, 239 OC: 249, 361	No data	5–20 ng/g lipid	50–1800 (4MBC) 40–2400 (OC)	15
Fish	PerfectSil 120 ODS-2 (125 mm × 3 mm, 3.5 μm)	4MBC: 255>105 EHMC: 291>161	86–108	2.4 ng/g muscle	214 (4MBC), 193–525 (BP3), 414 (EHMC), 300 (OC)	16

Table 2 (continued)

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
		BP3: 229>151 OC: 362>250				
Fish	Zorbax SB-C18 (150 mm × 3.0 mm, 3.5 µm) and OPTIMA-5-MS (50 m × 0.2 mm; 3.5 µm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PABA: 166>138 EHMC: 178, 290 3BC: 240, 197 4MBC: 254, 237 BP3 : 228, 227	76–99 (BP4 no extracted)	8–205 ng/g lipid	45–700 (EHMC)	17
	Zorbax SB-C18 (150 mm+3.0 mm, 3.5 µm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PAB: 166>138	80–99	1.8–10.7 ng/Kg body weigh		17
Fish	SE-54-CB (50 m × 0.55 mm; 0.25 µm)	IDM: 105, 147, 294 BM-DBM: 135, 161, 338 4MBC: 211, 239, 254 OD-PABA: 148, 165, 277 HMS: 109, 138, 262 EHMC: 161, 178,248 BP3: 165, 225, 242	89–106	50–90 ng/Kg body weigh	Muscle: 810 (4MBC), 310 (EHMC), 298 (BP3), 3100 (HMS); offal: 880 (4MBC), 283 (BP3), 185 (HMS); rest: 990 (4MBC), 50 (EHMC), 40 (BP3), 79 (HMS) whole fish: 930 (4MBC), 120 (EHMC), 150 (BP3), 791 (HMS); Muscle: 161 (4MBC), 41 (EHMC), 230 (BP3), 720 (HMS), 150(IDM); offal: 106 (4MBC), 270 (BP3), 970 (HMS), 210 (BM-DBM); rest: 60 (4MBC), 16 (EHMC), 22 (BP3), 41 (HMS), 9(IDM), 18 (TDM); whole fish: 78 (4MBC), 20 (EHMC), 78 (BP3), 237 (HMS), 29 (IDM), 44 (BM-DBM)	18
macrozoobenthos	Zorbax SB-C18 (150 mm+3.0 mm, 3.5 µm) and OPTIMA-5-MS (50 m × 0.2 mm; 0.35 µm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PABA: 166>138 EHMC: 178, 290	70–105	6–50 ng/g lipid	22–150 (EHMC)	19

Table 2 (continued)

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
		3BC: 240, 197				
		4MBC: 254, 237				
		BP3: 228, 227			91–133 (EHMC)	
Fish					23–79 (EHMC)	
					91–151 (BP3), 11–173 (EHMC)	
					9–337 (EHMC)	
					<LOQ (BP3), 30 (EHMC)	
Bird					16–701 (EHMC)	
Mussel[a]	SGE-BPX5 capillary column (30 m × 0.25 mm, 0.25 μm)	EHMC: 178>121, 132, 161 OC: 248>220, 219, 176	89–116	2 ng/g dw	3–256 ng/g (EHMC) 2–7112 ng/g (OC)	20
		OD-PABA: 165>91, 118, 148				

[a] Concentrations expressed in ng/g (not ng/g lipid), *nd* not detected

SIM mode, and with benzophenone-d_{10} and $^{13}C_6$-*p*-*n*-nonylphenol as surrogates. Analysis performed on samples of bluegill (*Lepomis macrochirus*) muscle, with only 0.4 % lipid content, provided good results. When this method was applied to samples of sonora sucker (*Catostomus insignis*), with an average of 4.9 % lipid content, GC–EI-MS failed to provide acceptable results. Analysis of spiked sample extracts that had not previously been subjected to GPC purification resulted in substantially compromised chromatographic performance. In this case, addition of a GPC step was essential. This additional purification enabled continuous analysis of sonora sucker samples with no compromise in chromatographic performance compared with GC–EI-MS analysis of spiked bluegill tissue. However, dramatic increases in background signal and/or reduction in analyte sensitivity were observed for several analytes. As a consequence some compounds, including 4MBC, were indistinguishable from the background. To increase the sensitivity and reduce the background signal observed, the method was improved by the application of tandem mass spectrometric detection. GC–EI-IT analysis was performed with a VF-5 MS capillary column (30 m×0.25 mm; 0.25 μm) under the same chromatographic conditions and with detection in selected reaction monitoring mode (SRM). The optimized transitions used are listed in Table 2. Nevertheless, this approach only slightly improved detection of 4MBC and a few other compounds, but to much less an extent than expected (this aspect will be further discussed in the section "Limits of detection").

Similarly, GC–EI-IT with an ion-trap mass spectrometer was used by Bachelot et al. [20] for determination of EHMC, OC and OD-PABA. In this work an SGE-BPX5 capillary column (30 m×0.25 mm, 0.25 μm) was used for the separation. Data were acquired in SIM mode for the isotopically labeled internal standard chrysene-d_{12} and in SRM mode for the UV filters.

LC–MS

This technique enables analysis of a wide range of compounds and significantly increases the possibility of analysis of metabolites, which are usually more polar that the parent compounds, without the need for derivatization.

For ionization of the UV filters three different techniques have been used—electrospray ionization (ESI) (which is by far the most commonly used for trace analysis of these pollutants in environmental samples), atmospheric-pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). All the approaches used for LC–MS analysis of UV filters in biota use ESI mode, which achieves efficient ionization of the analytes even though ESI is assumed to be susceptible to signal suppression or signal enhancement by components of sample matrix, as shown

by previous studies on UV filters in complex matrices such as sewage sludge [8]. Isotopically labeled compounds should be used as internal standards to compensate for the matrix effect. Quantification of UV filters in biota samples by external standard calibration is not recommended.

Meinerling and Daniels [16] developed an LC–MS–MS method for analysis of 4MBC, OC, BP3, and EHMC. Chromatographic separation was performed on a PerfectSil 120 ODS-2 (125 mm×3 mm) column with MeOH and water (each containing 0.05 % acetic acid) as mobile phase. Analysis was performed with a triple-quadrupole mass spectrometer fitted with electrospray interface operated in positive mode under SRM conditions (LC–ESI(+)–MS–MS). In this study external standard calibration was used.

Zenker et al. [17] analyzed nine UV filters by LC–ESI–MS–MS. Chromatographic separation was achieved on a Zorbax SB-C18 column (150 mm×3.0 mm, 3.5 μm) with a binary gradient prepared from a mixture of 0.1 % (*v*/*v*) formic acid in HPLC-grade water and 0.1 % (*v*/*v*) formic acid in ACN. Data acquisition was performed in SRM mode. Benzoic-d_5 acid was used as internal standard.

Method performance

Method recovery

High recovery was achieved in all the methods reported, especially when the lipid content of the biological sample analyzed was low.

Nagtegaal et al. [18] achieved recovery from 89 % to 96 % when extracting compounds with a Soxhlet-based procedure. Most studies analyzing lipophilic UV filters used solvent extraction and further clean up by GPC, and usually achieved good recovery (86–115 %). Mottaleb et al. [13] analyzed 4MBC and OC in fish tissue with low lipid content, with recovery of 98 and 99 %. In analysis of samples with higher lipid content lower recovery (57–79 %) was achieved.

Zenker et al. [17] used a mixture of ethyl acetate and *n*-heptane as extraction solvent; this enabled efficient extraction of eight of the nine UV filters with a wide range of polarity. Average recovery ranged from 76 to 99 % (SD from 0.3 to 4 %). However, extraction of the polar filter BP4 was feasible only with ACN and MeOH. Bachelot et al. [20] achieved even higher recovery, from 89 to 116 %, in analysis of lipophilic UV filters in mussel soft tissue extracted by MAE then further RP-HPLC purification.

Limits of detection

Method limits of detection were calculated by extraction of samples of fish spiked with low concentrations of analytes which can be detected in the presence of possible matrix effect. For biota samples MLODs are in the sub-ng g^{-1} range, although authors normalize their results differently, depending on the matrix, and express them in ng g^{-1} lipid or simply ng g^{-1}. The presence of UV filters in blanks is eventually reflected by higher MLODs.

MLODs are highly dependent on the matrix analyzed. Biological matrices may be quite different depending on the organism selected, the species, and the tissue chosen; even so, there is still great variability. As an example, Balmer et al. [14], in analysis of four lipophilic UV filters, obtained three significantly different MLODs ranges, 3–37, 10–56, and 7–380 ng g^{-1} lipid, as a function of the fish species analyzed. To compare MLODs between different methods and for different matrices is complicated. MLODs are usually lower when analysis is performed by GC–MS because matrix effects are usually smaller. Table 2 summarizes the MLODs obtained in each study.

Zenker et al. [17] developed a method for analysis of nine UV filters by GC–EI–MS and LC–ESI–MS–MS. In the first of these the limits of detection ranged between 8 and 36 ng g^{-1} lipid. For UV filters analyzed by LC–MS–MS limits of detection were between 86 and 205 ng g^{-1} lipid. These different MLODs are because of the greater matrix effect in analysis using electrospray interfaces, which can affect analyte ionization.

Mottaleb et al. [13] analyzed bluegill tissue (with low lipid content, 0.4 %) by GC–EI-MS and sonora sucker tissue (high lipid content, 4.9 %) by GC–EI-IT, which is, a priori, a more sensitive and selective technique. MLODs for most compounds in the GC–EI-IT study were higher than those obtained for bluegill tissue by use of GC–EI-MS (especially for 4MBC, 23-fold higher). Differences in detectability between the two approaches cannot be explained solely by differences in extraction efficiency. The authors suggest inefficient fragmentation of precursor ions in the ion trap. Because all MS–MS precursor ions are produced by EI, a relative hard ionization technique, it is likely that generation of product ions via collision-induced dissociation may eventually be problematic (precursor ions may be sufficiently stable, which makes further fragmentation unlikely). The MLODs afforded by GC–EI-IT exceeded the corresponding environmentally relevant concentration range identified in the literature. OC and 4MBC were not detected in any sample in the study.

Critical aspects in the analysis of UV filters

Background contamination is a common problem in the determination of UV filters at environmentally relevant levels. Therefore, several measures must be taken to prevent this problem. All glassware should be carefully cleaned. A typical procedure consists in washing and heating at 380 °C, then sequentially rinsing with different high-purity organic

solvents. Furthermore, gloves should be worn during sample preparation; separate solvents and only previously unopened packages of solvents, chemicals and other supplies, and glassware should be used.

In addition, a set of at least two operational blanks should be processed together with each batch of samples. Because many of the compounds analyzed undergo photodegradation, stock standard solutions should always be covered with aluminium foil and stored in the dark.

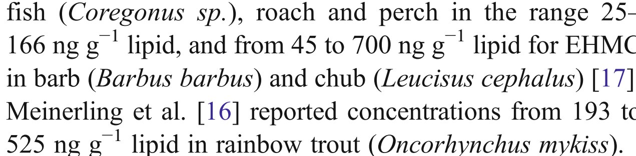
The presence of matrix effects has the potential to lead to compromised results, so precautions should be taken to minimize this effect. Measurements are further hindered by the lack of appropriate commercially available reference standards. Currently, only BP3-d_5 and 4MBC-d_4 are commercially available; none of the studies reported herein used these. Other isotopically labeled compounds, namely benzophenone-d_{10}, $^{15}N_3$-musk xylene, and $^{13}C_6$-*p*-*n*-nonylphenol were used for quantification. Development and further marketing of a wider range of isotopically labeled compounds for use as surrogate and internal standards is an important need for analysis of sunscreen agents in complex matrices.

Biota levels

UV filters enter the aquatic environment directly, as a result of swimming and other recreational activities or indirectly via wastewater treatment plants (WWTPs). Thus, it is expected that, because of the lipophilic properties of these compounds, they can reach and accumulate in tissues of aquatic organisms. Several fish species have been investigated together with, although to a lesser extent, mollusks, crustaceans, and birds. Table 2 summarizes UV filter occurrence data in biota.

A study carried out by Nagtegaal et al. [18] provided the first data on the occurrence of UV filters in fish. Perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from Maarfelder Lake (Eifel, Germany) were analyzed and the presence of seven UV filters with total concentrations of approximately 2000 ng g^{-1} lipid and 500 ng g^{-1} lipid, respectively, in whole fish were reported. This early study, besides providing the first data on bioaccumulation of UV filters in fish, reported prevalence profiles in different fish tissues (muscle, offal, the rest, and whole fish). Results indicated that 4MBC and HMS can be selectively accumulated depending on the species; perch accumulates 4MBC in muscle and HMS in offal. In contrast, roach had higher levels of 4MBC in offal and of HMS in muscle. In contrast, EHMC and BP3 had similar bioaccumulation profiles in both species.

In Switzerland similar concentrations were found in lake fish. 4MBC, BP3, EHMC, and OC were detected in white



fish (*Coregonus sp.*), roach and perch in the range 25–166 ng g^{-1} lipid, and from 45 to 700 ng g^{-1} lipid for EHMC in barb (*Barbus barbus*) and chub (*Leucisus cephalus*) [17]. Meinerling et al. [16] reported concentrations from 193 to 525 ng g^{-1} lipid in rainbow trout (*Oncorhynchus mykiss*).

Higher levels for 4MBC and OC (up to 1800 and 2400 ng g^{-1} lipid, respectively) were found in fish (brown trout, *S. Trutta fario*) from rivers downstream from a WWTP discharge [15], revealing its impact on the ecosystem. Buser et al. [28] demonstrated that the enantiomeric composition of 4MBC in perch was much different from that observed in the surrounding lake water. In contrast, the enantiomeric composition of 4MBC in roach was similar to that of the lake water indicating that bioconcentration or metabolism of a compound can be quite different from one species to another. The factors responsible for the differences in the enantiomeric composition of 4MBC found in fish remain unclear.

Concerning organisms other than fish, Fent et al. detected EHMC in crustaceans (*Dammarus sp.*) and mollusks (*Dreissena polymorpha*) at concentrations between 22 and 150 ng g^{-1} lipid. EHMC was also detected in different fish species at concentrations up to 337 ng g^{-1} lipid and in cormorants (*Phalacrocorax sp.*), at levels above 700 ng g^{-1}. BP3 was also detected, but at lower concentrations, in brown trout (*Salmo trutta*) and eel (*Anguilla anguilla*). These results suggest that biomagnification occurs through the food web; this aspect will be further discussed in the section "Bioaccumulation and biomagnification") [19].

Bachelot et al. proved the presence of UV filters residues in marine mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) of the Mediterranean French coast [20]. In that study all mussel samples contained EHMC, at concentrations up to 256 ng g^{-1} dw. In addition, 55 % of the samples contained OC also. In particular one of these samples had the highest concentration reported so far for an UV filter in biota, 7,112 ng g^{-1} dw.

Besides WWTP discharges, another important factor affecting accumulation in aquatic biota samples is the season of the year, with summer being the period when peak concentrations of sunscreens are observed. As an example, the concentrations reported for OC in fish in September were found to be three to five-fold higher than those corresponding to May, before the swimming period [15]. Similarly, a study carried out by Fent et al. showed that concentrations in freshwater mussels collected in a lake with recreational activity were higher after the summer [19].

UV filter 4MBC and other benzotriazole compounds have been analyzed in a great variety of aquatic species, for example tidal flat organisms, fish, coastal birds, and even hammerhead sharks of the Japan coast, by Nakata et al. [22]. 4MBC was not detected in any of the samples analyzed, even though this common sunscreen agent has been detected in samples in different studies in Europe. The authors of that

study suggested these results were evidence of the different production and usage profiles of UV filters among countries.

As shown in Table 2, of all the sunscreen agents investigated, EHMC is the most frequently found, but at lower concentrations that those usually observed for UV filters of similar log K_{ow}, e.g. HMS (log K_{ow} 6.16) and OC (log K_{ow} 4.95). On the other hand, other sunscreens with log K_{ow} in the same range, OD-PABA (log K_{ow} 6.15) and 3BC (log K_{ow} 4.49) were never detected, suggesting fast and effective metabolism.

Bioaccumulation and biomagnification

The net accumulation of a chemical by an organism from its combined exposure to water, food, and sediment is known as bioaccumulation. Species higher in the food web can be exposed to all the chemicals that lower-order species accumulate (biomagnification). Bioaccumulation models are useful tools for understanding the biomagnification of such substances [23]. The extent of biomagnification for a given contaminant is expressed in terms of the biomagnification factor (BMF), the ratio of the concentration of the contaminant in a predator to its concentration in prey. A BMF above 1 indicates biomagnification of the contaminant. However, the most conclusive evidence of the accumulation of chemicals by organisms and biomagnification in food webs is expressed by the trophic magnification factor (TMF) [23, 24], which is used to estimate the accumulation of contaminants through trophic levels of different food webs, for instance between fresh water and marine water systems.

Because of the lipophilic character (low water solubility) of most UV filters they may be expected to accumulate in biota and in humans and be stored rather faster than they are metabolized or excreted. A recent study by León-González et al. [25] revealed that the metabolites of OD-PABA were detected in human urine after 8 days of single cutaneous application of a cream, indicating slow metabolism. Despite this, only one field-based study has examined biomagnification through food webs [19]. EHMC bioconcentration was proven in macrozoobenthos, fish from different trophic levels, and cormorants (*Phalacrocorax sp.*) a species of fish-eating birds, in Switzerland. For example, estimated BCF for fish were far above unity, in the range 167–1500. Biomagnification was also assessed, but higher concentrations of EHMC in cormorants than in fish were not statistically significant. Despite this, estimated BMF, occasionally slightly higher than unity, were indicative of possible positive trophic magnification of EHMC. Nevertheless, a possible explanation of this finding may be found in feeding ecology; because birds and mammals are homeotherms their rates of feeding higher than for fish and invertebrates (poikilotherms) [26]. Higher BCFs were, however, reported by Balmer et al. [14] for 4-MBC in fish from Lake Zürich. In particular, BCFs for roach ranged from 2,300 to 9,700.

Biological and chemical factors, for example size, sex, age, life cycle, and metabolic activity, are important when assessing bioaccumulation and trophic transfer of contaminants in food webs. The lipid and protein content of tissue, which vary according to season, reproduction, migration, feeding rate, diet composition, growth rate, and food chain length, should be taken into account in analysis of UV filters [27].

The phenomenon of chirality exists in all biological systems. All proteins, enzymes, and carbohydrates are chiral. Because biological processes may be stereoselective (favor one structural form over the other), enantiomers of chiral compounds, both parent compounds and transformation products (metabolites, products of photodegradation, and disinfection byproducts) must be investigated separately to obtain reliable information about their bioavailability and biomagnification through the food web. It must be taken into account that metabolites of achiral compounds can also be chiral. Buser et al. [28] investigated the enantiomeric pattern of 4-MBC in lake fish. This compound exists as two stereoisomers, (*Z*) and (*E*), as a consequence of an exocyclic C=C bond. Both are chiral compounds with two stereocenters provided by the camphor moiety of their chemical structure. First, HPLC separation of the (*E*) and (*Z*) isomers of 4MBC was performed on a Nucleosil 120–5 C-18 reversed-phase column (250 mm×4 mm) with ACN–water 60:40 (*v*/*v*) as mobile phase. Enantiomeric separation of 4MBC was subsequently achieved by GC on a laboratory-prepared column containing 2,6-bis-(*tert*-butyldimethylsilyl)-γ-cyclodextrin in 70 % PS086. Detection was performed in full scan and SIM (GC–MS) or SRM (GC–EI-IT) modes.

Although the study did not reach a sound conclusion, the isomer ratio observed seemed to indicate preferential accumulation of the (*E*) isomer. With regard to potential enantioselective fish metabolism of 4-MBC, results were completely different for roach and perch; roach accumulates both enantiomers whereas perch is unable to metabolize the [1*R*,4*S*-(*E*)-4-MBC] stereoisomer only.

Ecotoxicological considerations

The ecotoxicological implications of exposure of biota to sunscreens have been addressed quite frequently. Despite this, the sparse studies available are conclusive [29]. Fish have long been regarded as tracers for assessing the extent of lipophilic contamination of aquatic ecosystems. As a consequence most ecotoxicological studies on the effect of UV filters have been conducted on different fish species for "in vivo" testing. Several sunscreens have been found to

have estrogenic hormonal activity, in particular, two which are extensively used 4-MBC and OMC; moreover, some have been shown to have multiple endocrine-disrupting activity, for example androgenicity or antiestrogenicity. Adverse effects on fecundity and reproduction have also been observed for BP3, benzophenone 2 (BP2), and 3 benzylidene camphor (3BC). Analysis of vitellogenin (VTG) in rainbow trout and Japanese medaka (*Oricias latipes*) after aqueous exposure to BP3 indicated, however, that high effective concentrations in the range 620–749 μg g^{-1} were needed to induce these effects [30]. These concentrations are greater than the reported level (19 ng L^{-1}) of BP3 in estrogenic fractions of effluent wastewater extracts [31]. When a similar test was conducted on 4MBC and EHMC [32], 4MBC had high estrogenic potency.

In male fathead minnows (*Pimephales promelas*), concentrations of BP2 of 1.2 mg L^{-1} and higher were found to induce VTG, modify gonad histology, and emasculate secondary sex characteristics; oocyte production in female fish was also inhibited significantly [33, 34]. In the same fish species, 3BC had high estrogenic potency, inducing VTG at doses of 435 μg L^{-1} and higher [35]; at concentrations near predicted environmental levels (3 μg L^{-1}) significant VTG induction, loss of secondary sexual characteristics, and inhibition of spermatogenesis were observed for male specimens. Loss of gender-specific mating behavior and cessation of milt production were, moreover, observed at 74 μg L^{-1} and 285 μg L^{-1}, respectively [34, 35]. In females, at the same concentrations, first oogenesis was inhibited, followed by cessation of egg production and release of mature oocytes.

Effects on algae have also been assessed. In a test on inhibition of reproduction of the green alga *Scenedesmus vacuolatus* OC and 4MBC had no activity whereas EHMC, BP3, and OD-PABA at predicted no-effects concentrations (PNEC; calculated from EC_{50} with a safety factor of 1000) in the range 0.17–0.76 μg L^{-1} were found to significantly inhibit algal growth [36]. In a similar study, exposure to BP3, 3BC, 4MBC, and EHMC resulted in inhibition of the growth of *Desmodesmus suspicatus*, with 72-h EC10 values in the range 0.21–0.56 mg L^{-1} [37]. Potential endocrine and toxic effects of BM-DBM, EHMC, and OC on infaunal and epibenthic invertebrates and zebra fish (*Danio rerio*) embryos was recently investigated by the same authors [38]. Test results revealed toxicity of these sunscreens was low (Table 3), with effect concentrations far higher than those reported in the environment. In particular, EHMC was found to have a toxic reproductive effect on *Potamopyrgus antipodarum* and on *Melanoides tuberculata* with no-observed effect concentrations (NOECs) of 0.08 mg kg^{-1} and 2 mg kg^{-1}, respectively; it also had sub-lethal effects on zebra fish with even higher NOEC, 100 mg kg^{-1}. Toxic effects on reproduction of the crustaceans *Daphnia magna* [19] and *Acartia tonsa* [39] have also been reported for BP1, BP3, and BP4.

In an early study by Donavaro and Corinaldesi [40] the affect of sunscreen agents on marine ecosystems was also demonstrated. These authors observed that UV filters increased virus production via prophage induction in marine bacterioplankton. Most recently the same authors also provided scientific evidence of the effect of these chemicals on hard corals and their symbiotic algae in the Celebes Sea, the Caribbean Sea, the Andaman Sea, and the Red Sea, by inducing rapid and complete coral bleaching even at extremely low concentrations [41]. Coral bleaching, the loss of intracellular endosymbionts (symbiodinium, also known as zooxanthellae, which impart specific colors, depending on the particular clade) as a result of expulsion under stress situations, has a negative effect on biodiversity and functioning of the great reef ecosystems of tropical seas.

Despite studies are mainly focused on solely one chemical, an organism is exposed not to single environmental chemicals but to mixtures of many. According to the literature, assessment of the effects of mixtures of chemicals has attracted increasing attention in recent decades. With regard to UV filters additive effects of mixtures are largely unknown, and are an important concern in environmental studies, because these substances are usually formulated as complex mixtures to achieve the high sun protection factors (SPF) currently demanded. Taking into account the large number of sunscreens used, and other endocrine-disrupting compounds, hormonally-active UV filters, may act additively. Indeed, cumulative interactions have been shown in a few studies [34, 42–44]; in particular, these papers report significant synergistic effects of combinations of UV filters mixed at NOECs of the individual compounds.

The lack of environmental occurrence and ecotoxicological data for most UV filters and matrices hinders reliable and integral environmental risk assessment for comprehensive protection of the environment. Moreover, for complete risk assessment metabolites produced by the organisms should also be considered and their prevalence and their ecotoxicity be. Nevertheless, preliminary ERA has recently been conducted by Fent et al. [34, 45] using the limited data available. According to calculated hazard quotients, a potential risk to aquatic ecosystems may be posed by 3BC, 4MBC, and EHMC.

Conclusions and future research perspectives

The biomonitoring data so far available have been provided by a limited number of research groups. Different sample characteristics, for example location, species, season, tissue, target analytes, and the analytical methods used, hinders comparison among studies, which in turn also hinders

Table 3 Summary of toxicity data available in the literature for UV filters

UV filter	Fish LOEC (mg L^{-1})	Aquatic invertebrates EC$_{50}$ (mg L^{-1})	Algae EC$_{50}$ (mg L^{-1})	Ref.
BP1	–	0.49–1.5[a] (AT)	–	[38]
	0.005[f]	–	–	[34]
BP2	0.001[f]	–	–	[34]
BP3	–	1.67 (DM)	–	[37]
	–	1.9 (DM)	–	[45]
	0.75[d]	–	–	[30]
	0.62[e]	–	–	[30]
	–	–	0.36 (EV)	[36]
BP4	–	50 (DM)	–	[45]
EHMC	–	0.57 (DM)	–	[37]
	–	–	0.19 (EV)	[36]
	–	0.29 (DM)	–	[45]
	9.87[e]	–	–	[32]
3BC	–	3.61 (DM)	–	[37]
	–	26.9–5.95[b,c] (LV)	–	[48]
	0.003[f]	–	–	[34]
4MBC	–	0.80 (DM)	–	[37]
	–	4.6[b] (PA)	–	[46]
	–	0.56 (DM)	–	[45]
	9.9[e]	–	–	[32]
IAMC	–	–	0.76 (EV)	[36]
OD-PABA	–	–	0.17 (EV)	[36]
Et-PABA	0.004[f]	–	–	[34]

[a] Under different experimental conditions
[b] μmol L^{-1}
[c] Different endpoints
[d] Vitellogenin induction in rainbow trout
[e] Vitellogenin induction in medaka
[f] Vitellogenin induction in fathead minnow
DM, *Daphnia magna*; AT, *Acartia tonsa*; EV, *Scenedesmus vacuolatus*; LV, *Lumbriculus variegatus*; PA, *Potamopyrgus antipodarum*; LOEC, lowest observed effect concentration

reliable assessment of the fate and effects of UV-absorbing compounds in aquatic ecosystems. Expression of the data in different units (body weight, lipids-basis) should be standardized, enabling comparison among similar studies. Researchers have used quite different analytical approaches, although most are based on solvent extraction, GPC clean-up, and analysis by GC–MS. The sensitivity and selectivity afforded are suitable for environmental trace analysis and recovery is also very good, with values close to 100 % when the lipid content of tissues is not high. Fish and, specifically, muscle has been the preferred sample for analysis, despite results which seem to indicate that individual compounds are selectively accumulated in muscle or offal, depending on the species.

Besides smart experimental design, for accurate comparison of contamination levels among different tissues, species, and locations, reporting of range and median values may be quite useful. Moreover, reporting of sunscreen concentrations in a specific organ, instead of a whole burden estimate approach, may lead to overestimation of BMFs and TMFs. Stable isotope analysis should be conducted to properly identify the trophic position of every species for further biomagnification considerations.

Ecotoxicological assessment of exposure to UV filters is a challenging task. Despite being scattered and limited, current ecotoxicological data indicate that the potential risk posed by these widely used chemicals requires further investigation. The estrogenic activity of most of the commonly used sunscreen agents is in the range of other well-characterized estrogenic chemicals. Findings indicate that some UV filters have endocrine-disrupting activity in, and/or affect reproduction of several species, although at concentrations higher than those measured in the environment. However, a propensity for rapid accumulation and temporal effects at environmentally relevant concentrations and the potential of mixture effects indicate the need for further studies to evaluate the effects of long-term exposure of biota to UV filters. Moreover, ecotoxicological studies indicate the need to consider multicomponent mixtures when evaluating hormonal activity of UV filters in aquatic organisms, for use in risk assessment to consider potential synergistic and/or antagonistic effects.

There should be greater emphasis on measurement of ecological, biological, and physicochemical variables in field studies conducted to analyze contaminants in species, and, more importantly, when comparing data between studies. In particular, the chemical characteristics of the UV filters should be considered on the basis of their different structural forms, including isomers and enantiomers. Preliminary findings indicate that stereochemical aspects of sunscreens should be included in future environmental and

toxicological research for proper characterization of their global prevalence in the environment and for elucidation of the processes of biodegradation of theses contaminants, because these properties may result in different potential for accumulation, as observed for other contaminants (perfluorinated compounds, halogenated flame retardants, etc).

Marine and, particularly, terrestrial environments should be more widely investigated in future studies to better understand the fate and effects of UV filters. The effect of climate change should also be considered, because seasonal changes in ice formation, temperature, drought–flood episodes, or food webs might have important effects on bioaccumulation and/or biomagnification of contaminants. For UV filters this is especially relevant, because higher levels of sunlight radiation, a consequence of increased depletion of the ozone layer, would increase the use of such chemicals.

Combining monitoring field studies with work on species biology, behavioral science, and exposure biomarkers, among others, would significantly contribute to improving our knowledge about these compounds.

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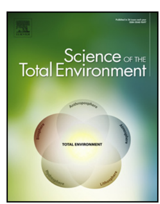


UV filters and benzotriazoles in urban aquatic ecosystems: The footprint of daily use products

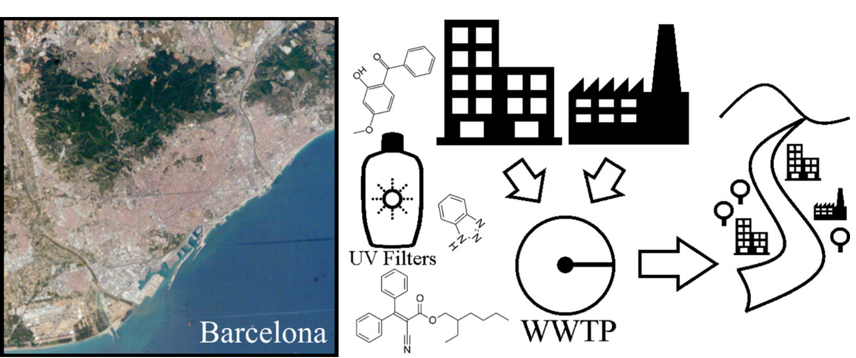
Daniel Molins-Delgado [a], João Távora [a], M. Silvia Díaz-Cruz [a,*], Damià Barceló [a,b]

[a] Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council of Scientific Research (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[b] Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona. C/ Emili Grahit, 101 Edifici H2O, 17003 Girona, Spain

HIGHLIGHTS

- UV-filters (UV-Fs) and benzotriazoles were analysed in two rivers from a densely-developed area.
- UV-Fs and benzotriazoles were detected in all water, wastewater, and solid matrixes.
- The removal rates of UV-Fs and benzotriazoles in the WWTPs were highly variable.
- WWTPs effluents are pointed out as the major source of contamination.
- Estimated hazard quotients for wastewaters showed that benzotriazoles are a risk.

GRAPHICAL ABSTRACT



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ABSTRACT

The increased use of beauty and other daily use products, in particular those containing UV filters (UV-Fs) and benzotriazoles, results in their introduction in significant amounts into the aquatic environment. In this study, we aim to assess the occurrence and impact of UV-Fs and benzotriazoles in aquatic ecosystems in the metropolitan area of Barcelona, Spain. River water samples from the Llobregat and Besòs Rivers were analysed together with sediment, suspended particulate matter, and wastewater samples from 6 wastewater treatment plants (WWTPs) along their basins. The analysis of 6 UV-Fs and 2 benzotriazoles in water samples was performed using an automatized on-line solid phase extraction coupled to liquid chromatography tandem mass spectrometry (SPE-HPLC-MS/MS) method. The analysis of the target compounds in the suspended solids and in the sediments was performed by HPLC-MS/MS. The analysis of the water samples showed the ubiquitous presence of UV-Fs. Benzotriazole (BZT; partition coefficient octanol-water Log $K_{ow} = 1.23$) and methylbenzotriazole (MeBZT; Log $K_{ow} = 1.89$) had the highest levels in both river water and wastewater. Removal rates in the selected WWTPs were highly variable (4–100%). Concentrations of lipophilic UV-Fs (Log K_{ow} 4.95–7.53) in suspended particulate matter from wastewaters were high (up to 1,031,868.2 ng g^{-1}dry weight (dw)), whereas in sediment the concentrations were always below 300 ng g^{-1} dw. The risk assessment expressed in terms of hazard quotients (HQs) revealed that most UV-Fs were not likely to produce adverse ecotoxicological effects against the living organisms assayed in river waters and influent wastewaters at the concentrations observed. However, HQs above 1 were obtained for BZT and MeBZT in effluent wastewaters discharged to the river.

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* Corresponding author.
E-mail address: sdcqam@cid.csic.es (M. Silvia Díaz-Cruz).

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1. Introduction

Excessive UV radiation exposure constitutes a well-known origin of pathological conditions such as skin burn, erythema, premature skin aging, photodermatoses, immunosuppression and skin cancer (Gasparro, 2000). As a consequence, nowadays UV radiation is considered a public health treat all over the world. UV filters (UV-Fs) are a wide group of chemicals that provide effective protection from UV radiation. These compounds are used in many consumers' goods, such as personal care products, and are also valuable additives in industrial products, where they prevent the deterioration of physical properties of materials, including decolouring and cracking (Fent et al., 2010). Benzotriazoles constitute a family of high production additives present in daily use products such as dishwasher detergents, corrosion inhibitors, and defrosting products as anti-icing agent. Benzotriazole is also a precursor of several derivatives which have UV light stabilizing capacities such as UV320 and UV327. These benzotriazole derivatives can be found for instance in textiles and plastic materials, including food and beverage containers, to preserve the integrity of materials and foodstuff (Molins-Delgado et al., 2015; Zhang et al., 2011).

These compounds are released into the environment through urban and industrial sewage waters or *via* recreational aquatic activities for UV-Fs or de-icing operations in aeronautics for benzotriazoles, making them ubiquitous. Previous studies have reported their occurrence in surface waters at concentrations in the range 1–862 ng l^{-1} (Ekpeghere et al., 2016; Gago-Ferrero et al., 2013a; Kasprzyk-Hordern et al., 2008; Tsui et al., 2014), in groundwater (Jurado et al., 2014; Serra-Roig et al., 2016) up to 55 ng l^{-1} and 1980 ng l^{-1}respectively, and in the range 1–8900 ng l^{-1} in wastewaters (Ekpeghere et al., 2016; Langford et al., 2015; Liu et al., 2012). UV-Fs have been found to adsorb onto sediments (Gago-Ferrero et al., 2011a; Langford et al., 2015) at concentrations in the range 3.2–870 ng g^{-1} dry weight (dw), and in sewage sludge (Gago-Ferrero et al., 2011b) between 260 and 970 ng g^{-1} dw. These studies also pointed out that the removal of these chemicals in wastewater treatment plants (WWTPs) was pretty variable, depending on the physical-chemical properties of the substances and the water treatment applied. The most lipophilic UV-Fs tend to bioaccumulate, for instance in fish (Fent et al., 2010; Gago-Ferrero et al., 2013c), mussels (Cunha et al., 2015), and dolphins (Gago-Ferrero et al., 2013b). The occurrence of these compounds has already been reported in human breast milk (Zafra-gómez et al., 2015), semen (León et al., 2010), and human placental tissue (Valle-Sistac et al., 2016; Vela-Soria et al., 2014).

There are little data currently available on the adverse effects the continuous exposure to these compounds may cause to living organisms. *In vitro* and *in vivo* assays have shown that these xenobiotics may interfere with the normal development in aquatic and terrestrial organisms (Klammer et al., 2007; Weisbrod et al., 2007). Some UV-Fs have been found to have similar estrogenic effects as those displayed by the natural sex hormone 17-β-estradiol (Fent et al., 2014; Klann et al., 2005; Kunz and Fent, 2006). Besides, the properties and environmental behaviour of the formed metabolites and other transformation products are yet mostly unknown. Data on the toxicity of benzotriazoles is scarce; they have been found to be toxic to aquatic organisms and slightly toxic to humans (Breedveld et al., 2002; Hem et al., 2003).

The aim of this study was to assess the occurrence of UV-Fs and benzotriazoles, in surface waters from the Besòs River and Llobregat River, in the wastewaters from 6 WWTPs along the two basins, in the suspended particulate matter and in the sediments from the urban aquatic environment of the large populated city of Barcelona, Spain. The compounds' removal rates in the WWTPs as well as the risk posed to selected aquatic species were also investigated.

2. Experimental

2.1. Reagents and materials

Highest purity (>99%) benzophenone-1 (BP1), benzophenone-3 (BP3), 4-hydroxybenzophenone (4HB), 4,4′-dihidroxybenzophenone (4DHB), ethylhexyldimethyl PABA (OD-PABA), octocrylene (OC), ethylhexyl methoxycinnamate (EHMC), ethyl PABA (EtPABA) and benzotriazole (BZT), were obtained from Sigma-Aldrich (Steinheim, Germany); 4-methylbenzylidene camphor (4MBC, 99% purity) was supplied by Dr. Ehrenstorfer (Augsburg, Germany) and 5-methyl benzotriazole (MeBZT, >99% purity) by TCI (Zwijndrecht, Belgium). Isotopically labelled standards 2-hydroxy-4-methoxy-benzophenone-2′,3′,4′,5′,6′-d_5 (BP3-d_5) and 3-(4-methylbenzylidene-d_4)camphor (99% purity) from CDN isotopes (Quebec, Canada) were used as internal standards.

Solvents HPLC-grade water, methanol (MeOH), acetone, ethanol (EtOH) and acetonitrile (ACN) were from J.T. Backer (Deventer, The Netherlands). Formic acid and alumina (aluminium oxide, 99%) were provided by Merck. The nitrogen (99% purity) used to evaporate samples was supplied by Air Liquide (Barcelona, Spain). The PURADISC syringe filters and the glass fibre filters (1 μm) and nylon membranes (0.45 μm) provided by Whatman International Ltd. (Maidstone, UK).

Standards and isotopically labelled internal stock standard solutions were prepared in MeOH at 200 mg l^{-1} and stored in the dark at −20 °C. From these solutions, a mixture standard solution containing all the UV filters and benzotriazoles was prepared in MeOH at 20 mg l^{-1}. Working solutions were freshly prepared by appropriate dilution of the mixed stock standards solution in MeOH. Table A1 in the Supporting Information summarises the name, abbreviation, and the Chemical Abstract Service (CAS) number of the selected UV-Fs.

2.2. Sampling area

In this study we performed a comprehensive analysis of environmental water and sediment samples collected in two Mediterranean river basins and in the WWTPs located in their basins close to Barcelona. Numerous cosmetic companies, as well as other kind of industries (pharmaceutical, pigments, textiles, plastic production…) are located along the basin of the studied rivers. The considered urban ecosystems constitute a good example of highly transformed environments, as a consequence of decades of human pressure, industrial contamination, resource depletion and natural ecosystem transformations.

Barcelona is the second biggest city in Spain, and one of the most industrialised cities in the Mediterranean coast (Palanques and Diaz, 1994). The city is enclosed by the mouths of the rivers Llobregat and Besòs and by the Serra de Collserola. Having 102.2 km^2, the metropolis has a permanent population of >1.6 million inhabitants (inhs) and a population density of 15,867 inhs. km^{-2} (Ajuntament de Barcelona, 2013). Besides, the surrounding Metropolitan Area of Barcelona (AMB), composed by 36 municipalities under direct influence of the city, has a population of 4.8 million inhs. with a density of 1926 inhs. km^{-2} (Institut d'Estadística de Catalunya, 2013).

The Llobregat River is a fast-flowing Mediterranean river that alternates from dry to torrential flows, with a mean flow rate of 19 m^3 s^{-1} (Agència Catalana de l'Aigua, 2013). Spreading along 5000 km^2, the river basin is composed of several tributary rivers, Cardener and Anoia Rivers being the most important ones, as shown in Fig. 1 (Rodríguez, 2001). The Llobregat's mouth constitutes the southwestern frontier of the city of Barcelona. Contamination is of great concern as this river is the main drinking water source to many municipalities of the AMB and part of Barcelona (Piedrafita, 1995).

Bounding Barcelona to the North, the Besòs River's mouth is at the end of a basin that occupies 1038 km^2. The main river has only 17.7 km, but its basin integrates several tributary drainage rivers, *i.e*. the Congost, Mogent, Tenes, Riera de Caldes and Ripoll Rivers which

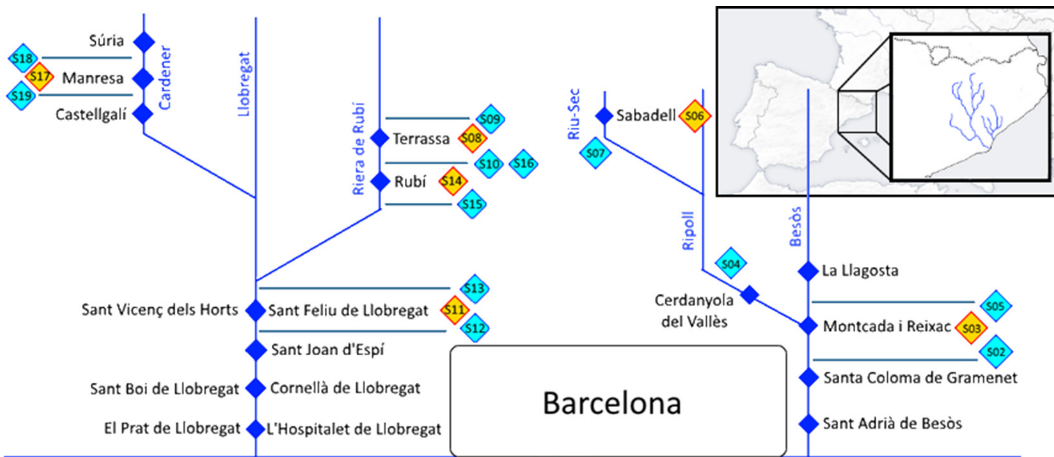


Fig. 1. Llobregat and Besòs basins and the sampling points along them (without scale).

make important contributions to its water flow (see Fig. 1). The Besòs basin has the typical characteristics of Mediterranean rivers similar to those of the Llobregat River.

The water quality of these two basins highly depends on weather conditions. River flows vary greatly from summer to winter. The decreased flows during summer lead to the aggravation of the water poor quality and increased concentrations of contaminants, due to the diminished or inexistent dilution factor that causes the increase the proportion of effluent waters in the total river flow (González et al., 2012). The water contamination of the mentioned basins is driven, to some extent, by a vast domestic and industrial WWTP network (pharmaceutical, pigments, textiles, plastic production…). However, the incomplete removal of many contaminants from the wastewaters results in their introduction into the water bodies receiving the effluent streams, jeopardizing the drinking water resources.

Currently, the Llobregat River has 70 WWTPs distributed along its basin, treating a total wastewater volume of 261,473 m^3 d^{-1} (9.5 million m^3 y^{-1}). In the Besòs River basin 25 WWTPs treated 203,815 m^3 d^{-1}wastewater (74.5 million m^3 y^{-1}) (Agència Catalana de l'Aigua, 2012). Six WWTPs from both basins were studied in this work. Table A2 lists the operational information about the selected WWTPs.

2.3. Sample collection

Samples were collected along the Llobregat River basin (Llobregat River and tributaries Cardener and Riera de Rubí) and the Besòs River basin (Besòs River and tributaries Ripoll and Riu-Sec) in February 2014. River water samples were collected upstream and downstream from WWTPs within a distance of 0.5 km. River water was gathered in pre-cleaned amber glass bottles, and sediments were collected in aluminium containers. Time-averaged (24 h) composite influent and effluent wastewater samples were also collected at the facilities in pre-cleaned amber glass bottles. All the water samples were shipped to the laboratory under cool conditions in portable refrigerators. Upon reception, 150 ml of each water sample was vacuum filtered through a 1 μm glass fibre filter, followed by a 0.45 μm nylon membrane filter to separate the suspended particulate matter from the water phase. Both glass fibre and membrane filters were dry weighted before and after the filtration, in order to quantify the suspended solids content in the corresponding water samples. For the analysis of the water samples, an aliquot of 25 ml of the filtered water was stored. Sediment samples were lyophilised and sieved. The particles <63 μm diameter were separated and preserved. All samples were then stored at −20 °C in the dark until analysis. Extended information on the samples is provided in Table A3.

2.4. Sample extraction and purification

2.4.1. Water samples

The analysis of the water samples was carried out by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry (SPE-HPLC-MS/MS) (Gago-Ferrero et al., 2013a, 2013b, 2013c) in a Symbiosis™ Pico (SP104.002, Spark, Holland) liquid chromatograph coupled to a hybrid quadrupole-linear ion trap 4000 QTRAP mass spectrometer (Applied Biosystems-Sciex; Foster City, CA, USA) using an electrospray ionization source (ESI). The internal standards were added into the water samples prior to analysis. The on-line SPE extraction of all samples, standard solutions and blanks was performed by loading 5 ml at 1 ml min^{-1} through a PLRP-s cartridge previously conditioned with 1 ml of MeOH, 1 ml of ACN, and 1 ml of water (flow rate of 5 ml min^{-1}). After loading, the cartridges were washed with water at 0.5 ml min^{-1} to complete the transfer of the analytes and remove potential matrix interferences. Further, the trapped compounds were eluted from the SPE cartridge to the HPLC-column by the mobile phase.

2.4.2. Suspended particulate matter and sediment samples

Solid samples were analysed following a method based on HPLC-MS/MS (Gago-Ferrero et al., 2011a, 2011b). Briefly, all samples were automatically extracted and purified by pressured liquid extraction (PLE) with an ASE-350 Accelerated Solvent Extractor from Dionex Corporation (Thermo Fisher Scientific, Sunnyvale, CA, USA). The sample (1 g of lyophilised sediment or the previously weighted filter containing the suspended particulate matter) was added to 1 g of activated neutral alumina, which had been put over a cellulose filter placed at the bottom of the PLE cell. Finally, the cell was filled until the top with alumina to avoid any empty volume. The PLE extraction was achieved by using MeOH and the mixture MeOH:H_2O (1:1 v/v) as extracting solvents. The obtained extracts were diluted to 25 ml with MeOH. A 2 ml aliquot of the diluted extract was then passed twice through 0.45 μm syringe filters to a LC-vial and evaporated to dryness under nitrogen in a TurboVap LV evaporator (Zymark, Hopkin, MA, USA). Finally, the residues were reconstituted with the internal standards solution to a final volume of 1 ml.

2.5. HPLC-MS/MS analysis

2.5.1. River water and wastewater samples

Chromatographic separation was achieved on a LiChorCART ® Purospher® STAR® RP-18 ec (125 mm × 2.0 mm, 5 μm) from Merck, preceded by a guard column LiChorCART® 4–4 Purospher® STAR® RP-18 ec (5 μm). The gradient of water and ACN, both with 0.1% formic acid, was as follows: it started with 5% of ACN, increasing until 75% in

the minute 7, and reaching 100% organic solvent during the next 3 min. Pure organic conditions were kept during 5 min., and then back to the initial conditions. The flow rate was set to 0.3 ml min^{-1}, and the run time was 23 min. The analytes were monitored in positive mode using ESI ionization under selected reaction monitoring (SRM) mode. Two transitions per compound were recorded for improved sensitivity and selectivity. Quantification of analytes was performed using the most abundant transition through the isotopic dilution approach and calibration curves were built by a series of freshly prepared matrix-matched standard solutions.

2.5.2. Suspended particulate matter and sediment samples

In the analysis of the PLE-extracts from sediments and particulate matter, the chromatographic separation was achieved using the same equipment, mobile phase and flow rate as described in the Section 2.5.1. However, in this case, the elution gradient was performed as follows: it started with 5% of ACN, increasing to 80% during the following 2 min, and reaching 100% during the next 9 min. Pure organic solvent was kept for 2 min. Prior returning to the initial conditions. The total run time was 16 min. The detection and quantification of the analytes were carried out by MS/MS under the same conditions that the ones described in the previous Section 2.5.1.

3. Results and discussion

3.1. Method performance

3.1.1. River water and wastewater samples

The goodness of the method was tested for linearity range, sensitivity, accuracy, repeatability, reproducibility and matrix effects. Table A4a summarises the instrumental quality parameters of the method. The instrumental limits of detection (ILODs), defined as the lowest analyte concentration with a signal to noise ratio (S/N) of 3, ranged from 0.2 to 14 pg and the instrumental limits of quantification (ILOQs), defined as the concentration with S/N ratio of 10 (U.S. Environmental Protection Agency, 2010), ranged from 0.7 to 47 pg. The intra-day and inter-day instrumental precision, both expressed as relative standard deviations (RSD), ranged between 3 and 5% and between 5 and 7%, respectively.

The method limits of detection (LODs) and method limits of quantification (LOQs) were calculated in the three different water matrices: river water, influent and effluent wastewater. Table 1a summarises the quality parameters of the method. High sensitivity was achieved in all matrices. In river waters LODs values ranged between 0.1 and 3.5 ng l^{-1}, whereas LOQs ranged between 0.5 and 13.3 ng l^{-1}. In influents the LODs range were 0.3–10 ng l^{-1} and the LOQs were comprised between 1.1 and 33.3 ng l^{-1}. For effluents LODs varied from 0.3 to 4 ng l^{-1} whereas LOQs ranged from 0.8 to 13.3 ng l^{-1}.

3.1.2. Suspended particulate matter and sediment samples

Table A4b compiles the instrumental quality parameters of the method applied in the analysis of the selected UV filters and UV blockers in suspended matter and sediment. ILODs ranged from 0.2 to 14 pg, whereas ILOQs were between 0.7 and 47.0 pg. Intra-day RSD varied in the range 3–5% and inter-day RSD values between 5 and 10%. Table 1b summarises the recovery rates (Gago-Ferrero et al., 2011a) and the LODs and LOQs for each compound. LODs were in the range 1.4–1.9 ng g^{-1} dw, whereas LOQs ranged from 4.6 to 6.5 ng g^{-1} dw.

Table 1
Method quality parameters for the different water matrixes (a) and sediments and suspended solids (b).

a)

Compounds	LOD (ng l^{-1})	LOQ (ng l^{-1})	LOD (ng l^{-1})	LOQ (ng l^{-1})	LOD (ng l^{-1})	LOQ (ng l^{-1})
	River water		*Effluent water*		*Influent water*	
BP1	1	3.3	2.5	8.3	8	26.7
BP3	0.7	2.3	1.5	5	5	16.7
4HB	1.1	3.7	1.5	5	8	26.7
4DHB	1.8	6	3.5	11.7	9	30
4MBC	3.5	11.7	4	13.3	10	33.3
EtPABA	1.5	5	2.5	8.3	5	16.7
BZT	0.3	0.9	1.1	3.7	0.3	1.1
MeBZT	0.1	0.5	0.3	0.8	1.1	3.7

b)

Compounds	Rec. (%) ± RSD	LOD (ng g^{-1})	LOQ (ng g^{-1})
	Suspended particulate matter and sediment		
BP1	30 ± 16	1.9	6.5
BP3	70 ± 10	1.9	6.4
4HB	95 ± 8	1.9	6.4
4DHB	96 ± 9	1.9	6.5
4MBC	102 ± 6	1.6	6.5
OC	70 ± 7	1.9	6.5
EHMC	90 ± 6	1.9	6.5
OD-PABA	85 ± 4	1.6	5.3
EtPABA	95	1.9	6.5
BZT	113	1.4	4.8
MeBZT	112	1.4	4.6

Rec. - recovery; RSD - relative standard deviation; LOD - method limit of detection; LOQ - method limit of quantification.

3.2. Occurrence of UV-Fs in the Barcelona's urban aquatic ecosystem

3.2.1. River water and wastewater samples

Fig. 2 represents the mean concentrations of the selected compounds in both river basins. The list of UV-F and benzotriazoles concentrations in river water samples are shown in Table A5. Benzophenone-type UV-Fs were ubiquitous, with concentrations ranging from 4.4 to 52.2 ng l^{-1}. The highest value corresponded to BP3 (also known as oxybenzone) (52.2 ng l^{-1}, S2) followed by its main metabolite BP1 (51.8 ng l^{-1}, S7). 4HB, another known BP3 metabolite was measured in three samples (10.1–12.1 ng l^{-1}), whereas another BP3 metabolite, 4DHB, was determined only in S15 at 9.2 ng l^{-1}. 4MBC was detected in all but one sample (S19), with concentrations ranging from 13.1 to 34.3 ng l^{-1}. EtPABA was observed at concentrations ranging from 5.5 to 111.9 ng l^{-1}. Considerably higher levels of benzotriazole were measured. BZT concentrations were between 23.7 and 8529.8 ng l^{-1}, and MeBZT was measured at concentrations ranging from 66.9 to 7181.4 ng l^{-1}. In both cases, maximum levels were observed in S15 and minimum in S10. Both sites correspond to Riera de Rubí, one of the tributaries of Llobregat River. S15 is in the lower part of the basin, in a highly developed industrial area, whereas S10 is located in the higher part of the river course, with low industrial impact.

The concentrations observed are similar to those reported in the literature. In a study carried out in the Llobregat River (October 2011) (Gago-Ferrero et al., 2013a), BP3 was observed at concentrations in the range 4.8–37.5 ng l^{-1}, BP1 was in the range 4.2–754 ng l^{-1}, and 4MBC was found at 112.6 ng l^{-1}. The differences between these values and the ones in the present study may be explained by the seasonal variations that these sunscreen residues register across the year, with increased levels in summer time (Balmer et al., 2005). In surface waters from Korea (Ekpeghere et al., 2016), concentrations of BP3 and 4MBC were always below 150 ng l^{-1}, and 50 ng l^{-1}, respectively. In river water from the United Kingdom (Kasprzyk-Hordern et al., 2008), BP1 was found at concentrations between below 0.3 and 13 ng l^{-1}, and BP3 was always below 15 ng l^{-1}. Concentrations in river water samples from Spain for BP1 and BP3 were in the range 3–221 ng l^{-1} (Gracia-Lor et al., 2012). In river waters from Italy, concentrations of BP3 were somewhat lower (3–112 ng l^{-1}) (Magi et al., 2012), and similar values were reported in river waters from Slovenia (47–114 ng l^{-1}) (Pedrouzo et al., 2010). The different levels observed among the studies are most likely due to the different regulations (ban and quantity allowed in

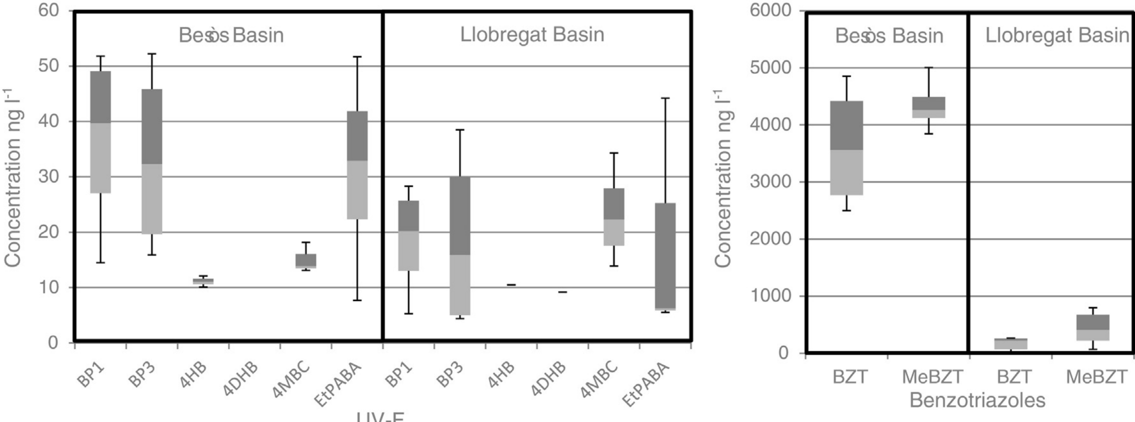


Fig. 2. Box plot of the concentrations (ng l^{-1}) for each compound in each river basin. The second and third quartile and the median concentration are represented. The whiskers represent values outside the middle 50%.

each formulation) regarding commercialised products in each country as well as to seasonal patterns of use (Balmer et al., 2005).

3.2.2. Wastewater samples

Influent and effluent wastewater samples were collected in 6 WWTPs (see Fig. 1): 4 in the Llobregat River basin (1 in the Llobregat River, 1 in the tributary Cardener and 2 in the tributary Riera de Rubí); and 2 in the Besòs River basin (1 at the Besòs River and 1 at the tributary Riu-Sec).

Table A5 lists the concentrations of the selected sunscreens in wastewater and Fig. 3 shows the mean values of influents and effluents. Similarly to river water, benzophenones were ubiquitous in wastewater, with concentrations between 7.7 and 687.9 ng l^{-1} for influent, and from 4.3 to 387.1 ng l^{-1} for effluent. BP3 concentrations ranged from 75.5 to 515.7 ng l^{-1} (influent) and from 9.4 to 217.8 ng l^{-1} (effluent). Benzophenone transformation products were detected in almost all samples; BP1 was measured between 94.6 and 687.9 ng l^{-1} (influent) and from 4.3 to 211.2 ng l^{-1} (effluent). 4HB was quantifiable only in S6 and S17 influent samples, at concentrations of 10.7 and 24.8 ng l^{-1}, whereas 4DHB was quantifiable in only one influent and one effluent sample (S14), at 7.7 and 10.9 ng l^{-1}, respectively. This suggests that 4DHB could be originated by the microorganisms during the biologic + nitrogen removal treatment applied at the Rubí WWTP. This facility is the only one among the studied that applies this treatment (see Table A2).

4MBC was detected in almost all samples. Influent concentrations were quite similar, between 29.3 and 30.1 ng l^{-1}, whereas effluent concentrations were in the range 22.0–58.1 ng l^{-1}. EtPABA was ubiquitous with concentrations ranging from 12.3 to 224.5 ng l^{-1} in influent, and from 22.4 to 129.1 ng l^{-1} in effluent. Likewise, high concentrations were observed for benzotriazoles. BZT influent concentrations were found between 1495.2 and 9481.6 ng l^{-1} (S3 and S6), whereas effluent concentrations were between 1084.1 and 16,933.1 ng l^{-1} (S11 and S14 respectively). MeBZT influent concentrations were in the range 3728.5–6366.2 ng l^{-1} (S17 and S6), whereas effluent concentrations were in the range 2173.6–8913.3 ng l^{-1} (S8 and S14). Both compounds are extensively used in different daily-use products as anticorrosive and antifreeze agents (Molins-Delgado et al., 2015).

The high concentrations of UV-Fs and benzotriazoles observed may be due to the intensive industrial activities carried out along both river basins. Taking into consideration the total amount of UV-Fs in each WWTP studied, we observe that in all cases but two (S11 and S14), the amount of UV-Fs is reduced to some extent. However, in S11 and S14 there is an increase in the amount of benzotriazoles present in the effluent stream. This may be due to the low biodegradability of some of these compounds (Jjemba, 2006; Ternes et al., 2004) or their formation as by-products of more complex benzotriazole compounds (Molins-Delgado et al., 2015).

UV-F concentrations in wastewater reported in the literature are similar to those observed in this study. In wastewaters from Korea,

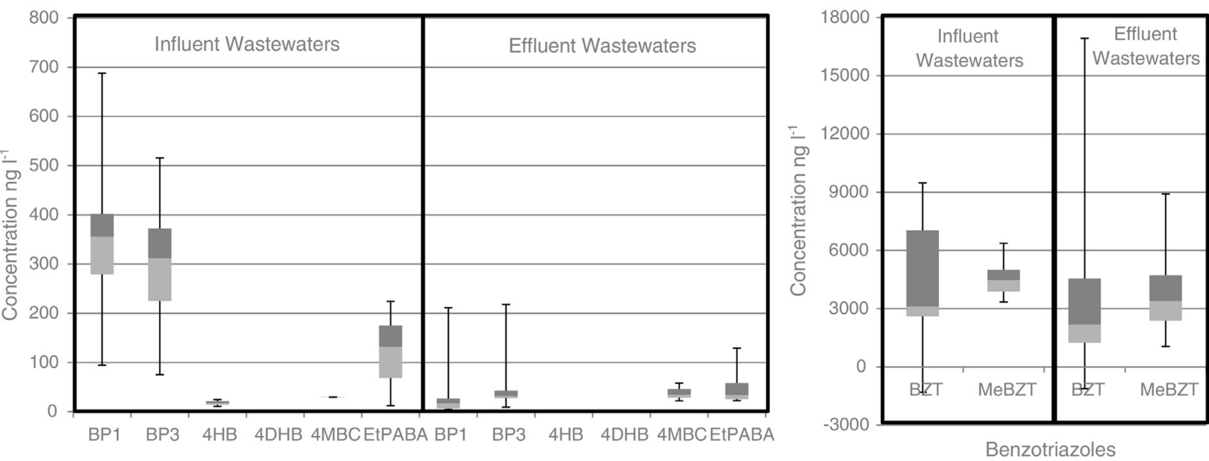


Fig. 3. Box plot of the concentrations (ng l^{-1}) of UV-Fs and benzotriazoles in influent and effluent wastewaters. The second and third quartile, and the median concentration are represented. The whiskers represent values outside the middle 50%.

concentrations of BP3 were between 500 and 6000 ng l^{-1}, and 4MBC concentrations were in the range 500–2000 ng l^{-1} (Ekpeghere et al., 2016). In wastewaters from Norway, BP3 concentrations were in the range 10–1915 ng l^{-1} (Langford et al., 2015) and in Australia reported influent and effluent wastewaters were 2086 and 153 ng l^{-1} (BP3), 90 ng l^{-1} (4MBC), 5706 and 2439 ng l^{-1} (BZT), and 6759 and 610 ng l^{-1} (MeBZT), respectively (Liu et al., 2012). In WWTPs across Spain (Bueno et al., 2012), BP3 effluent wastewaters levels were in the range 48–140 ng l^{-1}. In Catalonia (NE of Spain) (Molins-Delgado et al., 2015), concentrations of benzotriazoles in both influent and effluent wastewaters were high, with influent values in the range 76.5–47,142.9 ng l^{-1} for both benzotriazoles, and between 26.7 and 10,541.2 ng l^{-1} in effluent wastewaters, with the highest concentrations being located in the most urbanised and industrialised areas. These data suggest that, despite the differences in use of these compounds and the regulations in each country, the removal of most of these compounds is not effective. Sunscreens (especially those with low partition coefficient octanol-water; Log K_{ow}) are released back to the environment, likely making WWTPs the major sunscreens entrance pathway, regardless of the geographical area surveyed.

In order to gain more insight about the elimination and transformation of UV-Fs and benzotriazoles the removal rates (RE%) for the selected WWTP were calculated using the following equations:

$$Linf = V \times Cinf \quad (1)$$

$$Leff = V \times Ceff \quad (2)$$

$$RE\% = 100 \times \left(1 - \frac{Leff}{Linf}\right) \quad (3)$$

where, *Linf* and *Leff* are the influent and effluent daily loads (gd^{-1}), *V* is the average water flow rate per day (ld^{-1}) and *Cinf* and *Ceff* are the compound concentration (ng l^{-1}) observed in the studied samples, for influent and effluent wastewater, respectively (Molins-Delgado et al., 2015). Values calculated were highly variable among the WWTPs (see Table 2). RE% ranges observed were for BP1 (48–99%), BP3 (58–91%), EtPABA (71–81%); BZT (27–41%) and MeBZT (4–63%). 4HB was 100% removed in Sabadell (S6) and Manresa (S17) WWTPs, as well as 4MBC in Manresa WWTP. The differences in removal rates can be explained by the different physical-chemical properties of every single compound and by the treatments and operational conditions applied (Díaz-Cruz and Barceló, 2009; Kasprzyk-Hordern et al., 2008; Tsui et al., 2014). Four facilities had negative removal values, which may be attributed to the formation of biotransformation products during the treatment process, raising though, the concentration of certain compounds in effluent wastewaters. This behaviour was also observed in a previous study (Molins-Delgado et al., 2015), where the increased levels of BZT and MeBZT in effluent wastewaters were attributed to the transformation products formed from other more complex molecules or to the breakdown of conjugated derivatives of the benzotriazoles already present in the influent.

Table 2
Removal rates (%) of the studied wastewater treatment plants (WWTPs).

Sample	WWTP	Compound							
		BP1	BP3	4HB	4DHB	4MBC	EtPABA	BZT	MeBZT
S3	Montcada i Reixac	48	58	n.a.	n.a.	n.a.	81	41	4
S6	Sabadell	97	91	100	n.a.	n.a.	78	70	63
S8	Terrassa	92	88	n.a.	n.a.	−93	71	60	50
S11	St. Feliu de Llobregat	96	83	n.a.	n.a.	n.a.	−91	27	−30
S14	Rubí	95	88	n.a.	−42	n.a.	−142	−104	−73
S17	Manresa	99	91	100	n.a.	100	80	−56	36

n.a. – not applicable

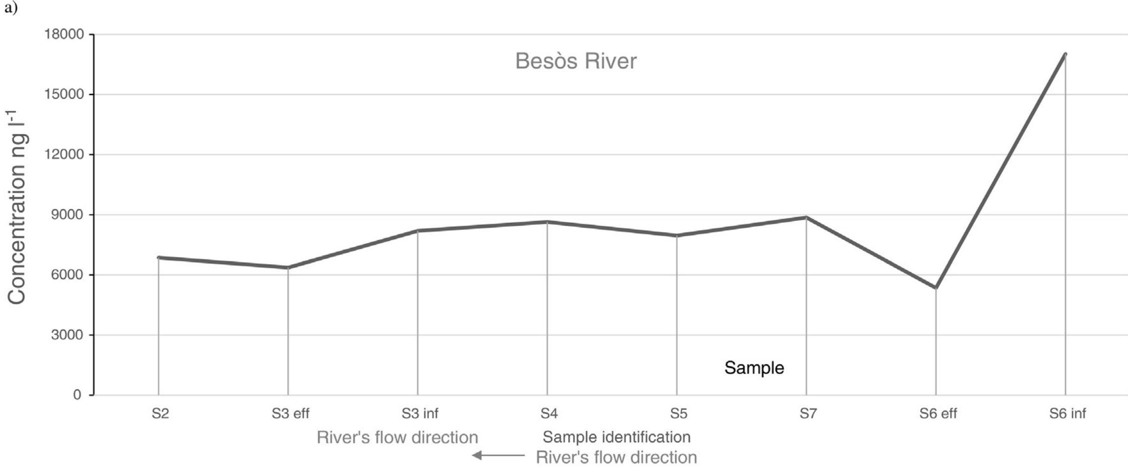
In order to have an estimation of the total loads of these chemicals in the aquatic environment, cumulative concentrations were calculated. Fig. 4 shows the total concentrations determined in the rivers and the wastewater streams. The Besòs River had a maximum total UV-Fs concentration of around 9000 ng l^{-1}, at the sampling site furthest from the sea (S7), whereas S2, the point closest to the sea, has a total load of nearly 7000 ng l^{-1}, likely due to the increase in the water flow of the river. Llobregat River has the highest and the lowest total loads of UV-Fs and benzotriazoles. The highest concentration of UV-Fs corresponds to S15, with almost 16,000 ng l^{-1}. This sampling point is located just after the WWTP (S14) with the highest influent and effluent total loads of sunscreens, pointing out that effluent discharge may be the principal contributor of sunscreens pollution in that area. Nevertheless, generally, water concentrations of sunscreens are at their lowest just after the WWTPs. On the other hand, at S18, in the Cardener tributary before the city of Manresa, the lowest total sunscreens loads were observed (around 100 ng l^{-1}). These low levels observed there may be due to the lower population density and the lower industrialization at the upper course of the Cardener River. In comparison, the mean concentrations at Besòs River are higher than those reported in Llobregat River. Taking into account the urbanisation and industrialisation of both basins, the difference may be explained in terms of dilution. Mean water flow in the Besòs River is 4.3 $m^3 s^{-1}$ and in the Llobregat River is almost 5-folds higher, 19 $m^3 s^{-1}$ (Agència Catalana de l'Aigua, 2013).

The UV-Fs and benzotriazoles daily mass loads in each WWTP were calculated using the formula described in Molins-Delgado et al. (2015) and are listed in Table A6. Sunscreens released into the rivers are between 0.07 and 0.25 mg d^{-1} Inh^{-1}. Most WWTPs reduce their mass load between 10 and 100-folds. However, WWTP S17 is only able to remove 0.01 mg d^{-1} Inh^{-1} during the process. This may be due to the poor removal of benzotriazoles, the largest contributors to the daily mass load. This fact is in line with the increased concentrations of UV-Fs detected in river waters downstream WWTPs. For instance, the Rubí WWTP (S14) had an estimated daily mass load of 0.77 mg d^{-1} Inh^{-1}; sample S16, upstream the WWTP had a total concentration of sunscreens of 3232.3 ng l^{-1}, whereas sample S15 (downstream) contained 15,901.1 ng l^{-1}, mostly attributable to benzotriazoles.

3.2.3. Suspended particulate matter samples

As summarised in Table 3a, the mass of particulate matter recovered from 150 ml of water samples was in the range 2.6–340.3 mg l^{-1} (S2 and S17I, respectively). The suspended solids from river waters were so low that the sunscreens were not detected or below the LOQ. Despite the overall low masses, influent particulate matter samples had quantifiable levels for most lipophilic sunscreens. The particulate matter from the wastewater streams showed extremely high load of BP3, EHMC, OC, BZT and MeBZT, with the highest values, as expected, corresponding to the influent wastewaters. The frequency of detection of the selected compounds was close to 100%, with the exception of EtPABA (Log K_{ow} = 1.86), which was detected only in 4 samples. This compound, as well as other *p*-aminobenzoic acid (PABA) derivatives, constitute and exception because, despite having low Log K_{ow}, aromatic amines, as they are, are readily reactive towards humus or organic matter and thus they can be retained in sediments and suspended particulate matter. BP3 (Log K_{ow} = 3.79) was found in one influent sample at 892.3 ng g^{-1} dw (S3), EHMC between 1.3 and 296.2 μg g^{-1} dw and 4MBC was measured at 9.0 ng g^{-1} dw (Log K_{ow} 5.80 and 4.95 respectively). OC, the most lipophilic compound in this study (Log K_{ow} = 7.53), was measured at concentrations between 1.9 and 1,031,868.2 ng g^{-1} dw, with the highest concentration by far achieved in this study corresponding to the influent at Rubí (S14). Rubí WWTP serves a population of nearly 80,000 inhs, and is located in a large industrialised area. These compounds have Log K_{ow} in the range 3.7–7.5; therefore, they show a strong tendency to be adsorbed onto the suspended matter. Regarding the benzotriazoles, the most polar

a)



b)

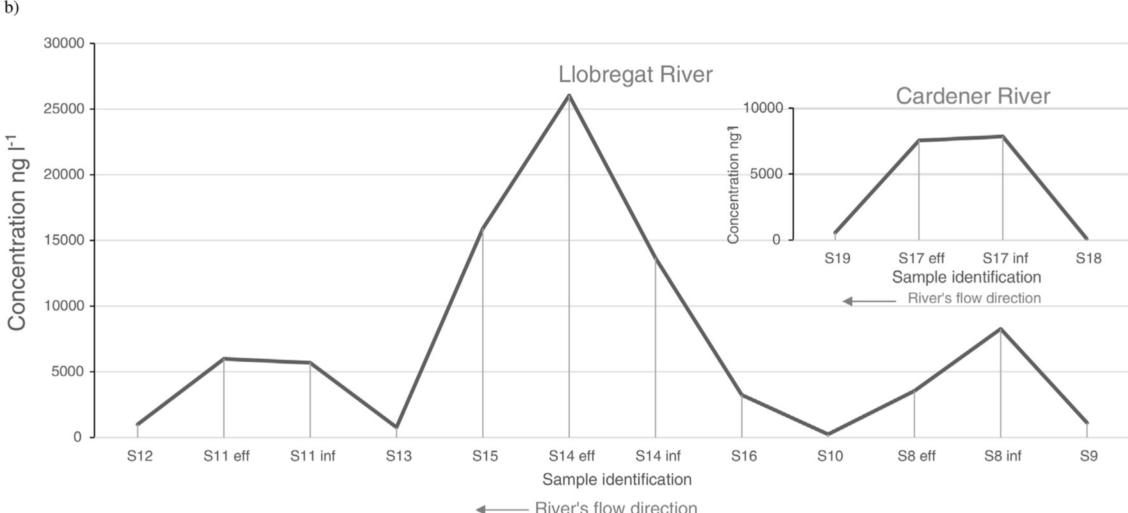


Fig. 4. Profile of the total concentration of UV-Fs and benzotriazoles in the Besòs River basin (a) and the Llobregat River basin (b).

compounds in this study (Log K_{ow} = 1.23–1.89), BZT was found in the range 1.4–21,832.1 ng g^{-1} dw and MeBZT in the range 1.4–16,026.1 ng g^{-1} dw. These high levels most likely have their origin in the large industrial activity of the area. Benzotriazoles, according to their low Log K_{ow}, were not expected to adsorb onto the suspended particulate matter. However, their high pKa (8.37–8.66) makes them be in the protonated form in most aqueous media, and this form is expected to bind strongly to solids' components since BZT and MeBZT, as a corrosion inhibitor agents, react with metallic and organic components. The rest of compounds were always below LOQ.

In Manresa WWTP suspended solid sample (S17) high levels of EHMC and OC were observed. As stated before, their high Log K_{ow} values indicate a trend to be adsorbed onto the suspended matter. The high levels of EHMC reported should be highlighted because this UV filter was included in the "Watch list" for European Union-wide monitoring in 2015. It is an endocrine disruptor and bioaccumulative compound (Tavazzi et al., 2016).

For effluent wastewaters, with low suspended matter content, only the sample S14 had OC at a quantifiable concentration (3977 ng g^{-1}), the rest were not detected or below the LOQ. WWTPs apply a filtration step during the wastewaters treatment process, which can explain the reduced amount of suspended particulate matter.

3.2.4. Sediment samples

Table 3b lists the UV-F and benzotriazole concentrations observed in the sediments. One sediment sample was collected together with each river water sample; however, from all them only six consisting in a fine particulate could be analysed. OC was measured in four out of the six samples, at levels between <LOQ and 226.6 ng g^{-1}, EHMC was detected in one sample and MeBZT in two, both below LOQ. In contrast, BZT was ubiquitous (<LOQ-230.9 ng g^{-1}). The adsorption onto the sediments observed for the polar benzotriazoles would be the result of their reaction with metals or organic matter present, as already observed in the suspended solids.

These results are in line with the concentrations reported in the literature. In sediments from Norway, concentrations of EHMC in the range 8.5–16.4 ng g^{-1} dw and of OC from 7 to 82.1 ng g^{-1} dw were determined (Langford et al., 2015). In sediments from the Ebro River basin in Spain, small amounts of BP3 (<LOQ-27 ng g^{-1} dw), EHMC (<LOQ-18 ng g^{-1} dw), OD-PABA (0.8–5.2 ng g^{-1}), and OC (7.3–540 ng g^{-1} dw) were determined (Gago-Ferrero et al., 2011a, 2011b). Additionally, low concentrations of BZT and MeBZT (below 33.4 ng g^{-1} dw and 165 ng g^{-1} dw, respectively) were found in sediments from China and USA (Zhang et al., 2011).

To the light of the results it appears that most lipophilic UV filters and benzotriazoles would be mainly distributed between the suspended particulate matter and the bottom sediments, whereas most polar sunscreens will be in the water column but can be also adsorbed onto the solid phases provided that their pKa be above the water pH. Therefore, due to its nature, some UV-Fs and benzotriazoles would remain trapped in the sediment, which act as a sink, as reported for other organic pollutants (Peck et al., 2004).

Table 3
Suspended solid (a) and sediment (b) UV-Fs and benzotriazoles concentrations. dw: dry weight.

a)

Concentration in suspended particulate matter (ng g^{-1} dw)

Sample			Weight (mg)	Compound										
				BP3	BP1	4HB	4DHB	EHMC	4MBC	ODPABA	OC	EtPABA	BZT	MeBZT
River														
S2	Besòs		2.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S4	Ripoll		8.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S5	Besòs		13.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S7	Riu-Sec		6.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S9	Riera de Rubí		6.2	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S10	Riera de Rubí		20.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S12	Llobregat		13.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S13	Llobregat		3.5	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S15	Riera de Rubí		196.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S16	Riera de Rubí		5.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S18	Cardaner		8.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S19	Cardaner		20.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
WWTP														
S3	Montcada i Reixac	inf.	80.6	892,3	<LOQ	<LOQ	<LOQ	2525,1	<LOQ	<LOQ	3183	<LOQ	<LOQ	416,8
		eff.	7.7	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S6	Sabadell	inf.	34.7	<LOQ	<LOQ	<LOQ	<LOQ	1344,9	<LOQ	<LOQ	2640,4	<LOQ	1684	<LOQ
		eff.	6.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S8	Terrassa	inf.	69.2	<LOQ	<LOQ	<LOQ	<LOQ	2273,3	<LOQ	<LOQ	1531	<LOQ	<LOQ	<LOQ
		eff.	8.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S11	St. Feliu de Llobregat	inf.	27.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5034,5	<LOQ	<LOQ	<LOQ
		eff.	9.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
S14	Rubí	inf.	17.9	<LOQ	<LOQ	<LOQ	<LOQ	296,233,2	9	<LOQ	1,031,868,2	<LOQ	21,832,1	16,026,2
		eff.	6.8	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3977	n.d.	<LOQ	<LOQ
S17	Manresa	inf.	340.3	<LOQ	<LOQ	<LOQ	<LOQ	31,214,8	<LOQ	<LOQ	92,400	n.d.	<LOQ	<LOQ
		eff.	1.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ

b)

Concentration in sediment (ng g^{-1} dw)

Sample			Compound										
			BP1	BP3	4HB	4DHB	EHMC	4MBC	ODPABA	OC	EtPABA	BZT	MeBZT
S2	Besos	Downstream	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	226,6	n.d.	128,4	n.d.
S17	Manresa	Downstream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
S11	St. Feliu de Llobregat	Downstream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	56,0	n.d.
		Upstream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	104,7	n.d.	109,4	n.d.
S14	Rubí	Downstream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	132,2	n.d.	148,8	<LOQ
		Upstream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	230,9	<LOQ

inf. – influent; eff. - effluent; <LOQ - below the limits of quantitation; n.d. – not detected.
<LOQ - below the limits of quantitation; n.d. – not detected;

3.2.5. Partitioning between phases

The partitioning of contaminants between the different phases was studied in order to gain more insight about the fate of the selected UV-Fs and benzotriazoles. In general, sunscreens' mean concentrations of influent and effluent wastewater are above those reported in river water; 8.2 ng ml^{-1} for influent, 6.2 ng ml^{-1} for effluent, and 2.2 ng ml^{-1}- for river waters. A similar trend is observed in the solid phases (see Fig. 5). The absence of detectable levels for most UV filters and benzotriazoles in sediment samples might be related to the physical-chemical characteristics and the abundance of the compound in river water, with only 4 substances detected (EHMC, OC, BZT and MeBZT) in the sediment. On the other hand, suspended solid samples from the rivers had concentrations <LOQ for all UV-Fs except EtPABA, which was not detected, which means that sediments are the final reservoir, having mean concentration of up to 231 ng g^{-1}. Looking into the wastewater suspended solid samples, as expected effluent wastewaters had less particulate in suspension and thus have lower concentrations of UV-Fs (<LOQ in most of them), than the influent wastewaters, where quantifiable amounts for EHMC, OC, BZT and MeBZT were observed. The only effluent particulate sample with quantifiable levels of UV-Fs and benzotriazoles had a concentration ten-fold lower than the mean concentration of influent suspended solid, but three-fold higher than the concentrations found in sediment. The presence of the four main contributors in both the suspended matter and the sediments may be explained by the lipophilic nature of EHMC and OC (Log K_{ow} of 5.8 and 7.53, respectively), whereas the high concentrations in the water samples together with the high pKa of BZT and MeBZT explains their occurrence in the suspended particulate matter, prevalence that is highly significant in the Rubí influent wastewater (S14). The microfiltration process carried out in the wastewater treatment helps to remove most of the suspended solid from the water by transferring them to the sludge, attenuating the release of these compounds into the environment. However, the use of such sludge as soil amendment in agriculture might facilitate their reintroduction into the water cycle.

3.3. Ecotoxicological risk assessment

The characterisation of the ecotoxicological risks associated to the occurrence of UV-Fs and UV blockers in the studied aquatic ecosystem was performed following the European Agency for the Evaluation of Medicinal Products (EMEA) guidelines (EMEA, 2006), as described in a previous work (Molins-Delgado et al., 2016). EC_{50} (50% effect concentration) or LC_{50} (50% lethal concentration) for the calculations were obtained from the literature (Park and Choi, 2008; Sanderson et al., 2003).

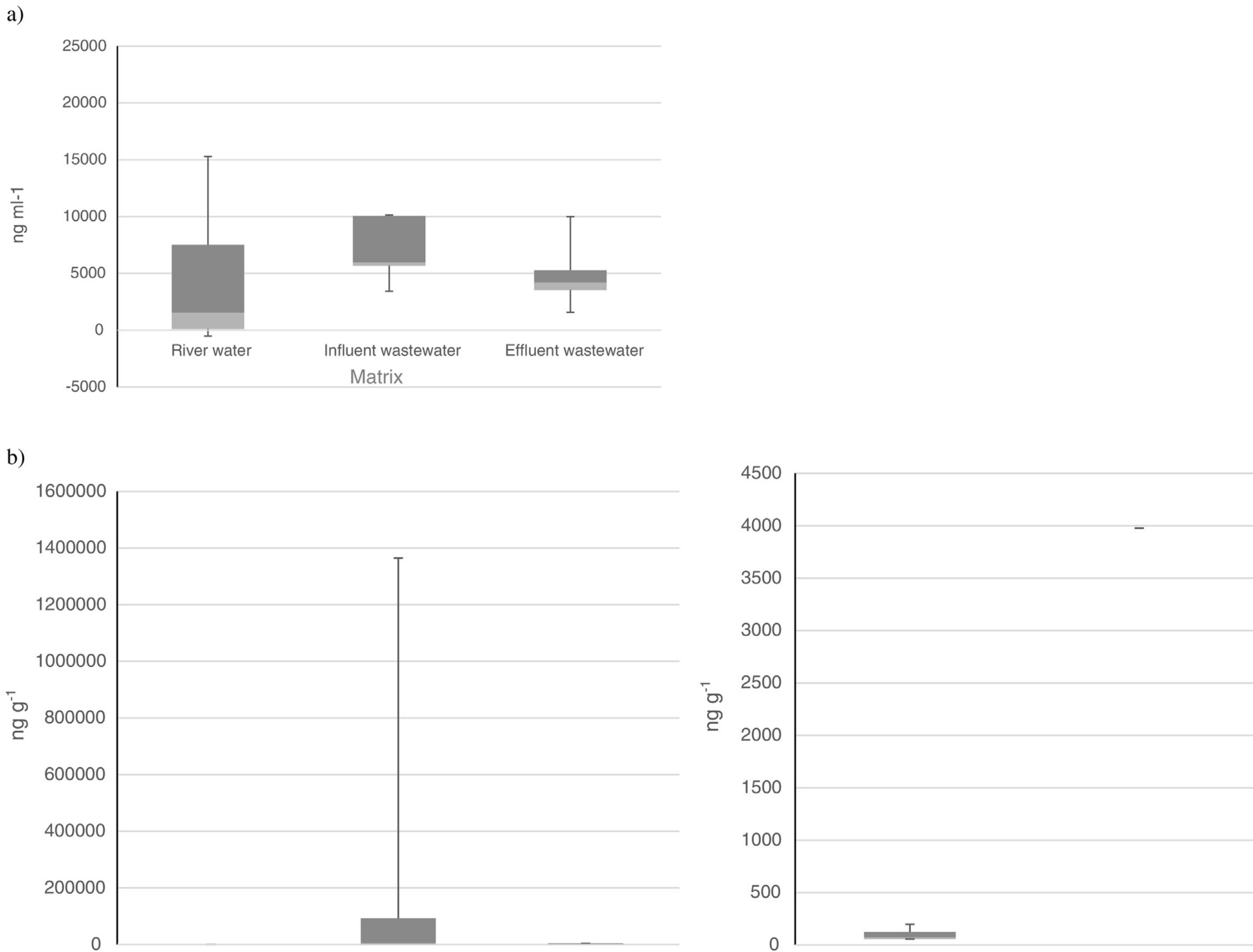


Fig. 5. Box plot of the concentrations of UV-Fs and benzotriazoles in the water compartment (a) and in the sediment and suspended particulate solid compartments (b). The second and third quartile and the median concentration are represented. The whiskers represent values outside the middle 50%.

The selection of EC_{50}-LC_{50} values was carried out in accordance to the minimum value available in the literature (Brausch and Rand, 2011), in order to consider the worst case scenario.

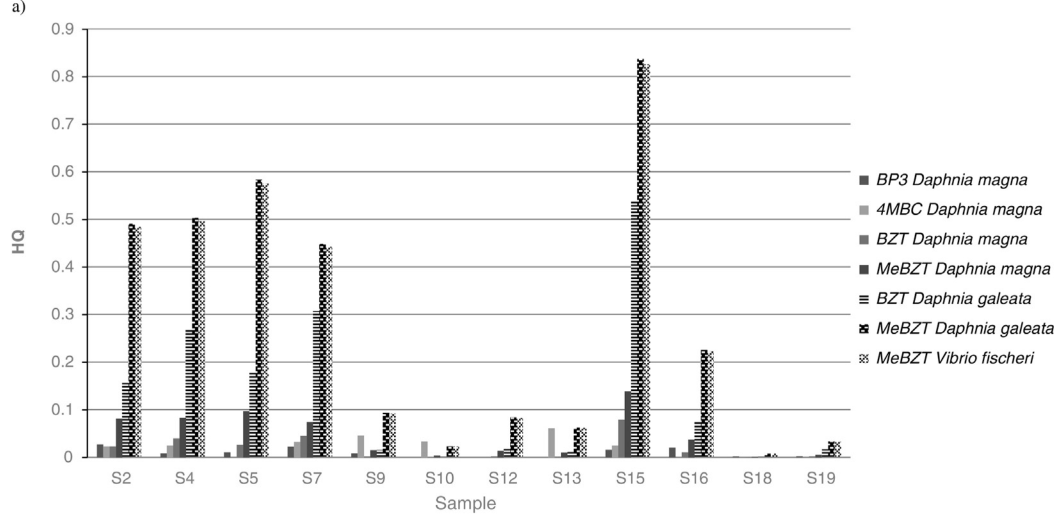
Hazard quotients (HQs) for acute toxicity were estimated for *Daphnia magna*, *Daphnia galeata* (invertebrates) and *Vibrio fischeri* (bacteria) in river water, and wastewaters for BP3, 4MBC, BZT and MeBZT. Obtained values were compiled in Table A7a and A8a. Figs. 6a and 7a represent the estimated HQs. As HQs were below 0.5, the associated ecotoxicological risk was medium-low for *Daphnia magna*. On the other side, *D. galeata* and *V. fischeri* appeared to be more sensitive to MeBZT, and in a lesser extent to BZT. These two compounds do not pose ecotoxicological risk, but the margin of safety is small, as result of HQ values >0.5 in some cases.

In river water (Fig. 6a), values for S15 (Riera de Rubí) pose a greater concern due to the HQs reached by MeBZT; 0.84 for *D. galeata* and 0.83 for *V. fischeri*. In influent wastewaters (Fig. 7a), estimated HQs were always <1, and maximum values were estimated for Sabadell WWTP, with HQ values of BZT for *D. galeata* of 0.6, and of MeBZT for *D. galeata* and *V. fischeri* of 0.74 and 0.73, respectively. On the other hand, effluent wastewaters (Fig. 7a) revealed HQ values <1 for all samples. The overall reduction of the sunscreen total mass load thanks to the water treatment is enough to reduce the risk that these compounds may pose in effluent streams. Nevertheless, the concentrations of both benzotriazoles in the Rubí WWTP (S14) showed the highest HQs values for effluent wastewaters; 1.07 for BZT to *D. galeata* and 1.04 and 1.02 for MeBZT to *D. galeata* and *V. fischeri*, values which indicate that the selected organisms are at risk.

For chronic toxicity, in the case of BP1, BP3, EtPABA and 4MBC, HQs were calculated for the fish species *Pimephales promelas* and *Oncorhynchus mykiss* (see Fig. 6b and 5b). HQs for river waters (Fig. 6b) showed values <0.1, indicating no risk. As regards wastewaters (Fig. 7b), HQs for the selected species were generally <1. However, BP3 reached values as high as 0.69 for *O. mykiss* in the Montcada i Reixac WWTP (S3 influent), narrowing the safety margin for this compound. The complete values of estimated HQs are listed in Table A7b and A8b.

In a recently published work by Serra-Roig et al. (2016), groundwater from the Besòs River aquifer were analysed for UV-Fs and other personal care products and the environmental risk posed by these compounds to certain species were calculated. The HQ values obtained were always <0.12 indicating low risk, but being BZT and MeBZT the highest contributors. The same study revealed that most of the compounds present in the river were removed during the natural infiltration process, being retained in the sediment and soil layers. However, the same work proved that some of these compounds, namely BZT,

a)



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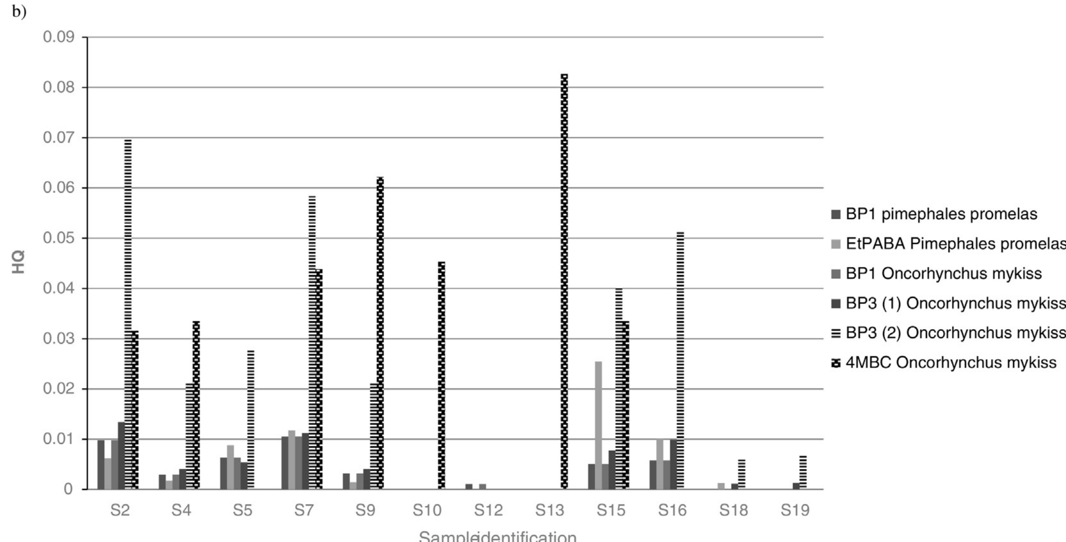


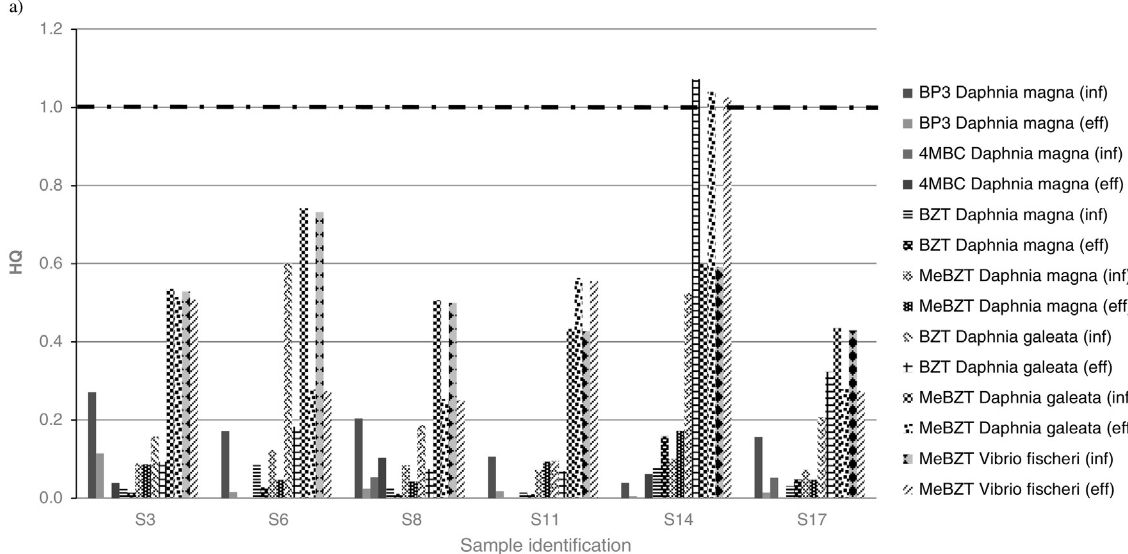
Fig. 6. Acute (a) and chronic (b) hazard quotients (HQ) for each compound in river waters. BP3 (1) and (2) indicate different EC_{50} values considered.

MeBZT, and 4MBC, were capable of reaching the aquifer, widening the contamination impact on the whole aquatic ecosystem. The fact that our results are similar to those reported in the literature may help to provide more insight on the potential risks that other river basins with similar concentration of residual UV-Fs and benzotriazoles may be suffering.

4. Conclusions

UV filters and benzotriazoles were determined in river waters, wastewaters, sediment and suspended particulate matter samples from a series of sampling sites along two river basins close to Barcelona city (Spain). These compounds were detected in all the studied water and wastewater samples. BP3's transformation products (4HB and 4DHB) were determined in both rivers, indicating biotic degradation of BP3 to some extent. The benzotriazoles, BZT and MeBZT, presented extremely high concentrations likely as a consequence of their wide use as sunscreens but also as anticorrosive and antifreeze agents. Mean concentrations of the target compounds in the Besòs River were overall higher than those in the Llobregat River, most probably due to the differences in flow regime leading to increased diluting effect. Both basin areas are highly industrialised which contribute to the high concentrations found in the selected sites. Removal rates estimated in the WWTPs along both basins showed overall good efficiencies for most compounds, with the notorious exceptions of BZT and MeBZT, whose concentrations increased, likely due to transformation processes of other more complex benzotriazoles or by the breakdown of benzotriazoles' conjugated forms (glucuronids, acetylated, …). UV-Fs and benzotriazoles concentrations in suspended particulate matter were below the LOQs in river samples, whereas significant values of some of them were found in particulate from wastewaters in S14, located in a densely industrialised area. Target UV-Fs and benzotriazoles were in most cases not detected in sediments, with the exceptions of OC and BZT, both compounds at concentrations up to 230 ng g^{-1}. As expected, mean concentrations of the analytes measured in river water and sediment are much lower than those observed in wastewaters and their corresponding suspended solids. Nevertheless, sediments appear to act as a sink for the most lipophilic and abundant compounds, whereas the wastewater treatment appear to remove most of the suspended solid. Additionally, the common practice of posterior use of the sewage sludge as fertiliser in agriculture is a matter of concern as it might further spread these compounds into the environment. According to the estimated HQs, the target compounds mostly do not pose risk for the aquatic environment in the frame of the described scenarios. However, BZT and MeBZT concentrations measured in some effluent streams do pose a risk for *D. galeata* and *V. fischeri*.

a)



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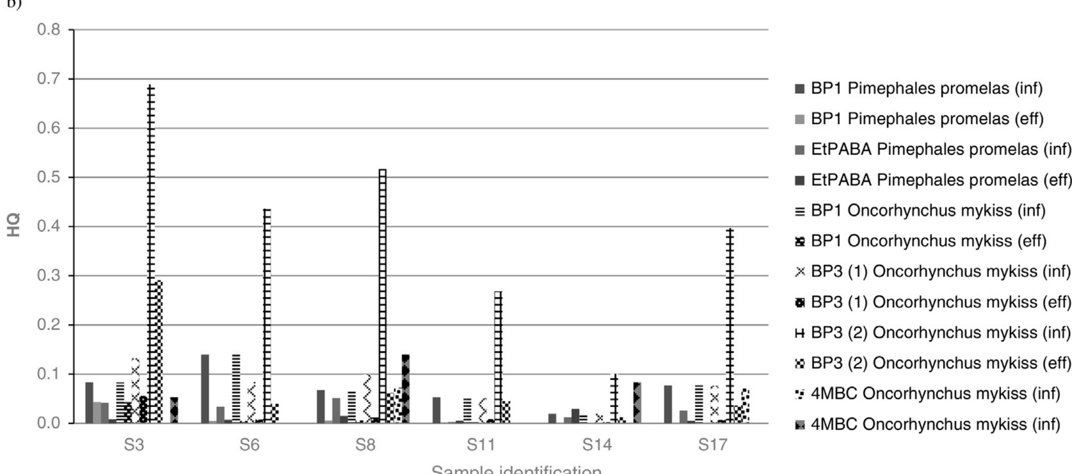

Fig. 7. Acute (a) and chronic (b) hazard quotients (HQ) for each compound influent (inf) and effluent (eff) wastewaters. BP3 (1) and (2) indicate different EC_{50} values considered.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.05.176.

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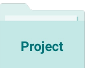

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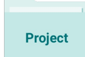

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Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil

Mariana B. Alonso [a, b, c], Maria Luisa Feo [d], Cayo Corcellas [d], Pablo Gago-Ferrero [d], Carolina P. Bertozzi [c], Juliana Marigo [c], Leonardo Flach [e], Ana Carolina O. Meirelles [f], Vitor L. Carvalho [f], Alexandre F. Azevedo [b], João Paulo M. Torres [a], José Lailson-Brito [b], Olaf Malm [a], M. Silvia Diaz-Cruz [d], Ethel Eljarrat [d, *], Damià Barceló [d]

[a] Radioisotopes Laboratory Eduardo Penna Franca (LREPF), Biophysics Institute Carlos Chagas Filho (IBCCF), Federal University of Rio de Janeiro (UFRJ), Brazil
[b] Aquatic Mammals and Bioindicator Laboratory (MAQUA), School of Oceanography, Rio de Janeiro State University (UERJ), Brazil
[c] Laboratório de Biologia e Conservação da Megafauna Marinha — Biopesca — Instituto de Biociências, Universidade Estadual Paulista, Unesp, Campus Litoral Paulista, Brazil
[d] Department of Environmental Chemistry, IDAEA, CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[e] Projeto Boto Cinza, Sepetiba, Rio de Janeiro, Brazil
[f] Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), Caucaia, Ceará, Brazil

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A B S T R A C T

Pyrethroids (PYR) and UV filters (UVF) were investigated in tissues of paired mother-fetus dolphins from Brazilian coast in order to investigate the possibility of maternal transfer of these emerging contaminants. Comparison of PYR and UVF concentrations in maternal and fetal blubber revealed Franciscana transferred efficiently both contaminants to fetuses (F/M > 1) and Guiana dolphin transferred efficiently PYR to fetuses (F/M > 1) different than UVF (F/M < 1). PYR and UVF concentrations in fetuses were the highest-ever reported in biota (up to 6640 and 11,530 ng/g lw, respectively). Muscle was the organ with the highest PYR and UVF concentrations ($p < 0.001$), suggesting that these two classes of emerging contaminants may have more affinity for proteins than for lipids. The high PYR and UVF concentrations found in fetuses demonstrate these compounds are efficiently transferred through placenta. This study is the first to report maternal transfer of pyrethroids and UV filters in marine mammals.

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1. Introduction

The number of organic contaminants found in the environment is constantly increasing. Among those that have emerged recently are synthetic pyrethroid insecticides and active ingredients in sunscreen products. Along with the emerging POPs (persistent organic pollutants, e.g. emerging flame retardants), these both classes of chemicals have been detected in biota (Alonso et al., 2012a, 2012b; Gago-Ferrero et al., 2012), in human matrices (Corcellas et al., 2012; Kunisue et al., 2012) as well as in other environmental samples (Feo et al., 2010b; Gago-Ferrero et al., 2011). The main concern for these emerging contaminants is endocrine disrupting activity in non-target organisms (Schreurs et al., 2005; Weybridge, 2012).

Synthetic pyrethroids (PYR) are hydrophobic, particle reactive, and are found in low concentrations in water (e.g. up to 70 ng/L in California creeks and 40 ng/L in Ebro river) (Amweg et al., 2006; Feo et al., 2010b). They are applied to land and/or around man-made structures for the control of arthropod-borne diseases, and are also used in agricultural, garden and veterinary products (Feo et al., 2010a; Santos et al., 2007). This group of insecticides has a high degree of toxicity in standard laboratory studies with fish and arthropods (Maund et al., 2002; Woudneh and Oros, 2006; You et al., 2008). Studies suggested carcinogenic, neurotoxic, immunosuppressive, allergenic and reproductive potential toxicity in mammals (Jin et al., 2012; Scollon et al., 2011; Shafer et al., 2008). Newborn rats were reported as 4 to 17 times more vulnerable to acute

* Corresponding author. Department of Environmental Chemistry, IDAEA, CSIC, C/ Jordi Girona 18-26, Barcelona, Spain.
E-mail address: eeeqam@cid.csic.es (E. Eljarrat).

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toxicity of PYR than adults (Ostrea et al., 2013; Shafer et al., 2004). Recent studies have reported their accumulation in biotic matrices, such as human breast milk (Corcellas et al., 2012; Feo et al., 2012) and in liver of marine mammals (Alonso et al., 2012b).

Ultra-violet filters (UVFs) are sunscreen agents that reduce the intensity of UV light incidence on cells. UVFs can be found in many products, including cosmetics (e.g. perfumes, shampoos, creams and make up products), as well as in industrial and/or commercial products as an agent to minimize photodegradation. UVFs produced estrogenic and androgenic activity in *in vivo* and *in vitro* studies (Díaz-Cruz and Barceló, 2009; Schreurs et al., 2005). Oral administration of UVF to mammals during pre and postnatal life, has shown that the central nervous system and reproductive organs are targets for the damaging effects on the development of the offspring; resulting in changes in gene expression in organs and regions of the brain related to sexual dimorphism (Schlumpf et al., 2004). Ethylhexyl methoxycinnamate (EHMC) can alter the gene expression in zebrafish (*Danio rerio*) and showed multiple hormonal activities at environmentally relevant concentrations (Zucchi et al., 2011). Recent studies showed that these chemicals are detected in the environment (Gago-Ferrero et al., 2011; Jurado et al., 2014), in biota (Buser et al., 2006; Gago-ferrero et al., 2013) and in human breast milk, placenta and semen (Jiménez-Díaz et al., 2011; León et al., 2010; Schlumpf et al., 2004).

Long-lived marine mammals such as cetaceans are exposed to multiple persistent bioaccumulative toxicants compounds throughout their life history. Dolphins in particular serve as excellent sentinel species for contamination in the marine environment (Alonso et al., 2014; Bossart, 2011; Kucklick et al., 2011). When concentrations reach high enough levels, these sentinels may experience neurotoxic, immunologic and multiple endocrine effects (McDonald, 2002; Zhou et al., 2002). The two primary exposure routes are via the diet and through maternal transfer that in turn occurs via lactation and gestation (Bossart, 2011; Kajiwara et al., 2008). Thus, the exposure of a pregnant female cetacean to toxicants may pose a health threat to the developing fetus, resulting in an increased susceptibility to disease in adulthood (Desforges et al., 2012; Park et al., 2010).

Few works have focused on maternal transfer of organohalogenated compounds in marine mammals. Most data are derived from studies of lactational transfer of contaminants in seals and sea lions (Debier et al., 2012; Vanden Berghe et al., 2012; Wang et al., 2013). Prenatal exposure of cetaceans to organohalogen contaminants was slightly recorded, mainly due to difficulty in obtaining such samples. Some reports recorded the transplacental transfer of organochlorines and organobrominated compounds in cetaceans (Desforges et al., 2012; Dorneles et al., 2010; Hoguet et al., 2013; Kajiwara et al., 2008; Weijs et al., 2013), and rare researches on perfluorinated compounds were conducted (Dorneles et al., 2008a; Van De Vijver et al., 2007), however with a very limited number of samples from mother-fetus pairs.

Our previous studies reported on residues of PYR and UVFs in free-ranging dolphins, including in placenta and milk samples (Alonso et al., 2012b; Gago-Ferrero et al., 2013). However, no direct evidence of maternal transfer was found in these studies. In this paper, we investigated for the first time the likelihood of maternal transfer of pyrethroids and UV filters in cetaceans by analyzing paired samples of mother-fetus of Franciscana and Guiana dolphins from the Southwestern Atlantic Ocean.

2. Materials and methods

2.1. Target species

The Franciscana dolphin (*Pontoporia blainvillei)* is a small odontocete that occurs exclusively in the southwestern Atlantic where it is considered to be among the most threatened cetacean species along the east coast of Latin America (Secchi and Wang, 2002). It has a small coastal home range due to its limited movement patterns (Wells et al., 2012). The site fidelity exhibited by *P. blainvillei* makes this species a particularly useful sentinel for regional contamination (Alonso et al., 2012a, 2012b). The Guiana dolphin (*Sotalia guianensis)* is another exclusively coastal species, ranging from south Brazil northward into Central America. This small delphinid inhabits shallow waters and is often found year-round in bays and estuaries (Batista et al., 2014). Studies have shown *S. guianensis* to reside in Guanabara and Sepetiba bays in Rio de Janeiro State, Brazil (de Freitas Azevedo et al., 2004; Flach et al., 2008). Their largely near-shore distribution render *S. guianensis* particularly vulnerable to impacts due to human activities, such as fishing by-catch and exposure to organohalogenated contaminants (Alonso et al., 2010; Dorneles et al., 2010; Lailson-Brito et al., 2010).

2.2. Sample collection

Fig. S1 (Supplementary Information) shows the coastal areas of Brazil where dolphins were sampled for this study. The southeast region of Brazil, where the states of São Paulo (SP) and Rio de Janeiro (RJ) are located, is one of the most developed areas of South America. The coastal ecosystems near these urban centers historically receive discharges of industrial, domestic and agricultural effluents that contain chemical contaminants (Buruaem et al., 2013), as reflected in residue analysis of biota (Lailson-Brito et al., 2012). In contrast, the land use and economy associated with Ceará state (CE), located in northeastern Brazil, specially where the samples where collected (C3 region, following Santos-Neto et al. (2014)), is based on agriculture, fishing and tourism and the population density is very low (44 hab./Km^2) (CEARA, 2014).

Tissue samples from five mother-fetus pairs of Franciscana dolphins from the São Paulo coast (Baixada Santista) in southeastern Brazil were collected, along with samples from three mother-fetus pairs of Guiana dolphins (two pairs from Sepetiba Bay, Rio de Janeiro coast and one pair from Canoa Quebrada Beach, Ceará coast) from 2004 to 2011 (Fig. S1). Pregnant female dolphins as by-catch were brought to research labs, where they were classified as carcasses in early decomposition stage. After dissection, biological samples were stored in aluminum foil and kept frozen (−20 °C) until processing for analysis. Total length of females and fetuses were measured and pregnancy stages were estimated assuming Franciscana dolphins average 10.2 months of gestation and length at birth is 72 cm (Bertozzi, 2009); for Guiana dolphins, gestation averages 11.4 months and length at birth is 106 cm (Ramos et al., 2000). Blubber and muscle from both mother and fetus were taken from both species. Maternal blubber ($n = 7$), fetal blubber ($n = 5$), maternal muscle ($n = 4$) and fetal muscle ($n = 4$), as well as umbilical cord ($n = 3$), placenta ($n = 4$) and milk ($n = 3$) from Franciscana dolphins; and maternal blubber ($n = 3$), fetal blubber ($n = 3$), maternal muscle ($n = 3$) and fetal muscle ($n = 3$) from Guiana dolphins.

2.3. Standards and reagents

A standard mixture of six PYRs containing cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin and tetramethrin, and single analytical standards of bifenthrin, lambda-cyhalothrin, esfenvalerate, tau-fluvalinate, tralomethrin. d_6-*trans*-permethrin and d_6-*trans*-cypermethrin, used as internal standard, were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The certified UVF standards 4-methylbenzylidene camphor (4MBC), 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), 2-ethyl-hexyl-4-dimethyl-

aminobenzoate (OD-PABA) and octocrylene (OCT) were supplied by Dr Ehrenstorfer and Sigma–Aldrich (Steinheim, Germany). Isotopically labeled 3-(4-methylbenzylidene-d_4)camphor (4MBC-d_4) used as internal standard was obtained from CDN isotopes (Quebec, Canada). Pesticide grade hexane, dichloromethane, ethyl acetate and acetonitrile were obtained from Sigma–Aldrich (St. Louis, MO, USA). Standard solutions were prepared in ethyl acetate for instrumental analysis. In order to check the linearity of the method, calibration curves were prepared at different concentrations ranging between 0.15 and 40 pg/μL for PYR and from 0.10 to 200 pg/μL for UVF. The solutions were stored in the dark at − 20 °C.

In order to prevent contamination and photodegradation of samples and standard solutions all glassware used was previously washed and heated overnight at 380 °C, and further sequentially rinsed with different organic solvents and HPLC grade water. Separate solvents and only previously unopened packages of solvents, chemicals and other supplies were used. In addition, a set of at least two operational blanks were processed together with each batch of samples. Standard solutions and samples were always covered with aluminum foil and stored in the dark. Furthermore, gloves were worn during the sample preparation process.

2.4. Sample preparation

The sample analyses were performed using previously described methods (Alonso et al., 2012b; Feo et al., 2012; Gago-Ferrero et al., 2013). Biological samples (0.1 g dry weight) were fortified with d_6-*t*-PERME (4 ng), d_6-*t*-CYPE (2 ng) and 4MBC-d_4 (4 ng) as surrogate standards and extracted by sonication with 20 mL hexane: dichloromethane (2:1) in a Raypa, UCI-200 bath for 15 min. Samples were centrifuged at 3500 rpm (20 min) and the organic phase was transferred to a vial and evaporated under a nitrogen stream. This extraction step was repeated twice and all the solvent residues were collected together. The lipid content was determined gravimetrically for each sample. Extracts were cleaned up by elution through C18 (2 g/15 mL) coupled to basic alumina cartridges (5 g/25 mL) and conditioned with 25 mL of acetonitrile. Samples were eluted with 30 mL of acetonitrile and evaporated under a nitrogen stream. The residue was dissolved in 100 μL of ethyl acetate for GC and LC analysis.

2.5. Instrumental analysis

The extracts were injected on a GC–NCI-MS/MS for pyrethroid analysis and on an HPLC-(ESI)-MS/MS for UV filters analysis. GC–MS–MS analysis were performed in negative chemical ionization mode on an Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quad. A DB-5 ms capillary column (15 m × 0.25 mm i.d., 0.1 μm film thickness) containing 5% phenyl methyl siloxane was used with helium as carrier gas at constant flow of 1 mL/min. The temperature program was from 100 °C (held for 1 min) to 230 °C at 15°Cmin−1, then from 230 to 310 °C (held for 2 min) at 10°Cmin−1, using the splitless injection mode during 0.8 min. Inject volume was 3 μL. The inlet temperature was set at 275 °C and ion source temperature at 250 °C. Ammonia was used as reagent gas at 2×10^{-4} Torr. More details on MS/MS and selected transitions were reported in Feo et al. (2011, 2010a). HPLC-MS/MS analysis were performed in positive electrospray ionization mode on an Agilent Technologies HP 1100 pump connected to a 4000 Q TRAP™ MS/MS system from Applied Biosystems-Sciex (Foster City, California, USA). The chromatographic separation was achieved on a Hibar Purospher® STAR® HR R-18 ec. (50 mm × 2.0 mm, 5 μm) from Merck, preceeded by a guard column of the same packaging material. A gradient using a mixture of HPLC grade water and ACN, both 0.15% formic acid was applied. The elution gradient started with 5% of ACN, increasing to 75% in 7 min, and then to 100% in 3 min. The mobile phase flow-rate was 0.3 mL/min. A detailed description of methods for both groups of pollutants can be found in Gago-Ferrero et al. (2013 a; 2013 b), Alonso et al. (2012b) and Feo et al. (2010a).

MS/MS analyses of both, PYRs and UVFs, were performed using selected reaction monitoring (SRM). Two major characteristic fragments of the protonated molecular ion $[M+H]^+$ were monitored per analyte to enhance method sensitivity and selectivity. The most abundant transition was used for quantification, whereas the second most abundant was used for confirmation. The quantification for both classes of contaminants was performed using internal standards. MS/MS identification was based on the following criteria: (i) simultaneous responses for the two monitored transitions (SRM1 and SRM2) must be obtained at the same retention time than those of available standards; (ii) signal-to-noise ratios must be > 3; and (iii) relative peak intensity ratio must be within ±20% of the theoretical values obtained with standard solutions.

2.6. Quality assurance

Blank tests were carried out to rule out possible contamination from the sampling, storage or instrumentation. In order to comply with internal quality control procedures, two control spiked samples, two solvent injections and two procedural blanks were inserted into each analytical batch made up of six samples. The individual values obtained for control samples were plotted on a process-behavior chart during the entire duration of the study to establish if the analysis was in a state of statistical control. The quality parameters were performed in the liver samples from individuals of the same population of Franciscana dolphins (SP coast) and were previously published in Alonso et. (2012b) and Gago-Ferrero et al. (2013). Briefly, procedural recovery was assessed by addition of each target contaminant to biological samples. These samples were previously analyzed in order to determine the compounds present before spiking (blank). Three replicates were prepared for the evaluation of the reproducibility of the method. The limits of detection of the method (mLOD) (3 times the signal to noise ratio level), and the limit of quantification of the method (mLOQ) (10 times the signal to noise ratio level), were calculated. To determine linearity, calibration curves were produced for all compounds. The linear calibration range studied was from 0.15 to 40 pg/μL for PYR and from 0.10 to 200 pg/μL for UVF at six concentration levels per compound class. Good correlations were obtained within the interval studied with correlation coefficients ranging between 0.998 and 0.999. Recoveries of spiked PYR ranged between 53 and 116%, with a relative standard deviation (RSD) < 20%; mLODs ranged between 0.02 and 0.71 ng/g lipid weight (lw), and mLOQs ranged between 0.08 and 2.38 ng/g lw. Recoveries of spiked UVFs ranged between 60% and 115% (RSD < 20%); mLODs ranged between 1.50 and 25 ng/g lw and the mLOQs ranged between 1.90 and 75 ng/g lw.

2.7. Statistical analyses

Statistical analyses were conducted using the R Core Team (Vienna, Austria, 2014) statistical package. The level of statistical significance was defined at $p < 0.05$. Non-parametric statistical tests were used since the data were found to have a non-normal distribution (Shapiro Wilk's W test). Kolmogorov–Smirnov tests were used to determine which concentrations were significantly different between the tissues in mother-fetus pairs according to each species and between species. Spearman's coefficients were calculated to understand the correlation between PYR and UVF concentrations and lipid content for both species.

Table 1
Insecticide pyrethroid concentrations (ng/g lw) in Franciscana and Guiana dolphins from Brazilian coast.

Mother/Fetus	Organs	Lipid (%)	Tetramethrin	Bifenthrin	*l*-Cyhalothrin	Delta/Tralomethrin	Fluvalinate	Es/Fenvalerate	Permethrin	Cyfluthrin	Cypermethrin	ΣPYR	References
Franciscana dolphin													
Pair 1													
Mother	*Blubber*	88	1.80	6.00	nd	nd	nd	nd	85.5	nd	7.60	101	this study
	Muscle	11	6.90	13.5	2.15	nd	nd	nd	113	nd	75.5	211	this study
	Placenta	1	37.0	38.0	21.5	nd	20.0	6.95	260	14.5	460	855	(Alonso et al., 2012b)
	Cord	4	47.0	nd	15.0	nd	nd	12.0	460	nd	325	860	this study
	Milk	58	0.60	0.70	nq	0.35	nd	0.35	2.30	0.20	0.30	4.80	(Alonso et al., 2012b)
Fetus	*Blubber*	9	18.5	55.0	29.0	nd	nd	40.5	1120	nd	700	1965	this study
	Muscle	8	77.0	55.0	35.5	nd	nd	nd	5280	nd	890	6340	this study
Pair 2													
Mother	*Blubber*	86	3.40	nd	nd	nd	nd	nd	24.5	nd	3.30	31.5	this study
	Muscle	2	41.0	nd	12.0	nd	nd	nd	355	nd	455	860	this study
	Placenta	12	25.0	51.0	9.25	13.0	7.15	12.5	105	15.0	95.5	331	(Alonso et al., 2012b)
	Cord	3	22.0	nd	29.5	nd	nd	nd	725	nd	420	1195	this study
Fetus	*Blubber*	44	1.45	nd	0.30	3.70	nd	nd	99.5	nd	30.0	130	this study
	Muscle	15	11.5	nd	7.65	nd	nd	2.15	73.5	nd	145	240	this study
Pair 3													
Mother	*Blubber*	82	0.35	2.55	nd	1.25	nd	nd	4.65	nd	3.65	12.5	this study
	Placenta	5	7.70	5.00	nd	22.0	nd	20.0	105	9.45	56.0	225	this study
Fetus	*Blubber*	81	6.55	nd	2.30	nd	nd	0.15	15.0	4.15	37.0	65.0	this study
	Muscle	28	1.30	nd	1.45	2.20	nd	0.90	21.5	0.00	5.85	33.5	this study
Pair 4													
Mother	*Placenta*	3	96.5	86.0	48.5	38.5	nd	10.1	385	41.5	1105	1810	(Alonso et al., 2012b)
	Cord	2	10.5	nd	33.5	12.5	nd	nd	530	15.5	475	1080	this study
Fetus	*Blubber*	71	4.65	1.50	0.45	nd	nd	nd	15.0	nd	32.0	54.0	this study
	Muscle	4	6.50	nd	8.75	nd	nd	nd	148	nd	350	511	this study
Mother 5													
Mother	*Blubber*	89	1.80	nd	nd	nd	nd	nd	nd	nd	15.0	16.5	this study
	Muscle	6	3.85	nd	2.45	nd	nd	10.1	225	42.5	117	400	this study
Mother 6													
Mother	*Blubber*	79	0.40	1.05	nd	nd	1.15	nd	3.95	nd	1.40	8.00	this study
Mother 7													
Mother	*Blubber*	82	0.70	1.15	nq	nd	nd	nd	5.15	nd	2.00	9.00	this study
	Milk	70	0.15	0.60	nq	0.55	nd	0.20	0.80	0.10	0.30	2.80	(Alonso et al., 2012b)
Mother 8													
	Milk	38	0.25	0.40	nq	0.45	nd	0.25	0.90	0.15	0.20	2.60	(Alonso et al., 2012b)
Calf-Mother-Fetus 9													
Calf	*Blubber*	80	0.55	20.0	1.50	nd	nd	nd	17.5	nd	20.5	60.0	this study
	Muscle	6	8.20	6.70	nd	nd	nd	nd	115	nd	33.5	165	this study

Mother	*Blubber*	80	0.65	14.0	1.35	4.50	nd	nd	17.0	nd	37.5	75.0	this study
	Muscle	2	8.20	6.70	nd	nd	nd	nd	115	nd	33.5	165	this study
Fetus	*Blubber*	22	1.15	nd	1.00	25.0	nd	nd	13.5	nd	44.5	90.0	this study
Guiana dolphin													
Pair 1													
Mother	*Blubber*	33	3.60	2.75	0.65	nd	5.45	nd	16.5	nd	9.8	40.0	this study
	Muscle	8	1.55	27.0	nd	nd	nd	17.0	99.5	nd	120	265	this study
Fetus	*Blubber*	14	1.50	4.05	nd	2.65	nd	2.95	25.5	8.60	12.5	58.0	this study
	Muscle	26	1.85	10.5	0.70	3.65	nd	0.00	41.5	nd	32.5	91.0	this study
Pair 2													
Mother	*Blubber*	25	4.35	12.0	0.20	12.0	0.60	0.90	82.0	nd	23.5	135	this study
	Muscle	5	4.10	17.5	41.0	nd	nd	nd	245	nd	315	620	this study
Fetus	*Blubber*	8	2.15	7.60	nd	nd	nd	nd	27.0	nd	19.5	57.0	this study
	Muscle	31	1.05	11.0	nd	nd	nd	nd	33.0	nd	10.5	55.5	this study
Pair 3													
Mother	*Blubber*	38	0.45	1.65	nd	0.35	nd	0.55	9.75	1.05	2.5	16.0	this study
	Muscle	4	2.00	nd	nd	nd	nd	nd	265	nd	300	570	this study
Fetus	*Blubber*	24	0.55	2.50	nd	nd	nd	nd	9.80	nd	9.5	22.5	this study
	Muscle	20	0.65	nd	nd	nd	nd	nd	43.0	nd	24.0	67.5	this study

nd – below mLOD.
nq – below mLOQ, mLOD and mLOQ were published in Alonso et al. (2012b).

3. Results and discussion

3.1. Biological parameters in females and fetus

Table S1 presents the biological data for individual animals, body length of females and fetus, collection date, fetus sex, estimated pregnancy stage for each species according to birth length and gestation period in the literature (Bertozzi, 2009; Ramos et al., 2000), number of corpus luteum and albicans to Franciscana (Bertozzi, 2009) and Guiana dolphins (Santos-Neto personal comm.) and age. The number of corpus albicans is correlated with the number of pregnancies in *Sotalia guianensis* (Rosas and Monteiro-Filho, 2002), however the presence of corpus albicans is temporary in *Pontoporia blainvillei* (Bertozzi, 2009). Lipid content in tissues of Franciscana and Guiana dolphin mother-fetus pairs were displayed in Tables 1 and 2.

3.2. Maternal transfer of pyrethroid insecticides

3.2.1. Concentration levels: fetuses vs. mothers

Pyrethroids were detected in all 44 samples analyzed, which included blubber and muscle of female and fetus, as well as placenta, milk and umbilical cord (Table 1). Mean ΣPYR concentrations found in Franciscana maternal blubber was 36.0 ng/g lw (SD ± 36.3); in maternal muscle 410 ng/g lw (SD ± 315); in fetal blubber 460 ng/g lw (SD ± 840); and in fetal muscle 1780 ng/g lw (SD ± 3045) (Fig. 1a). ΣPYR concentrations in Guiana dolphin in maternal and fetal tissues from Rio de Janeiro state (RJ) and Ceará state (CE) are in Table 1 and represented in Fig. 1b and c, respectively.

Fetus-mother ratios (F/M) ΣPYR concentrations in Franciscanas were 1.43, 2.67, 4.13 and to 19.5 (n = 4 pairs) in blubber tissue, and 0.28 and 30 (n = 2 pairs) in muscle. A significant difference ($p = 0.04$) was observed in blubber concentrations of PYR in Franciscana fetuses compared with their mothers (Fig. 1a). A higher proportion of F/M ratios >1 indicated that the fetuses contained a higher offload of PYR than their respective mothers in the blubber tissue (Fig. 1a). The same pattern (F/M > 1) was observed in relation to HCB in mother-fetus pairs of long-finned pilot whales (*Globicephala melas*) from Australia and in beluga whales (*Delphinapterus leucas*) from Alaska, demonstrating a strong offload tendency where a potential for bioaccumulation in fetuses is higher compared to their respective mothers (Hoguet et al., 2013; Weijs et al., 2013). Cetacean studies have shown a tendency to transfer from mother to fetus the less lipophilic organohalogens (lower chlorinated contaminants and with lower log K_{ow}), such as HCB that have six chlorines and log K_{ow} 5.24 (Desforges et al., 2012; Hoguet et al., 2013). The two predominant pyrethroids in fetal blubber in this study were permethrin and cypermethrin (log K_{ow} 6.5 and 6.6, respectively), which contain two chlorines in their molecular formulas, leading to suggest that these two pyrethroids may have a similar tendency to lower chlorinated pesticides, in relation to their transplacental transfers in cetaceans.

Fetus-mother ratios of blubber ΣPYR concentrations were 0.42 and 1.47 in Guiana dolphins from RJ and 1.39 in Guiana from CE, and muscle ΣPYR concentrations were 0.09 and 0.35 in Guiana dolphin pairs from RJ and 0.12 in a Guiana dolphin pair from CE (Table 1, Fig. 1b, c). Two different patterns of transference were observed in Guiana dolphins, accordingly to the tissue. F/M > 1 was similar to Franciscana and to PFOS (perfluorooctane sulfonate) in the same species from a close area (Guanabara Bay, F/M = 2.75 and 2.62) (Dorneles et al., 2008b), which characterizes an efficiently transfer of PYR in the blubber from mother to fetuses. In the other hand, F/M < 1 in all *Sotalia guianensis* muscles, indicated that mothers retained a higher load of PYR in muscle than their respective

Table 2
U.V. filter concentrations (ng/g lw) in Franciscana and Guiana dolphins from Brazilian coast.

Mother/Fetus	Organs	Lipid (%)	EHMC[a]	4MBC[b]	OD-PABA[c]	OCT[d]	ΣUVF
Franciscana dolphin							
Pair 1 (BP 106)							
Mother	*Blubber*	88	60.5	nd	nd	nd	60.5
	Muscle	11	43.0	nd	nd	nd	43.0
	Milk	58	120	20.0	nd	nd	140
Fetus	*Blubber*	9	66.5	nd	50.0	nd	115
	Muscle	8	250	110	45.0	11130	11,530
Pair 2 (BP 62)							
Mother	*Blubber*	86	35.5	47.5	nd	nd	83.0
	Muscle	2	42.5	355	nd	nd	400
Fetus	*Blubber*	44	nd	nd	5.90	nd	6.0
	Muscle	15	245	86.0	60.0	1780	2175
Pair 3 (BP 161)							
Mother	*Blubber*	82	nd	8.65	nd	nd	8.5
	Placenta	5	nd	nd	1385	nd	1385
Fetus	*Blubber*	81	68.5	nd	2.50	nd	71.0
	Muscle	28	69.0	nd	nd	nd	70.0
Pair 4 (BP 71)							
Fetus	*Blubber*	71	117	35.0	4.00	50.0	205
	Muscle	4	250	170	155	2090	2660
Mother 5 (BP 151)							
Mother	*Blubber*	89	43	13	nd	nd	56.5
	Muscle	6	54	110	nd	nd	165
Mother 6 (BP 152)							
Mother	*Blubber*	79	nd	nd	nd	nd	nd
Mother 7 (BP 153)							
Mother	*Blubber*	82	74.5	nd	nd	nd	74.5
	Milk	70	nd	17.5	8.50	nd	25.5
Mother 8 (BP 132)							
	Blubber	38	77.5	28.5	nd	113	219
Calf-Mother-Fetus 9 (BP 182)							
Calf	*Blubber*	80	67.0	nd	nd	nd	67.0
	Muscle	6	133	250	36.5	925	1345
Mother	*Blubber*	80	85.0	20.0	3.15	nd	110
	Muscle	2	67.5	855	nd	nd	920
Fetus	*Blubber*	22	70.0	97.0	67.5	nd	235
Guiana dolphin							
Pair 1 (RJ)							
Mother	*Blubber*	33	nd	nd	nd	nd	nd
	Muscle	8	155	230	50.0	970	1405
Fetus	*Blubber*	14	nd	nd	nd	nd	nd
	Muscle	26	83.5	80.0	26.0	165	355
Pair 2 (RJ)							
Mother	*Blubber*	25	205	48.0	34.0	220	505
	Muscle	5	545	570	1050	8310	10,475
Fetus	*Blubber*	8	nd	33.0	nd	nd	32.5
	Muscle	31	85.0	61.0	17.0	115	280
Pair 3 (CE)							
Mother	*Blubber*	38	48.0	18.5	nd	nd	67.0
	Muscle	4	70.0	395	nd	1350	1810
Fetus	*Blubber*	24	nd	34.0	nd	nd	34.0
	Muscle	20	40.0	60.0	25.0	240	365

nd – below mLOD.
[a] EHMC – ethylhexyl methoxycinnamate.
[b] 4MBC - 4-methylbenzylidene camphor.
[c] OD-PABA - 2-ethylhexyl-4-dimethyl-aminobenzoate.
[d] OCT – octocrylene.

fetuses, pointing to an opposite pattern than observed in blubber. F/M < 1 characterizes a lower input of the contaminants from mother to fetus in muscle tissue, but it is necessary to point out that ΣPYR concentrations in maternal muscle were up to 35 times higher than in maternal blubber. More studies are necessary to understand the exposition of fetuses to pyrethroids and their transplacental transport, however, is evident that PYR traverse placental membrane and bioaccumulate in fetal tissues in critical stages of development. The transplacental transfer of pyrethroids insecticides in dolphins was characterized by the fact that the pyrethroids were present in all fetus samples.

ΣPYR concentrations in a Franciscana fetus in muscle and blubber tissues (6340 ng/g lw and 1965 ng/g lw, respectively, Pair 1 in Table 2) and the placenta and cord (1810 ng/g/lw and 1080 ng/g lw, respectively, Pair 4 in Table 2) were the highest-ever reported in biota. These animals were taken from São Paulo coastal waters in which persistent organic pollutants in dolphins from the same population (Baixada Santista) show this to be one of the most contaminated coastal regions in Brazil (Alonso et al., 2012a). Pyrethroids are largely used in Brazil and their use is enforced by the Government Health Agency as a control of insect-borne diseases (Santos et al., 2007).

Concentrations of PYR in the maternal blubber of Franciscanas were between 1 and 2 orders of magnitude lower than DDTs and PCBs and similar to brominated flame retardants (PBDEs) and PFOS levels in females from the same population (Baixada Santista, SP)

(Alonso, 2008; Alonso et al., 2012a; Lailson-Brito et al., 2011; Leonel et al., 2008; Yogui et al., 2011). However, considering the highest pyrethroid levels in the same species that were in fetal muscle, PYR presented levels similar to PCBs, 1 order of magnitude higher than PBDEs and DDTs and 2 orders of magnitude higher than PFOS in females from the same population (Alonso, 2008; Leonel et al., 2008; Yogui et al., 2011). In addition, the lowest concentration among all matrices analyzed was found in milk (mean 3.40 ng/g lw, ±1.35, Fig. 1d). The mean level of PYR in breast milk of women from São Paulo was very similar to those found in the milk of dolphins from the same area (5.25 ng/g lw, ±3.00) (Corcellas et al., 2012), suggesting a similarity in PYR levels in marine and terrestrial mammals from the same region.

Pyrethroid concentrations in Guiana dolphins in the present study from Sepetiba Bay (RJ) were similar to DDTs and 1 order of magnitude lower than PCBs in females from the same population (Lailson-Brito et al., 2010), and were similar to PBDEs and PFOS values in Guiana females from RJ (Dorneles et al., 2010). Guiana dolphins from C3 region (CE) presented PYR levels between 5 and 6 orders of magnitude higher than DDTs and PCBs levels analyzed in mature females from the same population (Santos-Neto et al., 2014). The present data showed a pronounced concern in PYR accumulation in Franciscanas fetus from São Paulo coast and in Guiana dolphin females from Sepetiba Bay due to a number of other contaminants also present in both environments and in Ceará where pyrethroids had much higher contribution than organochlorines previously monitored. Therefore, our findings warrant a need for a regular monitoring of PYR in Brazilian coastal dolphin populations (especially Franciscana and Guiana dolphins), as well as pyrethroids are organohalogens (with exception of tetramethrin), and should be monitored along with other halogenated organic contaminants previously reported, in order to evaluate its long-term status in marine mammals as well in the marine environment.

3.2.2. Accumulation between tissues: blubber vs. muscle

A relevant point is that comparing the PYR levels in blubber and muscle of the two dolphin species analyzed in the present study, muscle was the organ that presented the highest concentrations ($p < 0.001$, Fig. 1a, b and c). To our knowledge, this is the first time that such observation is described. Total PYR concentrations were inversely proportional to the lipid content of the organs. Samples rich in lipids, such as blubber and milk, presented the lowest PYR concentrations, whereas organs with low fat content e.g. muscle, cord and placenta; showed the highest levels of the insecticides (Franciscana $p = 0.024$, $r = -0.66$; Guiana $p = 0.009$, $r = -0.91$). These data may indicate a pattern of preferential accumulation of PYR in muscle tissue, unlike other organohalogen contaminants that are highly lipophilic and tend to accumulate preferentially in tissues with higher percentage of lipids, such as blubber (Raach et al., 2011; Vetter, 2001). PFOS presented the same behavior in harbor porpoises from Red Sea, where muscle presented higher level than blubber and the highest concentration was measured in fetus (Van De Vijver et al., 2007). As it is known, PFOS has a different accumulation pattern from the other organohalogens, as it preferentially binds to blood proteins and accumulates in different tissues (e.g. liver, kidney and muscle) (Van De Vijver et al., 2007). As the results showed, pyrethroids seem to behave similarly with PFOS, since they are also organohalogen compounds, containing fluorine, bromine and/or chlorine (with exception of tetramethrin that is not an halogenated) (Feo et al., 2011).

3.2.3. Pattern of distribution

Permethrin was the PYR predominant in 68% of the dolphin samples in this study, at concentrations ranging from below mLOD (a blubber sample from a pregnant and lactating female) to 5280 ng/g lw (a muscle sample from a fetus) as showed in Table 1 and in Figure (Fig. S2). Permethrin is known as being more acutely toxic to children than to adults and female rats exposed to this insecticide during pregnancy generated calves who exhibited neurological effects (Cox, 1998; Horton et al., 2011; Nasuti et al., 2014; Sinha et al., 2004). The effects of pyrethroids in marine mammals are unknown, but the hypothesis of a synergistic effect of those entire chemical cocktail in dolphin fetuses cannot be discarded. More studies are needed in order to evaluate the toxicological effects of pyrethroids insecticides in marine mammals.

The next most abundant PYR was cypermethrin, which appeared in 32% of the samples as the predominant PYR (<mLOQ to 1110 ng/g lw). The highest levels of cypermethrin were found in placenta and muscle (Table 1, Fig. S2). USEPA (2002) and Assayed et al. (2010) reported abnormalities in the offspring and decreased calf survival percentage in mammals fed cypermethrin in their diets during pregnancy, which demonstrate the potential of cypermethrin to induce health risks for females and their progeny. From 1996 to 2003, cypermethrin was the mainly insecticide used in Brazil as malaria control (Santos et al., 2007) and it has been heavily used for dengue control since 1999 (Da-Cunha et al., 2005). However, the most abundant pyrethroid in the samples from this study was permethrin. Both pyrethroids have similar log K_{ow} (permethrin 6.5 and cypermethrin 6.6), though permethrin exhibits relatively longer half life in aerobic and anaerobic soils (39.5 days and 197 days, respectively) compared with cypermethrin (27.6 days and 55 days, respectively) (Feo et al., 2010b). Furthermore, permethrin is classified as Type I pyrethroid (lack of cyano group), and cypermethrin as Type II (containing a cyano group in its molecule), which raises the hypothesis that cypermethrin can be more readily degraded compared to permethrin. This hypothesis was supported in studies on sediments from Southern California estuaries, where permethrin was among the most abundant of pyrethroids analyzed; in contrast, the abundance of cypermethrin was half of its average usage, suggesting a relatively lower degree of persistence (Lao et al., 2012, 2010).

Milk was the compartment that presented the most diverse pattern among the matrices analyzed (Fig. S2). In this Figure is possible to observe a minor contribution of cypermethrin in milk samples compared with all other matrices, while delta/tralomethrin and es/fenvalerate had a higher contribution in milk samples compared to maternal blubber samples, as observed in Alonso et al. (2012b). Due to limited available milk samples ($n = 3$), it is difficult to assume, however one reason may be attributed to lipophilicity and persistence of delta/tralomethrin and es/fenvalerate (longer half-lives, up to 209 and 546 days, respectively) compared to cypermethrin, that could might influenced in the pyrethroid transport from blubber to milk.

Bifenthrin was detected in most Franciscana and Guiana tissue samples. In contrast, cyfluthrin was detected in blubber but not in muscle of Guiana dolphins. In urban creeks of California, bifenthrin found in sediments was the responsible for most amphipod mortality (Amweg et al., 2006; Weston et al., 2009). Weston et al. (2009) concluded that cyfluthrin, cypermethrin and permethrin individually were below acutely toxic concentrations; however together they have synergistically toxicity effects.

3.3. Maternal transfer of UV filters

3.3.1. Concentration levels: fetuses vs. mothers

UVFs were detected in all mother-fetus pair from Guiana and Franciscana dolphins in this study and the concentrations of 4-methylbenzylidene camphor (4MBC), 2-ethyl-hexyl-4-trimeth oxycinnamate (EHMC), 2-ethyl-hexyl-4-dimethyl-aminobenzoate

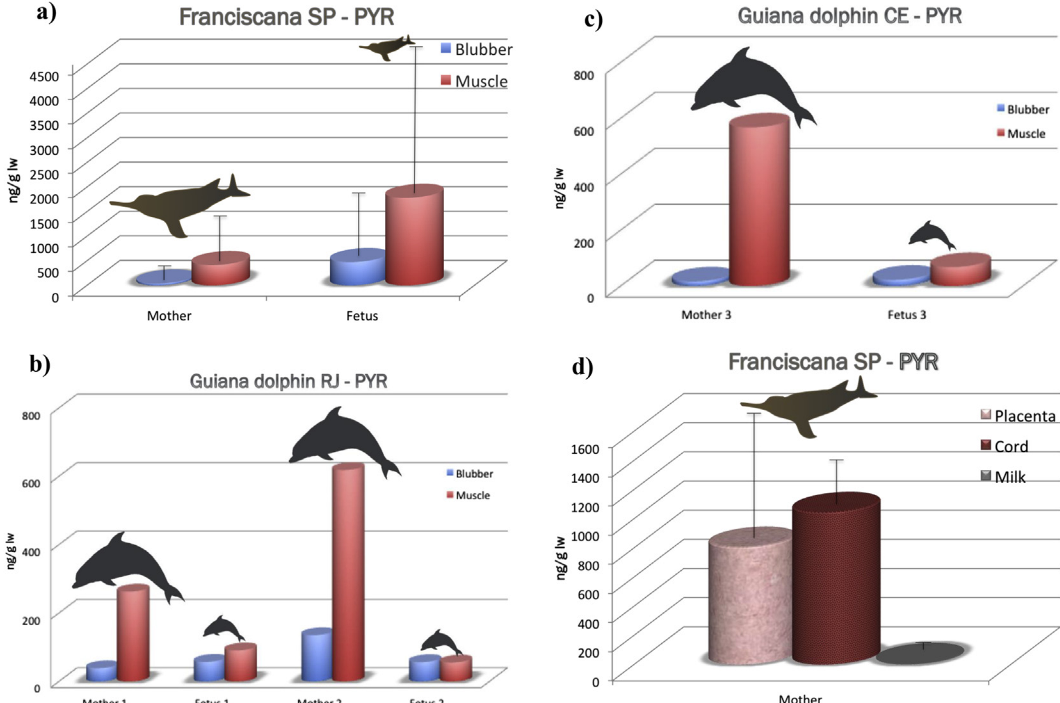

Fig. 1. a. ∑PYR concentrations (mean and standard deviation, expressed in ng/g lw) in maternal and fetal blubber and muscle of Franciscanas from Baixada Santista (SP), Brazil, South Atlantic. **b.** ∑PYR concentrations (two mother-fetus pairs, expressed in ng/g lw) in maternal and fetal blubber and muscle of Guiana dolphins from Sepetiba Bay (RJ), Brazil, South Atlantic. **c.** ∑PYR concentrations (one mother-fetus pair, in ng/g lw) in maternal and fetal blubber and muscle of Guiana dolphins from Canoa Quebrada (CE), Brazil, South Atlantic. **d.** ∑PYR concentrations in placenta, cord and milk (mean and standard deviation, ng/g lw) of Franciscanas from São Paulo coast, Brazil.

(OD-PABA) and octocrylene (OCT) are shown in Table 2. Mean ΣUVF concentrations in Franciscana maternal blubber was 55.8 ng/g lw (SD ± 39.0); in maternal muscle 381.7 ng/g lw (SD ± 389); in fetal blubber 126.3 ng/g lw (SD ± 95); and in fetal muscle 4108 ng/g lw (SD ± 6101). ΣUVF concentrations in Guiana dolphin from RJ and CE are reported in Table 2.

Fetus/mother ratios (F/M) of blubber and muscle ΣUVF concentrations were calculated, in order to assess the maternal transfer of these contaminants during gestation. F/M in Franciscanas were 0.07, 1.9, 3.52 and 8.19 in blubber tissue (n = 4 pairs) and 5.47 and 267 (n = 2 pairs) in muscle tissue, indicating a higher potential for bioaccumulation of sunscreen agents in the fetus than in their mothers. Similar findings in relation to HCB occurred in long-finned pilot whales (*G. melas*) from Tasmania (F/M from 1 to 1.5) (Weijs et al., 2013) and PFOS in Guiana dolphins from RJ (F/M = 2.75 and 2.62) (Dorneles et al., 2008a), but in a lower scale. A similar trend was observed for Franciscana in relation to PYR, suggesting that the fetus receives the major levels of these emerging organic pollutants in comparison to the adults.

In the other hand, a dramatic lower UVF input from female to fetus in Guiana dolphins occurred. F/M ratios of ΣUVF concentrations in *S. guianensis* were <mLOD and 0.06 for blubber and 0.25 and 0.03 for muscle in RJ dolphins, and 0.51 and 0.20 blubber and muscle, respectively in CE mother-fetus pair. ΣUVF concentrations in maternal muscle of Guiana dolphin were significantly higher ($p = 0.035$) than in Franciscana females. Guiana dolphins from both areas (RJ and CE) presented higher ΣUVF concentrations in maternal tissues (blubber and muscle) and lower in fetal tissues (F/M < 1) compared to Franciscanas. This fact may be due to Guiana dolphins could have a different (i) UVFs bioaccumulation and/or (ii) UVFs transplacental transfer and/or (iii) UVFs metabolic/detoxification rates and/or (iv) number of pregnancies, in relation to Franciscanas (Fig. 2a, b and c, Table S1). In Table S1 is possible to observe that Guiana females from Sepetiba Bay in this study had 17 and 13 pregnancies (Pair 1 and Pair 2, respectively) according to the number of corpus albicans and luteum in their ovaries, and Franciscanas probably had one or two pregnancies due to the number of corpus luteum, their age (2–4 years old), gestation period (mean of 10.22 months) and maturity age (1.2–1.8 years old) (Bertozzi, 2009). The lower transfer rates from mother to fetus in Guiana compared to Franciscana dolphins can be related to Guiana females have transferred their contaminant loads to previous offsprings, and Franciscanas were transferring for the first progeny that receives the higher amount of contamination compared with later birth order (Weybridge, 2012).

Although the higher UVF levels in Guiana dolphins compared with Franciscana can be related to (i) a higher bioaccumulation in Guiana tissues and/or (ii) higher biomagnification in Guiana trophic chain, and or (iii) a lower detoxification in Guiana, and/or (iv) females' age and/or (v) regional differences. Guiana dolphin females in this study were older (Mother 1 was 17 years old and Mother 2 was estimated to 18 years old) than Franciscanas (2–4 years old) and have accumulated UVF for a longer period of time in their tissues (Table S1 – Guiana dolphins estimative of age was based on 13 pregnancies, 11.4 months per gestation and maturity age of 6 years old (Ramos et al., 2000)). Another hypothesis might be a regional difference among dolphin populations, since Guiana dolphins were collected in RJ (22° S) and CE (5° S) that are tropical areas in Brazil where the use of sunscreen by the human population might be higher than in São Paulo (24° S) where Franciscanas were collected which is a subtropical area.

ΣUVF concentrations in muscle (11,530 and 10,475 ng/g lw, Franciscana fetus from Pair 1 and Guiana mother from Pair 2, respectively) were the highest-ever reported in biota. The two pregnant Guiana dolphins from Rio de Janeiro coast were found dead, and necropsy results revealed that both fetuses, although

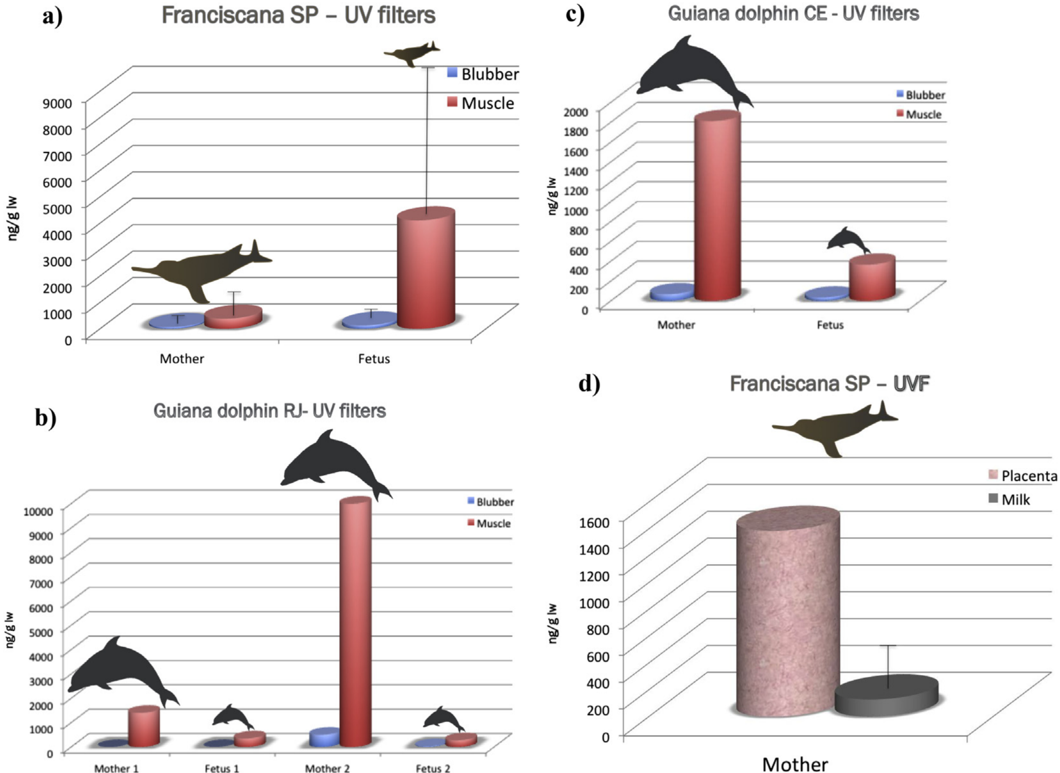

Fig. 2. **a.** ∑UVF concentrations (mean and standard deviation, expressed in ng/g lw) in maternal and fetal blubber and muscle of Franciscanas from Baixada Santista (SP), Brazil, South Atlantic. **b.** ∑UVF concentrations (two mother-fetus pairs, expressed in ng/g lw) in maternal and fetal blubber and muscle of Guiana dolphins from Sepetiba Bay (RJ), Brazil, South Atlantic. **c.** ∑UVF concentrations (one mother-fetus pair, in ng/g lw) in maternal and fetal blubber and muscle of Guiana dolphins from Canoa Quebrada (CE), Brazil, South Atlantic. **d.** ∑UVF concentrations in placenta and milk (mean and SD, ng/g lw) of Franciscanas from São Paulo coast, Brazil.

almost completely formed, had open abdominal cavity, incompatible with life (Marigo personal comm.). The agent responsible for the lesion in both fetuses is still unknown, but this developmental anomaly occurring in both animals from the same population, may suggest a teratogenic effect of the pollutants found in high concentrations in those pregnant females. Guiana dolphin populations that inhabit Rio de Janeiro state waters are highly exposed to all organic pollutants already targeted in samples from their tissues (e.g. PCBs, PBDEs, PFOS, PCDDs, PCDFs, OCPs and OTs) (Dorneles et al., 2013, 2010, 2008a, 2008b; Lailson-Brito et al., 2010), which can lead these individuals to be subject to adverse health effects associated with chronic exposure to a cocktail of POPs.

UVF concentrations in Franciscanas maternal blubber and muscle were similar to PBDEs, DDTs and PCBs (Alonso, 2008; Alonso et al., 2012a) and were higher than PFOS concentrations in blubber and liver of females from the same population previously analyzed (Alonso, 2008; Alonso et al., 2012a; Leonel et al., 2008; Yogui et al., 2011). Maternal blubber ∑UVF levels in Guiana dolphins from Sepetiba Bay (RJ) were 1 order of magnitude lower than PCBs, but in similar levels than DDT reported in mature female from the same population (Lailson-Brito et al., 2010). However, in maternal muscle from the same animals, ∑UVF concentrations were 1 order of magnitude higher than hepatic concentrations of PBDEs and PFOS in mature female from Rio de Janeiro (Dorneles et al., 2010, 2008a). Organochlorinated compounds were analyzed in Guiana female from C3 area in Ceará and the concentrations in blubber of mature females were 6–7 orders of magnitude lower than UVF concentrations found in this study (Santos-Neto et al., 2014). In other words, it is possible to observe that UVF levels verified in this study should be monitored in cetacean populations in future studies, in order to verify their behavior in a long range term.

In order to investigate the maternal transfer of UVFs in dolphins, placenta and milk samples of Franciscana dolphins were analyzed (Fig. 2d). All four UV filters analyzed in this study (OD-PABA, EHMC, 4MBC and OCT) were observed in Franciscanas' milk. ΣUVF concentrations in fetus from both cetacean species showed a transplacental discharge of these sunscreen agents, as well as they were present in milk.

3.3.2. Accumulation between tissues: blubber vs. muscle

The highest concentrations of sunscreen agents (UVFs) were observed in the muscle in both dolphin species analyzed from the Brazilian coast (Franciscana $p < 0.01$ and Guiana $p = 0.03$, Fig. 2). The lipid content was inversely proportional to UVF concentrations in both species (Franciscana $r = -0.66$ and Guiana $r = -0.83$). Log K_{ow} values of the four UVFs analyzed (OD-PABA, EHMC, 4MBC and OCT) ranged from 4.95 to 6.90 (Gago-Ferrero et al., 2012). In a previous study, 4MBC exhibited species-specific accumulation in fish and was detected at higher levels in muscle than in offal (Nagtegaal et al., 1997). And a review paper about organic UVFs in aquatic biota concluded that muscle has been the preferred sample for analysis in fish, despite that individuals showed preferential accumulation pattern according to species (Gago-Ferrero et al., 2012).

3.3.3. Pattern of distribution

The detection frequency of UVFs in the samples analyzed was as follows: EHMC 76%, 4MBC 68%, OD-PABA 47%, and OCT 34% (Fig. S3). Due to a lack of information on UVFs in marine mammals, concentration comparisons of the data in the present study to others cannot be made. The only other study was published by our group, and it comprised the determination of hepatic OCT in Franciscana dolphins, with levels in liver tissues up to 780 ng/g lw

(Gago-Ferrero et al., 2013).

The pregnant Franciscana females from this work correspond to individuals from the previously studied population (Gago-Ferrero et al., 2013). The currently presented OCT concentrations in fetal muscle are 15 times higher than those presented in liver from adult and juveniles animals. We previously suggested that UVFs have the potential to undergo maternal transfer based on the detection of OCT in one paired placenta and liver sample and in the calves analyzed (Gago-Ferrero et al., 2013). Further, the data presented here provides strong evidence for maternal transfer of UVFs based on detection in all paired samples of Franciscana (n = 5 pairs, 100%) and Guiana dolphins (n = 3 pairs, 100%), in the fetus and maternal bubbler and muscle, and in both the placenta and milk samples.

When OCT was detected in dolphin tissues, it corresponded to the highest contribution of ΣUVFs (Table 2, from 50 to 11,130 ng/g lw). Muscle was the predominant tissue to accumulate OCT.

The samples of Guiana dolphins collected from the Ceará coast belong to a region of marine algal banks and phanerogams (*Halodule wrightii*). This area is also known by the banks of calcareous algae (*Halimeda genus*), natural habitat of lobsters, one of the main fish stocks in the region (CEARA, 2014). This indicates that sunscreens are also present in this marine ecosystem in Brazil. Ecotoxicological studies have demonstrated estrogenic activity of the most commonly used sunscreen agents, additionally, with the potential for synergistic effects there is an urgent need for long-term occurrence studies in biota (Buser et al., 2006; Díaz-Cruz and Barceló, 2009; Gago-Ferrero et al., 2012; Schreurs et al., 2005).

4. Conclusions

This is the first time where tissue samples of mother-fetus pair from wild mammals were analyzed to evaluate the occurrence of PYR and UVF contaminants, proving the prenatal transfer of these compounds that are in heavy use worldwide. Muscle is the preferential organ for PYR and UVF bioaccumulation in comparison to the blubber. It differs from the former reports concerning well-known organic chlorinated and brominated contaminants, where the blubber was the target tissue for the analysis. However, they showed a similar behavior to PFOS, which preferentially bind to blood proteins. More studies are necessary in order to identify the ideal tissue for monitoring these novel contaminants in sentinel species such as dolphins.

Fetal tissue samples contained higher levels of PYR and UVF than their respective mothers in Franciscanas. Fetal blubber samples contained higher levels of PYR than their respective mothers and maternal muscle samples contained higher levels of UVF than their respective fetuses in Guiana dolphins. Fetal exposure to this cocktail of pesticides and chemical sunscreen agents may result in adverse teratogenic effects in calves, as the organ growth and development is at its maximum rate and vulnerability during the prenatal period.

The concentrations of PYR and UVFs detected in this study are of concern for those dolphin species from Brazilian coastal waters, as these compounds have been shown to be risk factors for cancer, immune deficiency, and reproductive abnormalities. Franciscana presented higher concentration of PYR than Guiana dolphins and the opposite is true for UVF. Additionally, both dolphin species inhabit anthropogenically disrupted environments they face a number of known and unknown threats, but also represent a good sentinel species for a regulated and targeted environmental monitoring program.

To our knowledge, this is the first study to evaluate the occurrence of UVFs in paired maternal and fetal tissues. Our results suggest prenatal transfer of these compounds, since ΣUVF concentrations were found in fetal tissues. Whereas ΣPYR concentrations were also observed in fetal samples, we can define PYRs and UVFs as transplacental contaminants.

Since dolphins are considered good sentinels for human exposure to marine pollutants, the same transfer might be occurring in humans. Future studies are warranted in order to demonstrate if the same pattern of the transplacental transfer occurs in humans as was indicated in the present study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.09.039.

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A Potential New Threat to Wild Life: Presence of UV Filters in Bird Eggs from a Preserved Area

Daniel Molins-Delgado,[†] Manuel Máñez,[‡] Ana Andreu,[‡] Fernando Hiraldo,[§] Ethel Eljarrat,[†] Damià Barceló,[†,∥] and M. Silvia Díaz-Cruz*[,†]

[†]Environmental Chemistry Department, Institute of Environmental Assessment and Water Research (IDÆA), Spanish Council of Scientific Research (CSIC), C/Jordi Girona 18-26, 08034, Barcelona, Spain

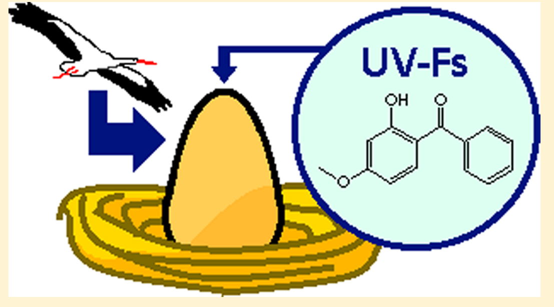
[‡]Natural Processes Monitoring Team, Doñana Biological Station (EBD-CSIC), C/Américo Vespucio s/n, 41092 Seville, Spain

[§]Department of Applied Biology, Doñana Biological Station (EBD-CSIC), Sevilla, Spain

[∥]Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona. C/Emili Grahit, 101 Edifici H2O, 17003 Girona, Spain

S *Supporting Information*

ABSTRACT: The present study uses bird eggs of seven wild species as a biomonitoring tool for sunscreens occurrence. Seven UV filters (UV-Fs), including 3 hydroxy-metabolites of oxybenzone (benzophenone 3, BP3) were characterized in unhatched eggs from Doñana Natural Space (Spain). High frequency of detection was observed for all UV-Fs, ranging from 95% to 100%. The oxybenzone metabolite 4-hydroxybenzophenone (4HB) was ubiquitous at concentrations in the range 12.0−3348 ng g^{-1} dry weight (dw). The parent compound, oxybenzone, was also present in all samples at lower concentrations (16.9−49.3 ng g^{-1} dw). Due to the three BP3′s metabolites, benzophenone 1 (BP1), 4HB, and 4,4′-dihydroxybenzophenone (4DHB) presence in unhatched eggs, it can be inferred that parent compounds are absorbed into the bird through the upper gut and the OH-derivatives formed are transferred by the mother to the egg before the lying. White stork (*Ciconia ciconia*) and western marsh harrier (Circus aeruginosus) were the most contaminated species, with mean total UV-Fs concentrations of 834 and 985 ng g^{-1} dw, respectively. Results evidenced that biomagnification process across the bird species studied cannot be ruled out.



1. INTRODUCTION

In the past decade, ingredients in personal care products (PCPs) have been described as chemicals of increasing environmental concern because of their extensive use, toxicity, persistence, bioaccumulation, and ubiquity.[1] Thousands of tons per year of PCPs are produced, which ultimately enter the environment through bathing, industrial and urban sewage effluents discharges and disposal of unwanted products.[2] As a consequence, PCPs have been frequently detected in water bodies,[3,4] sediment,[5,6] and aquatic biota,[7−9] raising environmental concern because of their endocrine disrupting activity.[10,11]

The current knowledge on these compounds reveals that PCPs cycling in the environment, particularly the correlation between the aquatic environmental occurrence and bioaccumulation profiles, appear to be similar to that of persistent organic pollutants (POPs). Among PCPs, UV filters (UV-Fs) have attracted the highest interest of scientists and of the public in general due to their extensive use, widespread occurrence and scarcity of data on their environmental impacts. UV-Fs are important ingredients in hygiene and beauty products, but also in plastics, paints, textiles and adhesives.[12] As a result of their lipophilicity (log $K_{ow} > 3$) these substances tend to accumulate in living organisms; for instance, 2-ethylhexyl-*p*-methoxycinnamate (EHMC) has been measured above 200 ng g^{-1} dry weight (dw), octocrylene (OC) at 30 ng g^{-1} dw, and oxybenzone (benzophenone 3, BP3) up to 24 ng g^{-1}dw in river fish from Spain.[9] These values are in good agreement with the concentrations reported in fish from other European rivers.[8,13−15] The highest levels of EHMC (>290 ng g^{-1}dw) were detected in the predator species Andalusian barbel (*L. sclateri*), suggesting that EHMC biomagnification may occur. The potential biomagnification capacity of EHMC was also pointed out by Fent et al.[8] when detecting EHMC in crustacean and molluscs from Switzerland in the range 22−50 ng g^{-1} lipid weight (lw), but above 700 ng g^{-1} lw, in aquatic fish.

The bioaccumulation of UV-Fs in marine biota has also been investigated. Gago-Ferrero et al.[16] recently provided the first data on the occurrence of UV filter residues in marine mammals. OC was measured up to 782 ng g^{-1} lw in liver tissue

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from *Franciscana* dolphin (*Pontoporia blainvillei*) from Brazil. In another study, the same authors evidenced the maternal transfer of UV-Fs in *Franciscana* and *Guiana* dolphins. UV-Fs were detected in all mother-fetus pairs from both species. Unexpectedly, tissues showed up to 20-folds higher burden in fetuses than in their respective mothers. Hence, transplacental discharge was suggested as the UV-Fs contamination pathway to fetuses.[17] In mussels from the French coast EHMC and OC were found up to 7000 ng g^{-1} dw and 200 ng g^{-1} dw, respectively.[18] Benzotriazoles have also been detected in aquatic species, including tidal flat organisms, fish, mussels, birds, and hammerhead sharks from the Japan coast, at concentrations in the range 7.9−720 ng g^{-1} dw.[6]

As the removal of unhatched eggs from the nests has no effect on the population level in the wild bird species, birds have been adopted as reliable indicators of persistent pollutants' bioaccumulation. Hence, the occurrence of POPs in bird eggs has been frequently addressed worldwide.[19−21] Nevertheless, to the authors' knowledge no previous study reported bioaccumulation profiles of UV-Fs in eggs. In this frame, the aim of the present study was to investigate the occurrence and to determine the concentration and maternal transfer of seven sunscreen agents in unhatched eggs of different wild bird species from Doñana, a preserved natural area located in southwest Spain. In addition, the stable isotopes $\delta^{15}N$ and $\delta^{13}C$ were determined and their ratios calculated in order to determine whether UV-Fs transfer along the food web occurs.

2. MATERIALS AND METHODS

2.1. Standards and Reagents. The sunscreens 2,4-dihydroxybenzophenone (BP1, 99%), 2-hydroxy-4-methoxybenzophenone (oxybenzone, BP3, 98%), 4-hydroxybenzophenone (4HB, 98%), 4,4′-dihydroxybenzophenone (4DHB, 99%), 2-ethylhexyl 2-cyano-3,3-diphenyl acrylate (OC, 97%), and 2-ethylhexyl 4-(dimethylamino)benzoate (ODPABA, 98%), and 2-(2H-benzotriazole-2-yl)-4-methyl-6-(2-propyl)-phenol (AllylBZT, 99%), used as internal standard for 2-(2-benzotriazolyl)-p-cresol (UVP) were purchased from Sigma-Aldrich (Steinheim, Germany). UVP (98%) was obtained from TCI (Antwerp, Belgium). BP3-d_5 (98% D), and 3-(4-methylbenzylidene) camphor-d_4 (4MBC-d_4, 98% D), used as internal standards, were supplied by CDN isotopes (Quebec, Canada). Supporting Information (SI) Table S1 lists the name, abbreviation, CAS number and log K_{ow} of the target compounds.

Ethyl acetate (AcEt), dichloromethane (DCM), methanol (MeOH) and water, all HPLC-grade, as well as the adsorbent Florisil (0.150−0.250 mm) were purchased from Merck (Darmstadt, Germany).

2.2. Sampling Area and Sample Collection. Egg samples were collected in the Doñana Natural Space, in the surrounding areas of La Puebla del Río and Almonte, as Figure 1 shows. This preserved area is located in southwest Spain and constitutes a sanctuary for thousands of bird species. Its location between the African and the European continents together with its proximity to the Mediterranean Sea and the Atlantic Ocean makes that area a must-step for migrating birds.[22]

In the present study, 39 unhatched bird eggs of 7 wild species and 4 different orders, that is, falconiformes (western marsh harrier (*Circus aeruginosus*), common kestrel (*Falco tinnunculus*)), ciconiiformes (white stork (*Ciconia ciconia*)), charadriiformes (slender-billed gull (*Chroicocephalus genei*), black

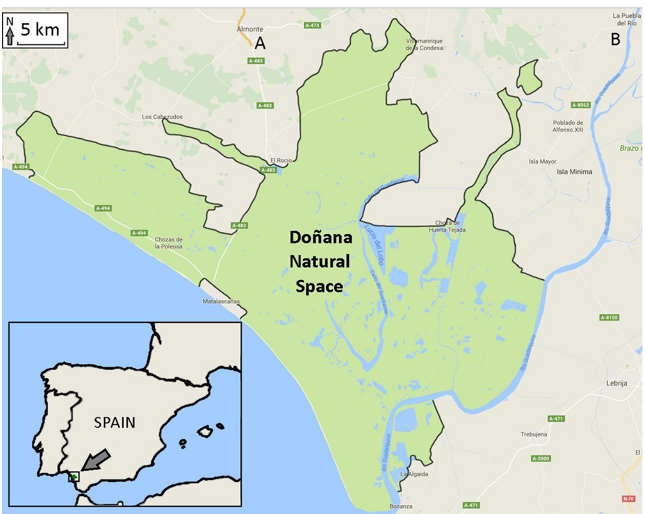

Figure 1. Map showing the location of the Doñana Natural Space (Spain) (in light gray) and the sampling areas of Almonte (A) and La Puebla del Río (B).

headed gull (*Chrococephalus ridibundus*), gull-billed tern (*Gelochelidon nilotica*)), and anseriformes (gadwall (*Anas Strepera*)) were collected during nest checking and ringing operations between 2010 and 2012. The eggs were frozen and shipped to the lab under cool conditions. Once in the lab the samples were thawed, homogenized, lyophilized and stored at −20 °C until analysis. No embryos were found in any of the studied eggs. Table 1 compile information related to the habitat, diet, and general ecology of the studied species.

2.3. Samples Pretreatment and Analysis. The pretreatment procedure and the analysis methodology applied were adapted from a previously developed method in our laboratory for the analysis of UV-Fs in fish.[23] A total of 0.1 g of freeze-dried sample was needed for the simultaneous analysis of the target UV-Fs. UV-Fs extraction from the samples was carried out by pressurized liquid extraction (PLE) using an ASE 350 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA) followed by a solid phase extraction (SPE) using Isolute C18 cartridges, from Biotage. The extracts were then prepared for the analysis. The separation and detection of the analytes were performed by liquid chromatography-tandem mass spectrometry in positive ionization mode using a Symbiosis Pico chromatograph from Spark Holland (Emmen, The Netherlands) attached to a 4000 Q TRAP MS/MS analyzer from Applied Biosystems-Sciex (Foster City, CA) (HPLC-QqLIT-MS/MS). A Hibar Purospher STAR HR R-18 ec. (50 mm × 2.0 mm, 5 μm) from Merck, preceded by a guard column of the same packaging material was used during the chromatographic separation. A detailed description of the pretreatment and analysis methods can be found in section A.1. of the SI.

The lipid content was determined gravimetrically. Lipids and water contents are listed in Table S2 in the SI.

2.4. Method Performance. For all target compounds a linear response was achieved in the range 0.1−500 ng g^{-1} dw. The instrumental limits of detection (ILODs) ranged from 0.1 to 5.7 pg, whereas the instrumental limits of quantification (ILOQs) were in the range 0.5−18.9 pg. Precision, expressed as relative standard deviation (RSD), were always below 18%.

Recovery rates were evaluated for five replicates at two concentration levels, 25 and 50 ng g^{-1} dw obtaining values

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Table 1. Order, Common Name, Scientific Name, Migratory and Feeding Behavior, Number of Samples Analyzed, And Sampling Point of Each Species

order	common name	scientific name	migratory behavior	feeding behavior	number of samples	sampling location
Falconiformes	Western marsh harrier	*Circus aeruginosus*	resident and dispersive	birds, small mammals, and eggs	1	La Puebla del Río
	Common kestrel	*Falco tinnunculus*	resident and dispersive	birds, small mammals, insects, and reptiles	10	Almonte
Ciconiiformes	White stork	*Ciconia ciconia*	mainly migratory but with a resident population	small fish and birds, insects, rodents, amphibians, reptiles, eggs and remains of human food	8	La Puebla del Río
Charadriiformes	Slender-billed gull	*Chrococephalus genei*	migratory but with a resident population	fish and invertebrates	1	La Puebla del Río
	Black-headed gull	*Chrococephalus ridibundus*	dispersive and partially migratory	fish, insects, earthworms, and urban and industrial wastes	4	La Puebla del Río
	Gull-billed tern	*Gelochelidon nilotica*	migratory	fish, insects, and crustaceans	4	La Puebla del Río
Anseriformes	Gadwall	*Anas strepera*	resident	roots, leaves, seeds, and algae	11	La Puebla del Río

Table 2. Maximum, Minimum, and Mean Concentrations, and Frequency of Detection for the Target UV-Fs in 0.1 g of Egg Sample of the Studied Species[a,b]

				concentration ngg^{-1} dw						
order	common name	species		BP1	BP3	4HB	4DHB	ODPABA	OC	UVP
Falconifoormes	Western marsh harrier	*Circus aeruginosus*	maximum	na	na	na	na	na	na	na
			minimum	na	na	na	na	na	na	na
			mean	40.6	46.7	895	<MLOQ	<MLOQ	<MLOQ	2.7
			frequency (%)	100	100	100	100	100	100	100
	Common kestrel	*Falco tinnunculus*	maximum	53.5	35.2	1200	132	<MLOQ	<MLOQ	3.0
			minimum	27.9	18.3	20.0	<MLOQ	<MLOQ	<MLOQ	0.4
			mean	39.3	24.8	210	132	<MLOQ	<MLOQ	1.5
			frequency (%)	100	100	90	100	100	100	100
Ciconiiformes	White stork	*Ciconia ciconia*	maximum	<MLOQ	29.2	3348	29.0	<MLOQ	26.6	<MLOQ
			minimum	<MLOQ	19.5	104	<MLOQ	<MLOQ	15.2	<MLOQ
			mean	<MLOQ	22.3	761	29.0	<MLOQ	20.9	<MLOQ
			frequency (%)	100	100	100	100	88	100	88
Charadriiformes	Slender-billed gull	*Chorocephalus genei*	maximum	na	na	na	na	na	na	na
			minimum	na	na	na	na	na	na	na
			mean	<MLOQ	22.5	266	<MLOQ	<MLOQ	<MLOQ	<MLOQ
			frequency (%)	100	100	100	100	100	100	100
	Black-headed gull	*Chorocephalis ridibundus*	maximum	677	44.4	111	<MLOQ	<MLOQ	65.2	8.4
			minimum	<MLOQ	24.7	72.8	<MLOQ	<MLOQ	11.4	1.4
			mean	433	34.8	88.4	<MLOQ	<MLOQ	33.5	4.9
			frequency (%)	100	100	100	100	100	100	75
	Gull-billed tern	*Gelochelidon nilotica*	maximum	66.1	49.3	472	<MLOQ	<MLOQ	<MLOQ	2.0
			minimum	29.0	18.2	12	<MLOQ	<MLOQ	<MLOQ	1.2
			mean	42.4	28.3	139	<MLOQ	<MLOQ	<MLOQ	1.6
			frequency (%)	100	100	100	100	100	100	100
Anseriformes	Gadwall	*Anas strepera*	maximum	87.3	31.5	560	<MLOQ	<MLOQ	<MLOQ	0.2
			minimum	23.3	16.9	13.5	<MLOQ	<MLOQ	<MLOQ	<MLOQ
			mean	38.1	22.1	122	<MLOQ	<MLOQ	<MLOQ	0.2
			frequency (%)	100	100	100	100	100	100	100

[a]na, not applicable. [b]<MLOQ, below the MLOQ.

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between 39 and 125%. Method limits of detection (MLOD) and quantification (MLOQ) were calculated following the IUPAC guidelines.[24] MLODs were in the range 0.04−0.5 ng g^{-1} dw, whereas MLOQ ranged from 0.1 to 1.8 ng g^{-1} dw. The complete list of values is presented in SI Table S3.

2.5. $\delta^{13}C$ and $\delta^{15}N$ Isotope Analysis. The determination of the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ is described in a previous work.[22] Briefly, lipids were extracted from the sample using a mixture of chloroform : MeOH (2:1, v:v). The samples were completely covered with the mixture for 24 h. before being removed. This process was repeated 3 times. After drying the samples at 50 °C, they were combusted at 1020 °C and determined using MS. The values, expressed using the standard δ−notation (‰) are listed in SI Table S2.

2.6. Statistical Analysis. Prior analysis, the differences between samples were assessed performing a *t*-student test of equal variances. The statistical significance was set at 5%. No significant difference was observed and thus the data was not normalized. All extend of association between two variables evaluated in the present study were carried out using Spearman's correlation for nonparametric data test.

3. RESULTS AND DISCUSSION

3.1. UV-Fs' Concentration Profiles in Bird Eggs. The results revealed that all the eggs contained residues of UV-Fs. All target analytes were very frequently detected in the samples (95−100% frequency), however 4DHB, ODPABA, OC and UVP were mostly at levels below the corresponding MLOQs. In particular, ODPABA was detected but could not be quantified. Maximum, minimum, and mean concentrations measured of UV-Fs are shown in Table 2. Oxybenzone, the parent compound of 4HB, 4DHB and BP1, was present in all the samples at much lower concentrations, from 16.87 to 49.28 ng g^{-1}dw. The hydroxylated (OH−) metabolite 4HB was ubiquitous and the most concentrated one, showing a maximum level of 3488 ng g^{-1} dw in white stork. Another of its metabolites, BP1, was also accumulated at high concentrations in all the species (23.3−677 ng g^{-1} dw) with the exception of the white stork, where the compound could not be quantified. In contrast, the dihydroxylated metabolite, 4DHB, was scarcely found. OC was detected in all samples, with the highest levels found in white stork and black-headed gull. UVP was the less frequently found compound (95%) and the one at lower concentrations (up to 8.4 ng g^{-1}dw). Tables S4, S5, and S6 in the SI show the complete list of concentrations with different units.

According to the EU Cosmetics Regulation (Regulation (EC) *N*° 1223/2009), the maximum concentrations of BP3, ODPABA and OC allowed for PCPs formulations are 10%, 8% and 10% (as acid), respectively. The lower allowable proportion of ODPABA, jointly with a relatively short half-life time in water in the presence of UV sunlight and free chlorine,[25,26] could, therefore, be the reasons behind why this compound was scarcely determined in the eggs analyzed.

Benzotriazoles (2-hydroxyphenyl benzotriazole derivatives) are employed as UV light blockers in paints, coatings, adhesives, polymeric surfaces, food packings, textiles and construction materials.[27] In the USA, up to 453 tons were produced/imported in 2005.[28] Similar to the UV-Fs used in PCPs, UVP is a sun-blocking agent, which prevents light-induced degradation reactions of host materials. It has exceptionally strong and broad-range UV light absorption properties being a highly efficient UV absorber of both UV A and UV B radiation, recommended for thermoset plastics and coatings that function as UV radiation screens for photosensitive substrates. Furthermore, UVP is very photostable and resistant to oxidizing and reducing agents. This compound is allowed in the EU as additive in food packing plastics.[29] In polystyrene UVP is added in 0.2−0.3% and up to 0.01% in PET.[30] These figures support the lower use and thus the lower presence of this compound in the eggs analyzed in this work.

The difference observed between the UV-Fs and the UVP concentration levels might be explained by the different uses and maximum percentages in formulations set by the regulations. For instance, UV-Fs such as BP1 and BP3 are allowed as additive in both the manufacture of food packing plastics and in cosmetics industry, whereas a sun-blocking agent such as UVP is not currently used in cosmetic formulations. Another feasible reason for such difference in concentrations might be the contamination source. According to the study by Kameda et al.,[31] on the presence of UV blockers in wastewaters, there was no correlation between domestic wastewaters and the occurrence of UVP, suggesting an industrial origin. However, it is well documented that UV-Fs are not efficiently removed under conventional wastewater treatments and the bioaccumulation capacity of these compounds allows them to be retained by the adipose tissue of aquatic organisms.[9,12,32] Hence, due to the absence of industrial activity in the area, the wastewater treatment plants effluents discharges, jointly with urban contributions from the villages surrounding Doñana Natural Space would be the responsible for the UV-Fs contamination.

This is the first study on the identification and quantification of UV-Fs in bird eggs, to the best of our knowledge. Therefore, there are no available values to compare with. However, when comparing egg concentrations with other organisms such as fish and mussels,[8,13−15,33] and other predators such as dolphins,[16,17] the determined concentrations lie within a similar range of concentrations. Nevertheless, regarding bird eggs in previous studies carried out in Doñana, other contaminants such as PBDEs and dechloranes,[22] and pyrethroids were investigated.[34] SI Table S7 shows the concentrations of the pollutants found in the common bird species surveyed in the Park in the three previous referenced studies. Results indicate that UV-Fs mean concentrations were notoriously higher (between 10-fold and 100-fold) in all species compared, whereas PBDEs concentrations reached mean values of up to 80 ng g^{-1} lw, dechloranes concentrations were almost 2-fold higher (161 ng g^{-1} lw), and similar for pyrethroids (162 ng g^{-1} lw). White stork, western marsh harrier, and black-headed gull were consistent among the most contaminated species overall. When correlating the mean concentrations of PBDEs, dechloranes, and pyrethroids with those of UV-Fs, it is observed that dechloranes and UV-Fs follow a similar distribution pattern (ρ = 0.964), suggesting similar accumulation mechanisms. No significant correlation was observed between UV-Fs and PBDEs or pyrethroids (ρ = 0.571 and 0.07, respectively). The measured concentrations of UV-Fs in eggs are in line with those reported for other organic pollutants such as PBDEs, determined in coastal zones worldwide (1.8 and 1400 ng g^{-1} wet weight (ww)).[35−38] However, UV-Fs concentrations were found to be 1 order of magnitude higher than those observed in bird eggs for PCDD/Fs and coplanar PCBs, which were in the range 1.2−1420 ng g^{-1} lw.[19,39−41]

Despite the scarcity of data available so far, some UV-Fs have recently been recognized as important organic contaminants of the aquatic environment by the European Parliament, due to its

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lipophilicity, endocrine disruption properties and potential for bioaccumulation (European Parliament, 2007).[42] As a tentative approach and due to the lack of information on PCPs, we compared our results with the limits set up by the EU for other organic pollutants of concern. The Directive 2000/60/EC (Water Framework Directive, WFD)[43] defines maximum allowable concentrations (MAC) for considered priority substances and other contaminants in water and biota. In view of this, the concentrations of BP3, BP1, 4HB and OC measured in this study exceed the MAC established as environmental quality standards for hexachlorobenzene, hexacyclohexane and mercury (10, 50, and 20 ng g^{-1} ww, respectively) in prey tissue of fish, molluscs, crustaceans, and other biota. It is noteworthy that toxicity between compounds and species can vary significantly, so these assessments must be taken with caution. UVP barely exceeded 2.4 ng g^{-1} ww. By contrast, ODPABA did not reach the MLOQ (0.03 ng g^{-1} ww) and 4DHB and OC seldom exceeded the MLOQ (0.12 and 0.10 ng g^{-1}ww, respectively), constituting a lower risk.

3.2. Bioaccumulation Patterns. Because egg concentrations reflect accumulated contaminants (i.e., parent compounds and metabolites) in food intake by mothers before the egg laying of the birds, species-related differences in contaminants of prey items, foraging ecology, and breeding cycles is reflected in egg contents. The seven analyzed bird species had comparable levels of BP3. However, the relative proportion of the hydroxylated metabolites (i.e., 4HB, 4DHB, and BP1) was variable. 4DHB was not quantifiable in any species, whereas 62% of the samples had superior concentration of 4HB than BP1. It has to be highlighted that the higher values reported of 4HB corresponded to white stork, species in which BP1 was not quantifiable.

The pattern of mean concentration values for BP3 and its metabolites vary across the species. On this basis, three groups could be distinguished: (i) 4HB > BP1 > BP3 (common kestrel, western marsh harrier, gadwall, and gull-billed tern), (ii) 4HB > BP3 and BP1 < MLOD (white stork and slender-billed gull), and (iii) BP1 > 4HB > BP3 (black-headed gull). Although the actual metabolic mechanism is unknown, main factors influencing the BP3 metabolic pattern in birds maybe exposure, type and affinity of ligand binding, and metabolism process and kinetics, as reported for polychlorinated biphenyls (PCBs).[44] Given that none of the analyzed eggs had embryos at any state of development, the potential contribution of metabolites due to the embryos development was discarded.

Regarding the other compounds, OC was only detected in species from groups (i) and (ii), and UVP was not observed in group (ii). It must be pointed out that, in the charadriiformes group each species presents a different concentrations pattern. with only black-headed gull having a different one to the other species of the same order. Of these three species, black-headed gull has a different pattern to that of other species from the same order. This could be related to its feeding behavior, as it is the only one that does not have fish in its diet and nourishes from urban and industrial wastes.

Regarding their migratory behavior, species in group (i) are resident species (except gull-billed tern), species in group (ii) are mainly migratory species, and the species in group (iii) is a dispersive and partially migratory species (Table 1). This suggests that the migratory behavior and the diet intake and further metabolisation of UV-Fs might be correlated. There is scarce information about the accumulation of ODPABA in biota, which may be due to its tendency to get adsorbed on sediment or to readily degrade in the presence of UV sunlight or free chlorine.[25,45]

The mechanisms driving the bioaccumulation of UV-Fs are not yet well understood. Organic lipophilic pollutants such as PCBs, with log K_{ow} between 5 and 7.5 are known to be absorbed through the birds' upper-gut, the same mechanism used to absorb lipids.[46] This study also pointed out that POPs distribution among tissues was not dependent on the compounds' log K_{ow}, but rather on the adipose content of the tissues. On the contrary, it is known that the excretion of these pollutants does rely on their log K_{ow}.[46] This is in agreement with what Gago-Ferrero et al.[9] discussed concerning lipophilic UV-Fs (log K_{ow} > 3) in fish and lipid content in the tissue.

Compounds with higher log K_{ow} are good candidates to undergo biomagnification through the food web; those more polar are expected to be metabolized in the gut and excreted. However, if the metabolism reactions take place within the organism tissues, contaminants, and metabolites may be protected from elimination processes, be accumulated, and then transferred to the offspring.[46,47] The most common metabolic processes involve the action of enzymes that react more easily with the alkyl-groups and facilitate the formation of low log K_{ow} hydroxylated derivatives.[44,46,48] A previous study analyzing PCBs and their OH-metabolites[49] pointed out that the generation of metabolites in unhatched eggs was highly unlikely due to the absence of embryonic development. The same study concluded that the transfer of the metabolites occurs through binding processes between the OH-metabolites and proteins.[49] This is in line with the fact that the highest exposure to pollutants in birds' life occur during hatching, whereas in mammals', exposure mainly occurs during the nursing period.[46,47] Nonetheless, the presence of UV-Fs in bird eggs could not only affect the avian species involved, but also has the potential to increase the exposure of these compounds to egg predators, as other organic pollutants do.[49] Along these lines, similar processes to those described for POPs may well be applicable for UV-Fs.

To assess the exposure and biomagnification of contaminants in organisms through the diet, the determination of stable isotopes present on a biological sample serves as a powerful tool. The δ^{15}N indicates the position in the food web, whereas the carbon food source is identified by δ^{13}C. The stable isotopes δ^{15}N and δ^{13}C of unhatched eggs were analyzed and results reflect the isotopic signature of mothers.[50]

Total concentrations of UV-Fs for each species are shown in SI Figure S1. The mean values of δ^{15}N and δ^{13}C are shown in SI Figure S2. As regards δ^{13}C, two species groups were observed: western marsh harrier, gull-billed tern, white stork, and common kestrel (mean δ^{13}C between −22.54 and −23.86) on one hand and black-headed gull, slender-billed gull, and gadwall (mean δ^{13}C between −17.82 and −19.20) showing two well-differentiated habitats. That is in agreement with what is known about their habitat-based diets, as the species in the first group are of land-based diet, whereas the species in the second group fall upon the aquatic-based diet category.[22] For unknown reasons, the exceptions to this would be gull-billed tern and black-headed gull, that shared a habitat-based diet opposite to the one showed here. δ^{15}N values are responsive to external sources of nitrogen such as sewage and agricultural activities in the area, as well as inputs through natural processes, being manure and sewage waters the biggest contributors of ^{15}N.[51] These values provide information about the position in the

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food web, with higher values of δ^{15}N indicating upper positions. In order to determine whether between δ^{15}N and UV-Fs occurrence hinted biomagnification through the food web, the mean values of δ^{15}N and UV-Fs concentrations were correlated using a Spearman's test, but the low coefficient (ρ = 0.04) indicated no correlation. However, as argued Barón et al.,[22] the human activities in the area of Doñana appeared to heavily impact the overall content of ^{15}N in the mothers, and thus in their eggs, misleadingly allocating the species at different levels of the food web. To avoid such issue, a larger number of specimens would be needed.

It has to be highlighted that the highest mean concentrations of BP3, 4HB, and the total UV-Fs load were observed in western marsh harrier. The black-headed gull is a dispersive and partially migratory species. When comparing the migration behavior of this species with others from the same habitat, and as suggested in a previous study by Huertas et al.,[52] migration allows the transfer of contaminants among food webs from different areas. Additionally, the feeding habits of the species should be considered, as the black-headed gull is the only reported species in this study that feeds on industrial and urban wastes.[22] Nevertheless, this assessment must be taken with caution, as contaminant concentrations can greatly vary between individuals of the same species, and an in-depth study would require a larger number of specimens of each species, as represented in SI Figure S2.

Although most of the species analyzed in this study feed on invertebrates, fish, or plant material, the white stork and the western marsh harrier diet contains eggs from other species, and the western marsh harrier together with the common kestrel feed on other birds. Given that, the transfer of UV-Fs through the diet from one species to another, propagating their presence through the food web, should not be ruled out, considering that the number of available samples for species is low. Because of many of the target UV-Fs display endocrine disrupting activity; these outcomes are indicative of the need for further risk assessment regarding wildlife to help interpret the implications for observed trends in UV-Fs bioaccumulation and evaluation on their potential biomagnification. The data provided in this first study corroborate that bird eggs are useful bioindicators to assess the occurrence of UV-F residues in the environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03300.

The in-depth description of the methods of analysis and the additional tables and figures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: silvia.diaz@idaea.csic.es.

ORCID

Daniel Molins-Delgado: 0000-0002-3105-8155
M. Silvia Díaz-Cruz: 0000-0003-3331-4076

Notes

The authors declare no competing financial interest.

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Effects of pH on weak and positive control mutagens in the Ames Salmonella plate assay.

Popkin DJ, Prival MJ.

Abstract
The effects of pH on the mutagenic activity of several chemicals were evaluated in the standard Ames Salmonella typhimurium plate-incorporation assay. The pH of the base agar was varied between 6.0 and 8.0. The positive control compounds routinely used in this laboratory, 2-aminoanthracene, 4-nitro-o-phenylenediamine, sodium azide and nitrofurantoin, showed increasing mutagenic activity as the pH was decreased to 6.0. However, the activity of two weakly mutagenic cosmetic ingredients, 2,2',4,4'-tetrahydroxybenzophenone and trans-4-phenyl-3-buten-2-one, was completely eliminated at pH levels near 6.0. It is concluded that plates poured with agar with pH levels below 7.0 can result in strong responses for the positive control chemicals but give negative results for some mutagens.

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Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W.
Erratum in
Environ Mutagen 1988; 11 Suppl 12:158.

Abstract
The results and data from the testing of 255 chemicals for mutagenicity in Salmonella are presented. All chemicals were tested under code using a preincubation modification of the Salmonella/microsome test in the absence of exogenous metabolic activation and in the presence of liver S-9 from Aroclor-induced male Sprague-Dawley rats and Syrian hamsters.

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Sunlight-induced mutagenicity of a common sunscreen ingredient.

Knowland J1, McKenzie EA, McHugh PJ, Cridland NA.
Author information
1 Department of Biochemistry, South Parks Road, Oxford OX1 3QU, UK.

Abstract
We have tested the mutagenicity of a UV-B sunscreen ingredient called Padimate-O or octyl dimethyl PABA, which, chemically speaking, is identical to an industrial chemical that generates free radicals when illuminated. It is harmless in the dark but mutagenic in sunlight, attacking DNA directly. A commercial sunscreen containing Padimate-O behaves in the same way. UV-A in sunlight also excites Padimate-O, although less than UV-B. Some related compounds, including a known carcinogen, behave similarly. As mutagens may be carcinogenic, our results suggest that some sunscreens could, while preventing sunburn, contribute to sunlight-related cancers.
Comment in
The molecular basis of UV-induced mutagenicity of sunscreens. [FEBS Lett. 1993]

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF BENZOPHENONE

(CAS NO. 119-61-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 2006

NTP TR 533

NIH Publication No. 06-4469

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

NTP TECHNICAL REPORT

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National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

M.C. Rhodes, Ph.D., Study Scientist
R.S. Chhabra, Ph.D., Study Scientist
J.C. Peckham, D.V.M., M.S., Ph.D., Study Pathologist
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
J.R. Hailey, D.V.M.
R.A. Herbert, D.V.M., Ph.D.
G.E. Kissling, Ph.D.
R.R. Maronpot, D.V.M.
D.P. Orzech, M.S.
S.D. Peddada, Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S.

Battelle Columbus Operations

Conducted studies and evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
J. Toft, II, D.V.M., M.S.
D.D. Vasconcelos, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology review

J.F. Hardisty, D.V.M., Principal Investigator
K.J. Cimon, D.V.M., M.S.
R.A. Miller, D.V.M., Ph.D.
J.C. Peckham, D.V.M., M.S., Ph.D.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology report on rats (April 15, 2003)

A.W. Suttie, B.V.Sc., Ph.D., Chairperson
ILS, Inc.
K.J. Cimon, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
R.A. Miller, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
A. Nyska, D.V.M.
National Toxicology Program
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.

Evaluated slides and prepared pathology report on mice (March 20, 2003)

C.A. Picut, V.M.D., J.D., Chairperson
ILS, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
G. Willson, B.V.M.S.
National Toxicology Program

Constella Group

Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

Biotechnical Services, Inc.

Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
P.A. Gideon, B.A.
B.F. Hall, M.S.
L.M. Harper, B.S.
D.C. Serbus, Ph.D.

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SUMMARY

Background
Benzophenone is an ingredient in a variety of chemical products including plastics, adhesives, insecticides, and pharmaceuticals. It is also used in fragrances and as a flavoring in foods. We studied the effects of benzophenone on male and female rats and mice to identify potential toxic or carcinogenic hazards to humans.

Methods
We gave feed containing benzophenone to groups of 50 animals for 2 years. Male and female rats and mice received 312, 625, or 1,250 parts per million of benzophenone in their feed (the highest concentration corresponding to 0.125%). Groups of animals receiving untreated feed served as controls. Tissues from more than 40 sites were examined for every animal.

Results
Almost all of the male rats receiving the highest concentration died before the end of the study. Male and female rats and female mice receiving benzophenone weighed less than the controls. Male rats receiving benzophenone had more severe kidney nephropathy than control animals and higher incidences of kidney tumors and leukemia. Female rats receiving benzophenone also had slightly higher rates of leukemia. Male and female mice had slightly increased rates of liver tumors and also increased severities of kidney nephropathy, metaplasia of the epithelium of the nose, and hyperplasia of the spleen. Some female mice also developed rare histiocytic sarcomas.

Conclusions
We conclude that benzophenone caused kidney cancer in male rats, liver tumors in male mice, and histiocytic sarcomas in female mice. Benzophenone may also have been associated with development of leukemia in male and female rats and with liver tumors in female mice.

ABSTRACT

BENZOPHENONE

CAS No. 119-61-9

Chemical Formula: $C_{13}H_{10}O$ Molecular Weight: 182.22

Synonyms: Benzene, benzophenone (8CI); benzoyl; benzoylbenzene; benzoylbenzenephenyl; diphenyl ketone; diphenylmethanone; methanone, diphenyl-(9CI); α-oxodiphenylmethane; α-oxoditane; phenyl ketone

Benzophenone is used as a photoinitiator, a fragrance enhancer, an ultraviolet curing agent, and occasionally as a flavor ingredient; it is also used in the manufacture of insecticides, agricultural chemicals, and hypnotics, antihistamines, and other pharmaceuticals; and it is used as an additive in plastics, coatings, and adhesive formulations. Benzophenone was nominated for study by the National Institute of Environmental Health Sciences based on its potential for occupational and consumer exposure and the lack of long-term toxicity data. Male and female F344/N rats and $B6C3F_1$ mice were exposed to benzophenone (greater than 99% pure) in feed for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium,* mouse bone marrow cells, and mouse peripheral blood erythrocytes. Results of 14-week toxicity studies in F344/N rats and $B6C3F_1$ mice were reported earlier (NTP, 2000).

2-Year Study in Rats

Groups of 50 male and 50 female rats were fed diets containing 0, 312, 625, or 1,250 ppm benzophenone (equivalent to average daily doses of approximately 15, 30, and 60 mg benzophenone/kg body weight to males and 15, 30, and 65 mg/kg to females) for 105 weeks. Survival of 1,250 ppm males was significantly less than that of controls. Mean body weights of 1,250 ppm males were markedly less than those of the controls during year 2 of the study, and weights of exposed females were consistently less than controls throughout the study. Feed consumption by 1,250 ppm males was less than that by the controls after week 70; feed consumption by 1,250 ppm females was generally less than that by the controls throughout the study.

There was a positive trend in the incidences of renal tubule adenoma in males, and the incidences in 625 and 1,250 ppm males exceeded the historical control range for all routes; these neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia. Due to these findings, additional kidney sections were evaluated; results indicated additional renal tubule adenomas in all groups of males and renal tubule hyperplasia in all groups of males and females. The incidences of pelvic transitional epithelium hyperplasia and the severity of nephropathy were significantly increased in all exposed groups of male rats.

Increased incidences of mononuclear cell leukemia in all exposed groups of females exceeded the historical

control range from feed studies, and the incidence in 625 ppm females was significantly greater than that in the controls. Male rats exposed to 312 or 625 ppm had significantly increased incidences of mononuclear cell leukemia. One 625 ppm female and two 1,250 ppm females had histiocytic sarcomas, and the incidence in the 1,250 ppm group exceeded the range in the historical controls.

Liver lesions included significantly increased incidences of hepatocytic centrilobular hypertrophy in all exposed groups of males and females, cystic degeneration in 625 and 1,250 ppm males, and bile duct hyperplasia in all exposed groups of females.

Incidences of mammary gland fibroadenoma in females exposed to 625 or 1,250 ppm were lower than expected after adjusting for body weight.

2-Year Study in Mice

Groups of 50 male and 50 female mice were fed diets containing 0, 312, 625, or 1,250 ppm benzophenone (equivalent to average daily doses of approximately 40, 80, and 160 mg/kg body weight to males and 35, 70, and 150 mg/kg to females) for 105 weeks. Survival of all exposed groups of mice was generally similar to that of the control groups. Mean body weights of exposed females were less than vehicle controls. Feed consumption by exposed males and females was similar to that by the controls.

In male mice, there were significantly increased incidences of hepatocellular adenoma in the 625 and 1,250 ppm groups, and these incidences exceeded the historical control range. All hepatocellular neoplasms combined occurred with a positive trend. In female mice, the incidences of hepatocellular adenoma in the 625 and 1,250 ppm groups were higher than expected after adjusting for the lower body weights in these groups. Incidences of centrilobular hepatocyte hypertrophy were significantly increased in all exposed groups of males and females. All exposed groups of male mice had significant increases in the incidences of multinucleated hepatocytes and chronic active inflammation. The incidences of cystic degeneration of hepatocytes in 625 and 1,250 ppm males were significantly increased. The incidence of histiocytic sarcoma in 625 ppm females was significantly increased and exceeded the historical control range.

The incidences of kidney nephropathy and mineralization in exposed groups of females and the severity of nephropathy in exposed groups of males were significantly increased.

The incidences of metaplasia of the olfactory epithelium were significantly increased in 1,250 ppm males and females. The incidences of hyperplasia of lymphoid follicles in the spleen were significantly increased in all exposed groups of males and in 312 and 625 ppm females.

Genetic Toxicology

Benzophenone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without hamster or rat liver activation enzymes. No significant increases in the frequencies of micronucleated polychromatic erythrocytes were seen in bone marrow samples from male mice administered benzophenone three times by intraperitoneal injection. In addition, no increases in micronucleated normochromatic erythrocytes were noted in peripheral blood of male or female mice administered benzophenone for 14 weeks in dosed feed.

Conclusions

Under the conditions of these 2-year studies, there was *some evidence of carcinogenic activity** of benzophenone in male F344/N rats based on increased incidences of renal tubule adenoma; mononuclear cell leukemia in male F344/N rats may have been related to benzophenone exposure. There was *equivocal evidence of carcinogenic activity* of benzophenone in female F344/N rats based on the marginally increased incidences of mononuclear cell leukemia and histiocytic sarcoma. There was *some evidence of carcinogenic activity* of benzophenone in male B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was *some evidence of carcinogenic activity* of benzophenone in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcoma; the incidences of hepatocellular adenoma in female B6C3F$_1$ mice may have been related to benzophenone exposure.

Administration of benzophenone in feed resulted in increased incidences and/or severities of nonneoplastic lesions in the kidney and liver of male and female rats

and in the liver, kidney, nose, and spleen of male and female mice.

Decreased incidences of mammary gland fibroadenoma in female rats were related to benzophenone exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Benzophenone

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm
Body weights	625 and 1,250 ppm groups less than the control group	625 and 1,250 ppm groups less than the control group	Exposed groups similar to the control group	312, 625, and 1,250 ppm groups less than the control group
Survival rates	22/50, 27/50, 31/50, 2/50	32/50, 38/50, 37/50, 34/50	44/50, 44/50, 44/50, 45/50	40/50, 42/50, 41/50, 31/50
Nonneoplastic effects	Kidney: renal tubule, hyperplasia (standard evaluation - 1/50, 5/50, 20/50, 23/50; standard and extended evaluations combined - 3/50, 11/50, 30/50, 40/50); pelvis, transitional epithelium, hyperplasia (1/50, 11/50, 29/50, 34/50); severity of nephropathy (1.3, 2.4, 3.3, 3.8) Liver: hepatocyte, centrilobular, hypertrophy (0/50, 17/50, 31/50, 19/50); degeneration, cystic (8/50, 11/50, 20/50, 15/50)	Kidney: renal tubule, hyperplasia (standard evaluation - 0/50, 1/50, 1/50, 1/50; standard and extended evaluations combined - 1/50, 8/50, 10/50, 7/50); severity of nephropathy - (1.1, 1.4, 1.7, 2.0) Liver: hepatocyte, centrilobular, hypertrophy (0/50, 27/50, 30/50, 33/50); bile duct, hyperplasia (10/50, 35/50, 39/50, 40/50)	Liver: hepatocyte, centrilobular, hypertrophy (0/50, 44/50, 50/50, 48/50); hepatocyte, multinucleated (0/50, 41/50, 47/50, 48/50); inflammation, chronic active (33/50, 47/50, 44/50, 42/50); hepatocyte, degeneration, cystic (0/50, 0/50, 5/50, 30/50) Kidney: severity of nephropathy (1.2, 1.4, 1.7, 3.0) Nose: olfactory epithelium, metaplasia (0/50, 2/50, 2/50, 24/50) Spleen: lymphoid follicle, hyperplasia, lymphoid (17/50, 31/50, 34/50, 32/50)	Liver: hepatocyte, centrilobular, hypertrophy (0/50, 29/50, 44/50, 37/50) Kidney: nephropathy (21/50, 33/50, 31/50, 30/50); mineralization (15/50, 31/50, 36/50, 49/50); severity of nephropathy - (1.2, 1.1, 1.5, 1.7) Nose: olfactory epithelium, metaplasia (0/50, 0/50, 0/50, 39/50) Spleen: lymphoid follicle, hyperplasia, lymphoid (24/50, 36/50, 37/50, 22/50)
Neoplastic effects	Kidney: renal tubule, adenoma (standard evaluation - 1/50, 1/50, 2/50, 4/50; standard and extended evaluations combined - 2/50, 2/50, 7/50, 8/50)	None	Liver: hepatocellular adenoma (11/50, 15/50, 23/50, 23/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (18/50, 20/50, 25/50, 29/50)	Histiocytic sarcoma: (0/50, 0/50, 5/50, 3/50)
Equivocal findings	Mononuclear cell leukemia: (27/50, 41/50, 39/50, 24/50)	Mononuclear cell leukemia: (19/50, 25/50, 30/50, 29/50) Histiocytic sarcoma: (0/50, 0/50, 1/50, 2/50)	None	Liver: hepatocellular adenoma (5/50, 4/50, 10/50, 8/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Benzophenone

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Decreased incidences	None	Mammary gland: fibroadenoma (27/50, 24/50, 15/50, 7/50)	None	None
Level of evidence of carcinogenic activity	Some evidence	Equivocal evidence	Some evidence	Some evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9		
Micronucleated erythrocytes				
Mouse bone marrow *in vivo:*		Negative		
Mouse peripheral blood *in vivo:*		Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on benzophenone on December 9, 2004, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Mary Anna Thrall, D.V.M., M.S., Chairperson
Department of Microbiology, Immunology and Pathology
Colorado State University
Fort Collins, CO

Diane F. Birt, Ph.D.
Department of Food Science & Human Nutrition
Iowa State University
Ames, IA

Kim Boekelheide, M.D., Ph.D.
Division of Biology and Medicine
Department of Pathology and Laboratory Medicine
Brown University
Providence, RI

Michael R. Elwell, D.V.M., Ph.D., Principal Reviewer
Pathology, Drug Safety Evaluation
Pfizer Global Research and Development
Groton, CT

Thomas A. Gasiewicz, Ph.D.
Department of Environmental Medicine
Environmental Health Sciences Center
University of Rochester School of Medicine
Rochester, NY

James E. Klaunig, Ph.D.
Division of Toxicology
Indiana University School of Medicine
Indianapolis, IN

Stephen M. Roberts, Ph.D., Principal Reviewer
Center for Environmental & Human Toxicology
University of Florida
Gainesville, FL

Richard D. Storer, M.P.H., Ph.D., Principal Reviewer
Department of Genetic and Cellular Toxicology
Merck Research Laboratories
West Point, PA

Mary Vore, Ph.D.
Graduate Center for Toxicology
University of Kentucky
Lexington, KY

Cheryl Lyn Walker, Ph.D.
Department of Carcinogenesis
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 9, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of benzophenone received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of benzophenone by describing the uses of the chemical and the rationale for the study, the design and dose selection for the feed studies, the survival and body weight effects, and the compound-related neoplasms and nonneoplastic lesions in rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* in male F344/N rats, *equivocal evidence of carcinogenic activity* in female F344/N rats, and *some evidence of carcinogenic activity* in male and female $B6C3F_1$ mice. The incidences of mononuclear cell leukemia in male rats and of hepatocellular adenoma in female mice may have been related to benzophenone exposure.

Dr. Storer, the first principal reviewer, commented that the study was well conducted. He asked for more discussion on the interpretation of the conflicting genetic toxicology data and if there was a link between the histiocytic sarcoma and the mononuclear cell leukemia.

Dr. Roberts, the second principal reviewer, agreed with the conclusions and noted that the dose formulations were often less than the target concentrations and were sometimes contaminated.

Dr. Elwell, the third principal reviewer, questioned the explanations for some of the choices of equivocal evidence versus some evidence, particularly for mononuclear cell leukemia in rats and histiocytic sarcoma in female rats.

Dr. J.R. Hailey, NIEHS, replied that there have been many studies in rats where there was an increased incidence of mononuclear cell leukemia with no increased incidence of histiocytic sarcoma. He also added that the mononuclear cell leukemias were considered an equivocal response because the incidences in the control and exposed groups were all unusually high but with no difference in severity. Dr. Hailey noted that the overall incidence of histiocytic sarcoma consisted of only three neoplasms among the four exposed groups, and it was possible that the distribution was spurious.

Dr. Storer moved that the conclusions be accepted as written. Dr. Roberts seconded the motion, which was approved unanimously with nine votes.

INTRODUCTION

BENZOPHENONE

CAS No. 119-61-9

Chemical Formula: $C_{13}H_{10}O$ Molecular Weight: 182.22

Synonyms: Benzene, benzophenone (8CI); benzoyl; benzoylbenzene; benzoylbenzenephenyl; diphenyl ketone; diphenylmethanone; methanone, diphenyl-(9CI); α-oxodiphenylmethane; α-oxoditane; phenyl ketone

CHEMICAL AND PHYSICAL PROPERTIES

Benzophenone, an aryl ketone, is a colorless crystalline solid with a geranium- or rose-like odor. There are two forms of benzophenone, α and β. These studies were conducted using the α, orthorhombic, stable form of the compound. Benzophenone has melting points of 49° C (α) and 26° C (β), a boiling point of 305.4° C, a flash point greater than 110° C, a vapor pressure of 1 mm Hg at 108.2° C, specific gravities of 1.0976 at 50° C/50° C (α) and 1.108 at 23° C/40° C (β), a refractive index of 1.60, and a log octanol:water partition coefficient of 3.18. It is insoluble in water and soluble in organic solvents such as alcohol, acetone, ether, acetic acid, chloroform, and benzene (Hansch and Leo, 1979; *Merck Index*, 1996; Lewis, 1997). Benzophenone is photochemically reactive, incompatible with strong oxidizing and reducing agents, and may attack some plastics. Decomposition of benzophenone produces toxic fumes of carbon monoxide and carbon dioxide (*Sigma-Aldrich*, 1988).

PRODUCTION, USE, AND HUMAN EXPOSURE

Benzophenone is prepared in 66% yield by a Friedel-Crafts acylation using benzoyl chloride with an excess of benzene in the presence of anhydrous aluminum chloride (Furia and Bellanca, 1975; *Kirk-Othmer*, 1978). It is classified as a high volume chemical, with production exceeding 1 million pounds per year in the United States (USEPA, 2003).

Benzophenone is used primarily as a photoinitiator and fragrance enhancer (Anonymous, 1990; CBNB, 1991), and it is also used in the manufacture of insecticides, agricultural chemicals, and hypnotics, antihistamines, and other pharmaceuticals; as an ultraviolet curing agent in sunglasses and ink; as an additive in plastics, coatings, and adhesive formulations; and, occasionally, as a flavor ingredient. Concentrations of benzophenone in food products range from 0.57 ppm in nonalcoholic beverages to 3.27 ppm in frozen dairy products; it may also be an ingredient in baked goods, soft candy, gelatins, and puddings (NAS/NRC, 1979).

Because of its high octanol:water partition coefficient and its insolubility in water, benzophenone will partition in soil and sediment (USEPA, 1984); the adsorption of benzophenone to soil is proportional to the organic content of the soil (OHMTADS, 1991). Although benzophenone has been identified in the atmosphere, it is difficult to determine whether its presence is due to its being a direct product of combustion or a secondary

product of atmospheric degradation (Helmig *et al.*, 1989). Leary *et al.* (1987) found that benzophenone is a component of emissions from a standard residential oil burner. It has also been detected in surface and groundwater samples, primarily from the discharge of raw sewage and wastewater into waterways. Based on the use of benzophenone as an additive in fragrances, cosmetics, toiletries, pharmaceuticals, insecticides, and flavor ingredients, consumer exposure may be significant. Additionally, surveys showed that 41,520 workers in the United States were potentially exposed to benzophenone between 1981 and 1983 (NIOSH, 1990).

Absorption, Distribution, Metabolism, and Excretion

In rhesus monkeys, percutaneous absorption of benzophenone was found to be 44% and 69% for unoccluded and occluded sites, respectively (Bronaugh *et al.*, 1990). The primary pathway of benzophenone metabolism following dietary administration in rabbits was reported to be reduction of the keto group to yield benzhydrol, which was excreted at concentrations of 41% to 61% of the administered dose as a labile glucuronide in the urine (Robinson and Williams, 1957; Robinson, 1958). In male Sprague-Dawley rats that received benzophenone by gavage, 1% of the administered dose was detected as *p*-hydroxybenzophenone in enzyme-treated urine samples but not in unhydrolyzed urine (Stocklinski *et al.*, 1980). No *p*-hydroxybenzophenone was detected in the feces.

The metabolism of benzophenone was investigated in isolated rat hepatocytes at a low toxic level of 0.25 mM. Benzophenone was enzymatically converted to at least three metabolites: benzhydrol, *p*-hydroxybenzophenone, and a sulfate (Nakagawa *et al.*, 2000) (Figure 1). Benzhydrol is produced by a reduction of the carbonyl group to the corresponding secondary alcohol. Benzophenone is converted to *p*-hydroxybenzophenone probably by a cytochrome P450 enzyme. The amount of free *p*-hydroxybenzophenone was less than that of the sulfate, which accumulated in hepatocyte suspensions with time. In a subsequent study (Nakagawa and Tayama, 2002), 6 hours after a single oral dose of 100 or 400 mg benzophenone/kg body weight, female Sprague-Dawley rats (four per group) displayed serum concentrations of benzhydrol > benzophenone > *p*-hydroxybenzophenone.

Toxicity

Experimental Animals

Median lethal (LD_{50}) doses of benzophenone given by oral, intraperitoneal, and dermal routes of administration are given in Table 1; these data indicate that benzophenone is only slightly toxic.

In 14-week studies, benzophenone was administered to groups of 10 male and 10 female F344/N rats and $B6C3F_1$ mice in feed at concentrations of 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm (NTP, 2000). The estimated daily dose ranged from 75 to 850 mg benzophenone/kg body weight for male rats; 80 to 1,000 mg/kg for female rats; 200 to 3,300 mg/kg for male mice; and from 270 to 4,200 mg/kg for female mice. Benzophenone was unpalatable for both rats and mice at 20,000 ppm. All 20,000 ppm rats had significant body weight loss and were terminated on days 39 or 40; all other rats survived to the end of the study. All male mice and four female mice in the 20,000 ppm groups died or were sacrificed moribund prior to the end of the study. There was no exposure-related mortality in the remaining groups. Significantly decreased body weights relative to the controls occurred in all exposed groups of female rats and in all exposed groups of male rats, except the 1,250 ppm group. Lower body weights were apparent in 10,000 ppm male male mice and in 5,000 ppm or greater female mice.

In the 14-week study in rats, benzophenone toxicity occurred in the liver, kidney, and hematopoietic system of males and females (Table 2; NTP, 2000); exposure-related increases in liver weights were attributed to centrilobular hypertrophy and cytoplasmic vacuolization of hepatocytes. Exposure-related increases in alanine aminotransferase and bile salt concentrations indicated a hepatic effect consistent with the gross and microscopic liver changes. These alterations were accompanied by benzophenone-induced increases in pentoxyresorufin dealkylase, an enzyme activity linked to the cytochrome P450 2B isomer. Exposure-related increases in kidney weights were associated with renal changes in exposed male and female rats. These lesions included tubule dilatation, protein casts, tubule epithelial regeneration, mineralization, and necrosis in renal papillae. Unique lesions were well-demarcated, wedge-shaped areas of prominent tubule dilatation. Renal tubule dilatation occurred in 2,500 ppm or greater males and in 10,000 and 20,000 ppm females. Incidences and/or severities of focal tubule regeneration were increased relative to the

Benzophenone
Ketone Reductase
Benzhydrol
Mono-oxygenase
p-Hydroxybenzophenone
Sulfotransferase
p-Hydroxybenzophenone-sulfate

Figure 1
Proposed Metabolism of Benzophenone
(adopted from Nakagawa *et al.*, 2000)

Table 1
Summary of Selected Acute Animal Toxicity Data for Benzophenone

Species	Route of Administration	LD_{50} (mg/kg)	Reference
Rat	Oral	>10,000	Opdyke, 1973
Rat	Oral	1,900	Eastman Kodak Company, 1991
Mouse	Oral	2,895 (2,441-3,434)	Caprino *et al.*, 1976
Mouse	Intraperitoneal	727 (634-833)	Caprino *et al.*, 1976
Rabbit	Dermal	3,535 (2,007-6,226)	Opdyke, 1973

TABLE 2
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
Liver[b]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[c]	0	0	0	0	5* (1.2)[d]	7** (1.0)
Hepatocyte, Vacuolization Cytoplasmic	1 (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.4)	10** (1.2)
Kidney	10	10	10	10	10	10
Renal Tubule, Dilatation	0	0	6** (1.0)	8** (1.0)	9** (1.3)	8** (1.8)
Renal Tubule, Protein Casts	0	8** (1.0)	8** (1.0)	9** (1.2)	10** (1.3)	0
Renal Tubule, Regeneration	10 (1.0)	10 (2.0)	10 (1.5)	10 (2.0)	10 (2.0)	8 (1.6)
Mineralization	0	0	0	5* (1.0)	10** (1.1)	0
Papilla, Necrosis	0	0	0	0	2 (1.0)	6** (1.2)
Female						
Liver	10	10	10	10	10	9
Hepatocyte, Hypertrophy	0	2 (1.0)	8** (1.0)	10** (1.1)	10** (1.0)	7** (1.0)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	9** (1.1)	10** (1.0)	7** (1.1)
Kidney	10	10	10	10	10	9
Renal Tubule, Dilatation	0	0	0	0	3 (1.0)	5* (1.6)
Renal Tubule, Protein Casts	0	0	2 (1.0)	0	4* (1.0)	0
Renal Tubule, Regeneration	3 (1.0)	8* (1.0)	6 (1.0)	6 (1.0)	9** (1.2)	7 (1.6)
Mineralization	10 (1.5)	10 (1.6)	10 (1.6)	10 (1.1)	10 (1.2)	9 (1.2)
Papilla, Necrosis	0	0	0	0	0	3 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] NTP, 2000
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

controls in exposed males and females. Secondary lesions due to inanition resulted in atrophy of bone marrow in both sexes and testicular hypoplasia in males. Hematology results indicated mild anemia, altered circulating erythroid mass, and transient exposure concentration-related increases in platelet counts. At necropsy, three males had small seminal vesicles; microscopic examination revealed that these were immature seminal vesicles.

Mice exposed to benzophenone in the 14-week study were less sensitive to the effects of exposure compared to rats, requiring higher doses on a body weight basis to display benzophenone toxicity (NTP, 2000). Thinness and lethargy in the high dose groups were the only clinical signs reported. There were no gross lesions observed at necropsy related to exposure to benzophenone. The liver weights were increased up to 100% in males and 62% in females. Significant microscopic findings for both sexes were limited to the liver (Table 3). Significantly increased incidences of centrilobular hypertrophy of hepatocytes that corresponded to increased liver weight were observed in the liver of all exposed groups. The severity of hepatocyte hypertrophy increased in an exposure concentration-related manner, with moderate to marked severity in all 20,000 ppm mice. Male mice exhibited evidence of anemia in the 5,000 and 10,000 ppm groups, demonstrated by minimal decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. Liver contents of cytochrome P450 of male mice in all exposed groups, except the 10,000 ppm group, were significantly greater

TABLE 3
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hypertrophy[b]	3 (1.0)[c]	8* (1.0)	10** (2.0)	10** (3.0)	10** (3.0)	10** (3.2)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	0	0	3 (2.0)
Inflammation, Chronic Active	5 (1.0)	4 (1.0)	8 (1.0)	8 (1.0)	5 (1.0)	1 (1.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hypertrophy	3 (1.0)	9** (1.0)	10** (2.0)	10** (3.0)	10** (3.0)	10** (4.0)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	2 (1.0)	9** (2.4)	1 (1.0)
Inflammation, Chronic Active	8 (1.0)	9 (1.1)	9 (1.0)	9 (1.0)	9 (1.0)	3 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] NTP, 2000
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

than in the vehicle controls. The testis and epididymis weights of male mice in the 10,000 ppm group were significantly less than those of controls, but sperm motility parameters were normal. As in rats, kidney weights were increased in all exposed animals, except in the 1,250 ppm group. However, unlike in rats, there were no microscopic effects to account for the increased weights.

Benzophenone induced histological alterations in the liver of guinea pigs (Dutta *et al*., 1993). An unspecified number of male guinea pigs received intraperitoneal injections of 5 mg benzophenone/kg body weight daily for 15 days. Microscopic evaluation of the liver revealed disorganization of lobular architecture and hepatic cords, nuclear hyperchromatia, and hepatocellular necrosis.

Groups of male rats (strain not specified) were fed diets containing 0.1% or 1.0% benzophenone for 10 consecutive days (USEPA, 1984). Feed consumption and body weights were slightly reduced in the 1.0% group. Exposure concentration-dependent increases in absolute and relative liver weights and relative kidney weight were observed. Serum alanine aminotransferase activity of rats in the 1.0% group was increased compared to that of the controls. Mild degenerative effects were observed in the liver and bone marrow of rats in the 1.0% group, suggesting that the liver may be the primary target of the toxic effects of benzophenone and that the bone marrow may also be a target.

Benzophenone was administered in feed to Sprague-Dawley rats at concentrations of 20 mg/kg body weight per day for 90 days or 100 or 500 mg/kg per day for 28 days (Burdock *et al*., 1991). Decreases in hematocrit values, erythrocyte counts, and hemoglobin concentrations were observed in 100 and 500 mg/kg females; a decrease in hemoglobin concentration was also evident in 500 mg/kg males. Males in the 100 and 500 mg/kg groups had increased urea nitrogen concentrations; total bilirubin and protein were increased in 500 mg/kg males and in 100 and 500 mg/kg females. Males and females exposed to 100 or 500 mg/kg had increased albumin concentrations and absolute and relative liver and kidney weights. Histopathologic examination of the liver revealed hepatocellular enlargement with associated clumping of cytoplasmic basophilic material around the central vein in rats in the 100 and 500 mg/kg groups.

Slight skin irritation, evidenced by slight erythema and desquamation and slight to moderate edema, was observed in guinea pigs that received dermal applications of benzophenone on the abdomen for 24 hours under an occlusive wrap or uncovered on the back for 10 days (USEPA, 1984). Additional exposures to benzophenone failed to exacerbate the irritation. In a dermal study using the Draize method, benzophenone was determined to have medium irritation potential, with a primary cutaneous irritation index of 2.0 in rabbits (Calas *et al.*, 1977). Additional experiments were conducted by these investigators in guinea pigs to determine skin irritation and contact hypersensitivity induced by benzophenone; in the open epicutaneous test, the Draize test, the maximization test, and a test with Freund's complete adjuvant, benzophenone did not induce allergenicity in guinea pigs.

Humans

Benzophenone in sunscreen produced an allergic skin reaction in one patient, as assessed by photopatch testing (Cook and Freeman, 2001). This compound was positive in patch test results in 1% to 2% of patients tested by the North American Contact Dermatitis Research Group (Mitchell *et al.*, 1982). Derivatives of benzophenone, particularly 2-hydroxy-4-methoxybenzophenone (benzophenone-3) and 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (benzophenone-4), are skin irritants that cause photoallergy and have been associated with allergic contact dermatitis (Alanko *et al*., 2001) and facial erythema (Nedorost, 2003). No epidemiology studies related to benzophenone exposure in humans were found in the literature (HSDB, 2004).

Reproductive and Developmental Toxicity

Experimental Animals

In an NTP developmental toxicity study, benzophenone was administered by gavage to timed pregnant Sprague-Dawley (CD®) rats (22 to 25 per group) at doses of 100, 200, or 300 mg/kg per day on days 6 to 19 of gestation (NTP, 2002). While there were no treatment-related maternal deaths, maternal toxicity occurred in all dosed groups. Clinical signs observed at all doses of benzophenone included lethargy, piloerection, weight loss, and rooting in bedding after dosing. Maternal liver and kidney weights were significantly increased in all dosed groups. Reduced maternal body weight gain and decreased feed consumption were observed in the 300 mg/kg group. Benzophenone had no adverse effects on prenatal viability or overall incidences of fetal malformations or variations. However, average fetal body weight per litter in the 300 mg/kg group was significantly lower than that in the vehicle controls. The incidences of unossified sternebrae were increased at all doses of benzophenone, and the incidences of extra rib on Lumbar I were increased in the 200 and 300 mg/kg groups. Although a no-observed-adverse-effect-level (NOAEL) was not achieved for developmental toxicity, the effects described above are limited to mild developmental delays with a high probability of recovery during early postnatal development.

In another NTP (2004a) study, New Zealand White rabbits (24 per group) were administered benzophenone by oral gavage at 5, 25, or 45 mg/kg on gestational days 6 to 29. Maternal body weight and feed consumption showed decreasing trends. There were no changes in the weights of the gravid uterus, liver, or kidney of treated does. Benzophenone had no significant adverse effect on prenatal viability in litters that were carried until scheduled termination on gestational day 30. Nevertheless, the ability of does to successfully carry their pregnancies was clearly compromised in a dose-related manner (0 mg/kg, 24/24; 5 mg/kg, 24/24; 25 mg/kg, 19/22; 45 mg/kg, 12/19). Fetal body weight was significantly decreased in the 45 mg/kg group only. Similar to the rat studies, developmental toxicity was noted only in the presence of well-defined maternal toxicity. Thus, there was no evidence for selective susceptibility of the conceptus relative to the pregnant dam in either the rat or the rabbit.

Immature, 21-day-old female Sprague-Dawley rats (unspecified number of animals per group) were used to compare uterotrophic effects of 17 β-estradiol, benzophenone, and two metabolites of benzophenone, *p*-hydroxybenzophenone and benzhydrol (Nakagawa and Tayama, 2001). Animals were dosed with 100, 200, or 400 mg benzophenone, *p*-hydroxybenzophenone, or benzhydrol per kg body weight via subcutaneous injection once per day for 3 days and sacrificed 6 hours after the last dose. Neither benzophenone nor benzhydrol affected uterine weight or morphology of the uterus or vagina. *p*-Hydroxybenzophenone elicited increases in absolute and relative uterine weights in a dose-dependent manner and increased the luminal epithelium height and thickness of the stromal layer of the uterus at 400 mg/kg. In the vagina, *p*-hydroxybenzophenone

increased the thickness of the epithelial cell layer, accompanied by cornification.

A subsequent study examined the effects of benzophenone on ovariectomized rats (Nakagawa and Tayama, 2002). Female Sprague-Dawley rats (five per group) were ovariectomized at 4 weeks of age, acclimated for 3 weeks, orally administered 100 or 400 mg benzophenone per kg body weight for 3 days, and sacrificed 24 hours after the last dose. The 400 mg/kg dose of benzophenone elicited approximately 1.9-fold increases in absolute and relative uterine weights. The uterine response was accompanied by increased luminal epithelium height and thickness of the stromal layer of the uterus. Additionally, benzophenone (400 mg/kg) increased the thickness of the vaginal epithelial cell layers with cornification.

The developmental and teratogenic effects of benzophenone were also studied in Japanese newts. Seven days after amputation of the forelimb at a position proximal to the elbow, benzophenone (~5 µg) was inserted in the anterior part of the regeneration blastema. No retardation of regeneration was observed, and growth continued normally in the dosed group (Tsonis and Eguchi, 1982).

Humans

No studies of reproductive or developmental effects of benzophenone in humans were found in a review of the literature.

Carcinogenicity

Experimental Animals

The carcinogenicity of benzophenone has been studied in female Swiss mice (Stenbäck and Shubik, 1974) and New Zealand White rabbits (Stenbäck, 1977). In lifetime studies, animals received twice-weekly topical administrations of 0.02 mL of 5%, 25%, or 50% benzophenone in acetone. Benzophenone was applied to a 1-inch square area on the dorsal skin between the flanks of mice; for rabbits, the dose was applied to the inside of the left ear. All mice died by week 110. The incidences of skin neoplasms in dosed mice were similar to those in the controls (Stenbäck and Shubik, 1974). Benzophenone had no effect on survival rates or on the incidences of neoplasms or nonneoplastic lesions in rabbits after 160 weeks of treatment (Stenbäck, 1977).

Humans

No epidemiology studies or case reports examining the relationship between exposure to benzophenone and human cancer were found in the literature.

Genetic Toxicity

Benzophenone was not mutagenic in the standard Ames test using various strains of *Salmonella typhimurium* (Mortelmans *et al*., 1986) or in the *Escherichia coli* Pol A assay (Fluck *et al*., 1976). In addition, negative results were reported with benzophenone in the mouse lymphoma L5178Y/tk$^{+/-}$ cell test for induction of trifluorothymidine resistance (CCRIS, 1991). All three of these *in vitro* assays were performed with and without rodent liver S9 metabolic activation enzymes. Results of a recent investigation of the genotoxic potential of benzophenone showed no induction of DNA damage as measured by *umu* gene expression in *S. typhimurium* strain TA1535/pSK1002 in the absence or the presence of microsomes from rat, mouse, or human liver (Takemoto *et al*., 2002). However, when various human P450 preparations, including human P450 2A6 and P450 family I enzymes, were tested for ability to activate benzophenone, significant dose-related increases in *umu* gene expression were seen in TA1535/pSK1002 (Takemoto *et al*., 2002); the benzophenone metabolites benzhydrol and *p*-benzoylphenol were also activated by human P450s to produce an increase in *umu* gene expression in this test system. The positive results reported for benzophenone in the *umu* gene expression assay do not directly conflict with the negative results obtained in *Salmonella* gene mutation assays because the endpoints measured by the two assays differ, as do important aspects of the test protocols. Briefly, the *umu* assay indirectly detects DNA damage induced anywhere in the *Salmonella* genome by analyzing fluorescent signals produced by expression of the *umu*-beta-galactosidase gene complex carried in the pSK1002 plasmid (genes in the *umu* operon control SOS error-prone DNA repair which is expressed in response to induced damage). The *Salmonella* assay, in contrast, measures fixed damage induced specifically within defined regions of the histidine operon, resulting in heritable changes in the bacterial DNA directly observable as mutant colonies. Primary DNA damage, such as that detected in the *umu* assay, may or may not result in mutation. In addition to the endpoint differences, the activation systems contained different liver enzyme mixtures, and the human

cytochrome preparations used in the *umu* assay had specific enzymatic cofactors added to the mixture to ensure the availability of a sufficient number of electrons for metabolic activities to proceed. The pretreatments used to induce rodent S9 liver enzymes in standard bacterial mutation assays may not induce the P450 2A6 and specific other cytochromes that were shown to be effective in transforming benzophenone into a DNA-damaging agent in the *umu* assay.

Study Rationale

Benzophenone is a component of many widely used commercial products, such as plastics and pharmaceutical products, and it is used as a flavor ingredient. It was nominated by the National Institute of Environmental Health Sciences for toxicity and carcinogenicity testing based on the potential for occupational and consumer exposure and the lack of chronic toxicity data. The results of subchronic toxicity studies of benzophenone have been published (NTP, 2000). This report describes the results of 2-year toxicity and carcinogenicity studies conducted in male and female F344/N rats and B6C3F$_1$ mice, along with toxicokinetic studies (Appendix J). Feed was chosen as the route of exposure because this mimics exposure to humans consuming benzophenone as a flavoring agent. The highest dose was set at 1,250 ppm based on the minimum toxicity observed at this level in the 14-week studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BENZOPHENONE

Benzophenone was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (10803KG) that was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) and the study laboratory, Battelle Columbus Operations (Columbus, OH) (Appendix F). Reports on analyses performed in support of the benzophenone studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white crystal with a geranium- or rose-like odor, was identified as benzophenone by melting point determination; infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; x-ray crystallography; and low and high resolution mass spectrometry. Karl Fischer titration indicated a moisture content of 0.426%. Gas chromatography indicated one major peak that accounted for 100% of the total peak area. The overall purity of lot 10803KG was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at approximately 25° C in sealed, amber-glass containers. The study laboratory performed periodic purity reanalyses of the bulk chemical using gas chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared at least once a month by mixing benzophenone with feed (Table F1). Formulations were stored in five-gallon, white plastic buckets lined with plastic can liners at approximately 5° C for up to 35 days.

Homogeneity studies of the 312 and 1,250 ppm dose formulations and stability studies of the 312 ppm dose formulation were performed by the analytical chemistry laboratory using high performance liquid chromatography (HPLC). Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in sealed glass containers protected from light at up to 5° C. Under simulated animal room dosing conditions, there were small losses after 3 days and significant losses after 7 days. To confirm these findings, the study laboratory performed simulated animal room stability studies of the 312 and 1,250 ppm dose formulations using HPLC. Results indicated that feeder changes twice weekly should be acceptable, though there would be a slight decrease in concentration of benzophenone.

Periodic analyses of the dose formulations of benzophenone were conducted by the study laboratory using HPLC. During the 2-year studies, the dose formulations were analyzed at least every 11 weeks (Table F2). Of the dose formulations analyzed and used, all 63 for rats and all 60 for mice were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; 11 of 24 samples analyzed for rats and 8 of 48 samples analyzed for mice were within 10% of target concentrations. The decline in benzophenone concentration was not anticipated from animal room simulations with air and light performed during pre-study developmental work. After the decline was observed, additional experiments were performed in which benzophenone feed formulations were spiked with rodent urine and feces. Declines were approximately 5% with light and air and increased to approximately 15% in the presence of urine and feces. Contamination occurs when the animals crawl into or onto the feeders. The problem increases in cages where multiple animals are housed and are worst with female mice. Feeders were changed twice per week during the study to minimize the problem, but some contamination was unavoidable.

2-Year Studies

Study Design

Core study groups of 50 male and 50 female rats and mice were fed diets containing 0, 312, 625, or 1,250 ppm benzophenone for 105 weeks. The highest dose was set at 1,250 ppm based on the minimal toxicity observed at this level in the 14-week studies (NTP, 2000). In the 14-week study, body weight gain was reduced by 12% in female rats exposed to 2,500 ppm benzophenone. Because of the body weight reduction, 1,250 ppm was selected as the high dose for female rats in the 2-year study. This dose was also selected as the high dose for male rats because exposure to 2,500 ppm benzophenone for 14 weeks caused a 7% reduction in body weight gain, significant increases in liver weights (males 43%, females 28%), and increased incidences of kidney lesions. In mice, exposure to 2,500 ppm benzophenone for 14 weeks caused dramatic increases in liver weights (males 55%, females 56%). Therefore, 1,250 ppm benzophenone was also selected as the high dose for the 2-year study in mice.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 or 12 days (rats) or 25 or 26 days (mice). Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were approximately 6 weeks old and mice were approximately 8 weeks old on the first day of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Rats were housed 2 or 3 (males) or 5 (females) per cage, and mice were housed 1 (male) or 5 (females) per cage. Feed and water were available *ad libitum*. Feed consumption was measured one week out of every four weeks beginning the first week of the study. Cages were changed twice weekly, and cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 4. Information on feed composition and contaminants is provided in Appendix H.

Clinical Examinations and Pathology

Animals were observed twice daily and were weighed initially, on day 8, at 4-week intervals thereafter, and at the end of the studies. Clinical findings were recorded on day 36 and at 4-week intervals (rats had one interval each at 3 and 5 weeks; mice had one interval each at 2, 3, 5, or 6 weeks).

Complete necropsies and microscopic examinations were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. To perform an extended evaluation of renal tubule proliferative lesions, additional sections of both kidneys in the residual formalin-fixed wet tissues from each male and female rat were embedded in separate paraffin blocks and step sectioned at 1 mm intervals. Up to eight step sections were examined for each animal. Tissues examined microscopically are listed in Table 4.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified; and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the kidney and liver of rats and the kidney, liver, nose, and uterus of mice. Slides of spleen, liver, and lung were graded for severity of mononuclear cell leukemia. Stomach, heart, adrenal gland, and parathyroid gland of male rats and ovary, bone marrow, lung, and mammary gland of female rats were reviewed. Spleen of male and female mice, testes and preputial gland of male mice, and bone marrow and thymus of female mice were reviewed.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

During the microscopic evaluations of the additional kidney sections for renal tubule proliferative lesions, the step section lesions were compared with lesions observed during the initial standard evaluation to ensure consistency between evaluations and prevent duplication of diagnoses for lesions already diagnosed in the standard evaluations. The findings and slides from the step section evaluations were reviewed by the PWG chairperson using procedures for the reviews of standard sections. Final diagnoses for the step sections were recorded separately from the standard sections.

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Benzophenone

Study Laboratory
Battelle Columbus Operations (Columbus OH)

Strain and Species
F344/N rats
B6C3F_1 mice

Animal Source
Taconic Laboratory Animals and Services (Germantown, NY)

Time Held Before Studies
Rats: 11 (males) or 12 days (females)
Mice: 26 (males) or 25 days (females)

Average Age When Studies Began
Rats: 6 weeks
Mice: 8 weeks

Date of First Exposure
Rats: August 16 (males) or August 17 (females), 1999
Mice: September 14 (males) or September 13 (females), 1999

Duration of Exposure
105 weeks

Date of Last Exposure
Rats: August 13 or 14 (males) or August 14, 15, or 16 (females), 2001
Mice: September 12, 13, or 14 (males) or September 10, 11, or 12 (females), 2001

Necropsy Dates
Rats: August 13 or 14 (males) or August 14, 15, or 16 (females), 2001
Mice: September 12, 13, or 14 (males) or September 10, 11, or 12 (females), 2001

Average Age at Necropsy
Rats: 110 weeks
Mice: 112 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
Rats: 2 or 3 (males) or 5 (females)
Mice: 1 (males) or 5 (females)

Method of Animal Identification
Tail tattoo

Diet
NTP-2000 irradiated open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed twice weekly

Water
Tap water (City of Columbus) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Benzophenone

Cages
Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly, rotated every 2 weeks

Bedding
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least twice weekly

Cage Filters
DuPont 2024 spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every two weeks

Racks
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Exposure Concentrations
0, 312, 625, or 1,250 ppm in feed

Type and Frequency of Observation
Observed twice daily. Animals were weighed initially, on day 8, at 4-week intervals thereafter, and at the end of the studies. Clinical findings were recorded on day 36 and at 4-week intervals (rats had one interval at 3 and 5 weeks; mice had one interval each at 2, 3, 5 or 6 weeks) throughout the study. Feed consumption was recorded for 1 week out of every 4 weeks beginning the first week of the study.

Method of Sacrifice
CO_2 inhalation

Necropsy
Necropsies were performed on all animals.

Histopathology
Complete histopathology was performed on rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, harderian gland, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver (2 sections including left lateral lobe and median lobe), lung, lymph nodes (mandibular and mesenteric), mammary gland with adjacent skin, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic

evaluation or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all 23 studies that use the NTP-2000 diet with histopathology findings completed up to the present. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison.

Quality Assurance Methods

The 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in

the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of benzophenone was assessed by testing the ability of benzophenone to induce mutations in various strains of *Salmonella typhimurium,* micronucleated erythrocytes in mouse bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database, permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 2). Survival of 1,250 ppm males was significantly less than that of the control group. Survival of exposed females was similar to that of the controls.

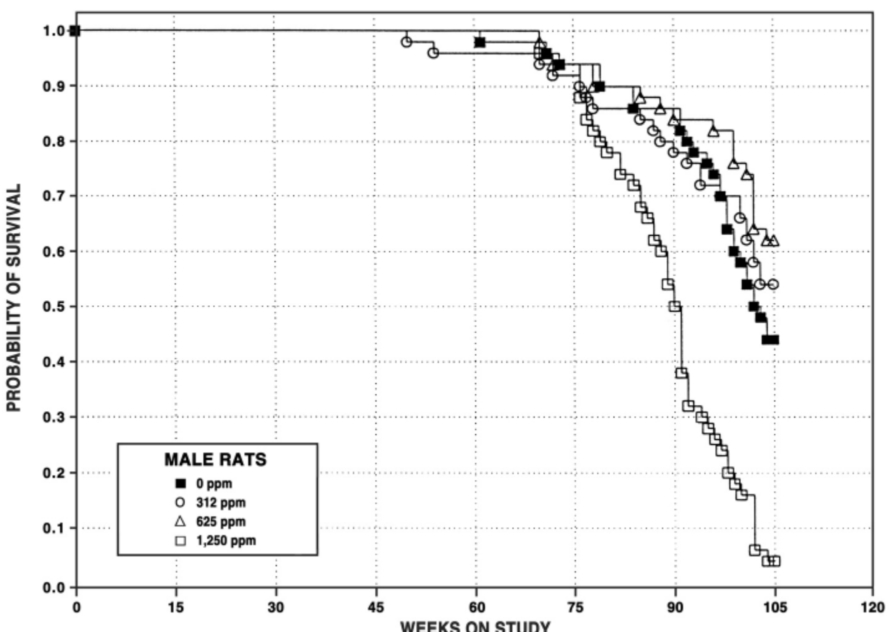
Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 1,250 ppm males were less than those of the controls after week 62, and those of 625 ppm males were less after week 86 (Figure 3 and Table 6). Mean body weights of 625 and 1,250 ppm female rats were generally less than those of the controls after week 10 (Figure 3 and Table 7). Feed consumption by 1,250 ppm males was less than that by the controls after week 70 of the study; feed consumption by

TABLE 5
Survival of Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	25	12	16	44
Natural deaths	3	11	3	4
Animals surviving to study termination	22	27	31	2
Percent probability of survival at end of study[a]	44	54	62	4
Mean survival (days)[b]	681	674	694	622
Survival analysis[c]	P<0.001	P=0.517N	P=0.099N	P<0.001
Female				
Animals initially in study	50	50	50	50
Moribund	16	10	9	13
Natural deaths	2	2	4	3
Animals surviving to study termination	32	38	37	34
Percent probability of survival at end of study	64	76	74	68
Mean survival (days)	688	715	713	704
Survival analysis	P=0.985N	P=0.246N	P=0.354N	P=0.809N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.



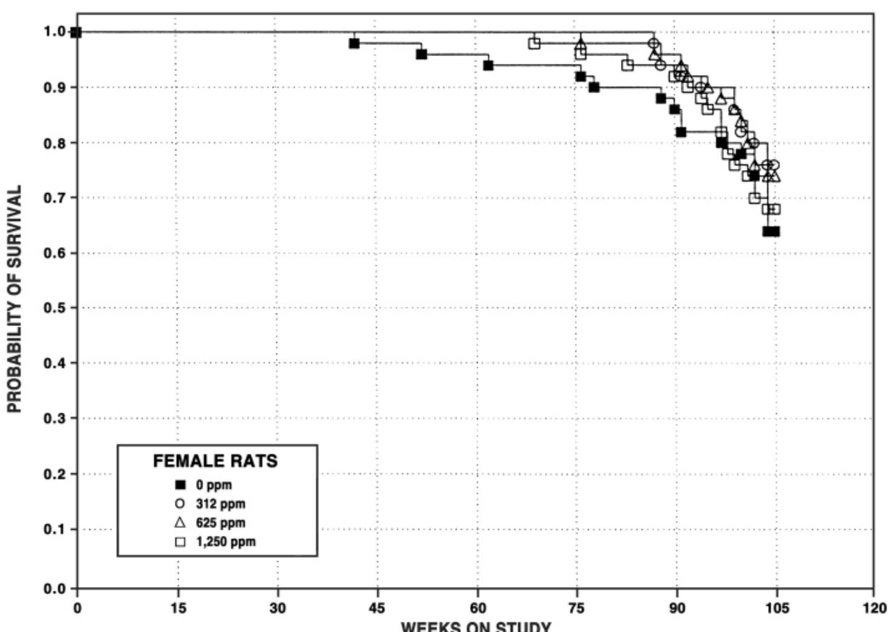
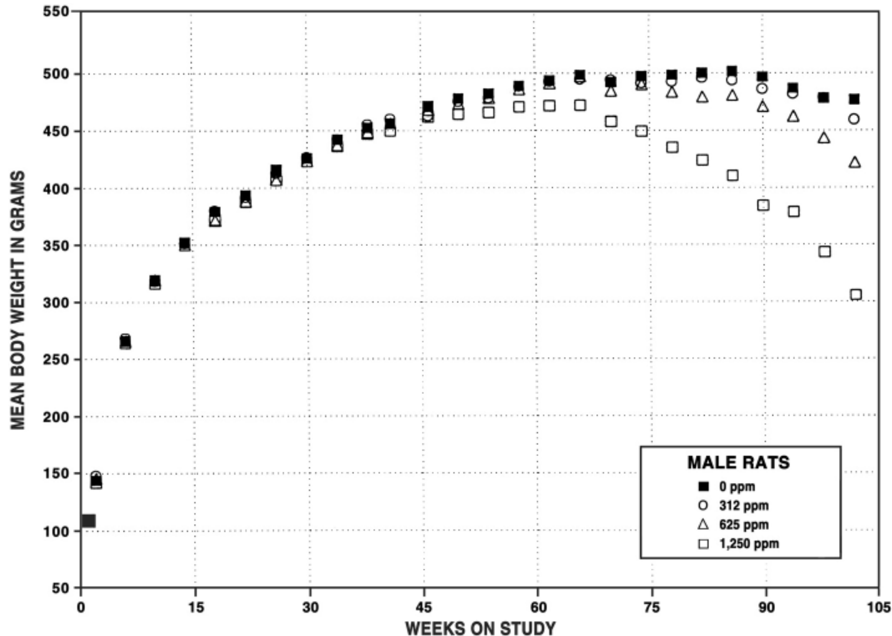


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Benzophenone in Feed for 2 Years



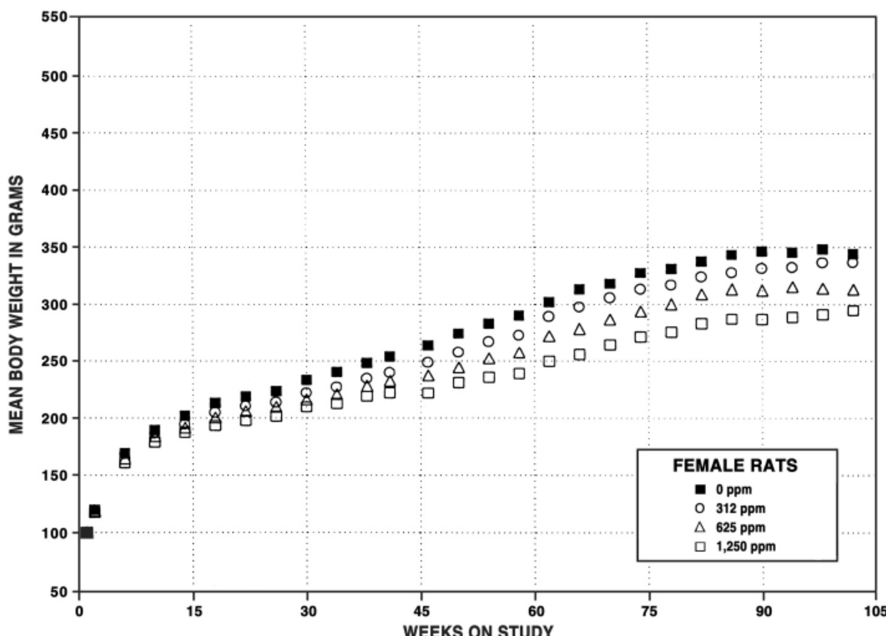


FIGURE 3
Growth Curves for Male and Female Rats Exposed to Benzophenone in Feed for 2 Years

Table 6
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Benzophenone

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	108	50	109	101	50	109	100	50	110	102	50
2	144	50	148	103	50	146	101	50	142	99	50
6	266	50	268	101	50	266	100	50	264	99	50
10	319	50	318	100	50	319	100	50	316	99	50
14	352	50	352	100	50	350	100	50	353	100	50
18	379	50	380	100	50	372	98	50	372	98	50
22	394	50	392	99	50	388	99	50	388	99	50
26	416	50	413	99	50	407	98	50	407	98	50
30	426	50	427	100	50	424	99	50	423	99	50
34	442	50	443	100	50	438	99	50	436	99	50
38	453	50	455	101	50	449	99	50	447	99	50
41	457	50	460	101	50	457	100	50	450	99	50
46	472	50	463	98	50	468	99	50	462	98	50
50	478	50	476	100	50	473	99	50	465	97	50
54	482	50	479	99	49	479	99	50	466	97	50
58	489	50	488	100	48	486	99	50	471	96	50
62	494	49	493	100	48	491	100	50	472	96	50
66	499	49	495	99	48	497	100	50	472	95	50
70	492	49	494	100	47	485	98	50	458	93	50
74	498	47	491	99	46	490	99	47	449	90	47
78	499	47	493	99	44	484	97	47	436	87	42
82	500	45	496	99	43	479	96	45	424	85	39
86	502	43	494	98	42	481	96	44	411	82	34
90	497	43	486	98	40	471	95	43	384	77	27
94	487	39	482	99	38	463	95	42	379	78	16
98	478	34	478	100	35	444	93	41	343	72	11
102	477	27	460	96	31	422	89	37	306	64	8
Mean for weeks											
1-13	209		211	101		210	100		208	100	
14-52	427		426	100		423	99		420	98	
53-102	492		487	99		475	97		421	86	

Table 7
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Benzophenone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	312 ppm Av. Wt. (g)	312 ppm Wt. (% of controls)	312 ppm No. of Survivors	625 ppm Av. Wt. (g)	625 ppm Wt. (% of controls)	625 ppm No. of Survivors	1,250 ppm Av. Wt. (g)	1,250 ppm Wt. (% of controls)	1,250 ppm No. of Survivors
1	101	50	101	100	50	100	99	50	100	99	50
2	120	50	120	100	50	119	99	50	118	98	50
6	169	50	169	100	50	165	97	50	161	95	50
10	189	50	186	98	50	184	97	50	179	95	50
14	202	50	195	96	50	191	95	50	187	93	50
18	214	50	205	96	50	201	94	50	193	91	50
22	219	50	210	96	50	206	94	50	198	90	50
26	224	50	214	96	50	210	94	50	202	90	50
30	234	50	222	95	50	216	93	50	210	90	50
34	241	50	227	95	50	221	92	50	213	89	50
38	249	50	235	95	50	228	92	50	219	88	50
41	254	50	240	95	50	233	92	50	222	88	50
46	264	49	249	94	50	237	90	50	222	84	50
50	275	49	258	94	50	245	89	50	231	84	50
54	283	48	268	95	50	253	89	50	236	83	50
58	290	48	273	94	50	258	89	50	239	82	50
62	302	48	290	96	50	272	90	50	250	83	50
66	313	47	298	95	50	279	89	50	256	82	50
70	318	47	306	96	50	287	90	50	265	83	49
74	328	47	313	96	50	294	90	50	272	83	49
78	331	46	317	96	50	300	91	49	276	83	48
82	338	45	324	96	50	309	92	49	284	84	48
86	344	45	328	95	50	313	91	49	287	84	47
90	347	43	332	96	47	312	90	48	287	83	46
94	346	41	333	96	46	315	91	46	289	84	45
98	349	40	337	97	45	314	90	44	291	84	41
102	345	37	337	98	41	313	91	40	295	86	37
Mean for weeks											
1-13	145		144	99		142	98		140	97	
14-52	238		226	95		219	92		210	88	
53-102	326		312	96		294	90		271	83	

1,250 ppm females was generally less than that by the controls throughout the study (Tables G1 and G2, respectively). Dietary concentrations of 312, 625, and 1,250 ppm resulted in average daily doses of approximately 15, 30, and 60 mg benzophenone/kg body weight to males and 15, 30, and 65 mg/kg to females. No clinical findings other than those associated with morbidity (e.g., nasal/eye discharge, thinness, ruffled fur) were attributed to benzophenone exposure.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and histiocytic sarcoma and neoplasms and/or nonneoplastic lesions of the kidney, liver, thyroid gland, mammary gland, and skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Kidney: Initially, the standard single sections of the left and right kidneys from each rat were examined microscopically. There was a positive trend in the incidences of renal tubule adenoma in males (Tables 8 and A3). No renal tubule adenomas have been reported in feed study controls given NTP-2000 diet, with a single exception found in this study. The incidences in 625 and 1,250 ppm males exceeded the historical range in controls from all routes (Tables 8 and A4a). One renal tubule carcinoma was observed in a 312 ppm male rat. These renal tubule neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia in 625 and 1,250 ppm males (Tables 8 and A5). Two females in the 625 ppm group and one in the 1,250 ppm group had renal tubule adenomas; this neoplasm has occurred in only one historical feed control female (Tables 8, B1, and B4a). Renal tubule hyperplasia was not significantly increased in exposed females. Renal tubule hyperplasia, adenoma, and carcinoma are thought to represent a continuum in the progression of proliferative lesions of the renal tubule epithelium. Because the incidences of renal tubule adenoma in exposed males and females and renal tubule hyperplasia in males were increased in the single sections, additional kidney sections were evaluated. After the extended evaluation (Tables 8, A3, and B3), a significant increase in the incidence of renal tubule adenoma was observed in 1,250 ppm males, and increased incidences of hyperplasia were observed in all exposed groups of males. As a result of the extended evaluation, three renal tubule adenomas were observed in the control females; no additional neoplasms were observed in exposed females. Incidences of renal tubule hyperplasia in all exposed female groups were significantly greater than that of the control group when the single and step section evaluations were combined (Table 8).

Renal tubule hyperplasia consisted of one or more tubules having multiple layers of polygonal epithelial cells with slightly varied sizes. Nuclei were generally round, stained slightly basophilic, and had prominent nucleoli. The cytoplasm was clear, eosinophilic or basophilic with a granular to foamy appearance. Cystic and solid patterns were formed. Hyperplastic tubules with lumens partially or totally filled by epithelial cells were enlarged two to four times normal diameter. Renal tubule adenomas were larger, discrete lesions, ranging from greater than four tubule diameters to 1 mm or more in size. They often consisted of a solid mass of large, relatively normal appearing, closely packed tubular epithelial cells. Cells within adenomas were mildly to moderately pleomorphic, sometimes had vacuolated cytoplasm, and tended to form complex patterns, particularly microtubular structures. The renal tubule carcinoma was differentiated from the adenomas in that it was larger, had a prominent vascular supply, and had more anaplasia and cellular atypia. Cells of this carcinoma were characterized by vesiculated nuclei with prominent nucleoli and increased numbers of mitotic figures.

Oncocytic hyperplasia was observed in the single sections of one male exposed to 625 ppm. During the extended evaluation, this lesion was observed in a few additional exposed male rats (Tables 8 and A5). This lesion was characterized by individual tubules or small clusters of tubules that were somewhat dilated and totally filled by large polygonal cells with abundant, brightly eosinophilic granular or reticulated cytoplasm and small, centrally located, basophilic nuclei (oncocytes). These lesions are thought to arise from the distal tubule epithelium. One male exposed to 1,250 ppm had a benign oncocytic neoplasm (oncocytoma) at the extended evaluation.

Significantly increased incidences of pelvic transitional epithelial hyperplasia were observed in all exposed male

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Renal Tubule, Hyperplasia[a]	1 (1.0)[b]	5 (1.4)	20** (1.5)	23** (1.3)
Renal Tubule, Hyperplasia, Oncocytic	0	0	1 (2.0)	0
Pelvis, Transitional Epithelium, Hyperplasia	1 (1.0)	11** (1.2)	29** (1.5)	34** (1.7)
Nephropathy	50 (1.3)	45 (2.4)▲	50 (3.3)▲	50 (3.8)▲
Renal Tubule, Cyst	0	0	1	9**
Pelvis, Transitional Epithelium, carcinoma	0	0	0	1
Renal Tubule, Adenoma[c]				
Overall rate[d]	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate[e]	2.4%	2.4%	4.5%	12.1%
Terminal rate[f]	0/22 (0%)	1/27 (4%)	1/31 (3%)	0/2 (0%)
First incidence (days)	709	729 (T)	687	537
Poly-3 test[g]	P=0.046	P=0.758	P=0.519	P=0.114
Renal Tubule, Carcinoma	0	1	0	0
Step Sections (Extended Evaluation)				
Renal Tubule, Hyperplasia	2 (1.0)	8* (1.1)	26** (2.0)	37** (2.2)
Renal Tubule, Hyperplasia, Oncocytic	0	1 (1.0)	3 (1.3)	1 (2.0)
Oncocytoma	0	0	0	1
Renal Tubule, Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	5/50 (10%)	4/50 (8%)
Adjusted rate	2.4%	2.4%	11.2%	12.1%
Terminal rate	0/22 (0%)	1/27 (4%)	4/31 (13%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	624
Poly-3 test	P=0.034	P=0.757	P=0.114	P=0.113
Renal Tubule, Carcinoma	0	1	0	0
Single Sections and Step Sections (Combined)				
Renal Tubule, Hyperplasia	3 (1.0)	11* (1.3)	30** (1.8)	40** (2.1)▲
Renal Tubule, Hyperplasia, Oncocytic	0	1 (1.0)	4 (1.5)	1 (2.0)
Oncocytoma	0	0	0	1
Pelvis, Transitional Epithelium Carcinoma	0	0	0	1
Renal Tubule, Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	7/50 (14%)	8/50 (16%)
Adjusted rate	4.7%	4.8%	15.6%	23.3%
Terminal rate	0/22 (0%)	2/27 (7%)	5/31 (15%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	537
Poly-3 test	P=0.004	P=0.688	P=0.093	P=0.017
Renal Tubule, Carcinoma	0	1	0	0

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Renal Tubule, Hyperplasia	0	1 (4.0)	1 (3.0)	1 (4.0)
Pelvis, Transitional Epithelium, Hyperplasia	1 (1.0)	2 (1.5)	2 (2.0)	4 (1.0)
Nephropathy	47 (1.1)	49 (1.4)	48 (1.7)▲	49 (2.0)▲
Renal Tubule, Adenoma, Multiple[h]	0	0	2	1
Step Sections (Extended Evaluation)				
Renal Tubule, Hyperplasia	1 (1.0)	7* (1.1)	9* (2.1)	6 (1.7)
Renal Tubule, Adenoma	3	0	2	1
Single Sections and Step Sections (Combined)				
Renal Tubule, Hyperplasia	1 (1.0)	8* (1.5)	10** (2.2)	7* (2.0)
Renal Tubule, Adenoma, Multiple	0	0	1	1
Renal Tubule, Adenoma (includes multiple)	3	0	2	1

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$
▲ Significantly different ($P \leq 0.05$) from the control group by the Mann-Whitney U test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 1/459 (0.3% ± 0.8%), range 0%-2%; all routes 5/1,152 (0.5% ± 0.9%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with kidney examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: feed 1/460 (0.1% ± 0.4%), range 0%-1%; all routes 1/1,205 (0.1% ± 0.2%), range 0%-1%

groups; a slight increase in the incidence of this lesion was observed in females exposed to 1,250 ppm (Tables 8, A5, and B5). Hyperplasia of the transitional epithelium lining the pelvis and overlying the renal papilla frequently accompanies severe nephropathy, and the increased incidences in the current study may reflect the enhanced nephropathy. One male exposed to 1,250 ppm had a transitional epithelial carcinoma. Transitional epithelial hyperplasia was characterized by small papillary fronds or nodules of normal appearing transitional epithelial cells protruding into the pelvis lumen.

In males, the severity of chronic nephropathy increased with increasing exposure concentration, and the increases in all exposed groups were significant (Table 8). In exposed females, the severity of nephropathy was significantly increased in the 625 and 1,250 ppm groups. Nephropathy is an age-related disease process characterized by a spectrum of lesions, including varying degrees of tubule dilation; proteinaceous tubule casts; atrophy, degeneration, regeneration, and hypertrophy of the tubule epithelium; thickening of tubule and glomerular basement membrane; glomerulosclerosis; interstitial fibrosis; and varying numbers and aggregates of mononuclear inflammatory cells within the interstitium. Minimal nephropathy was characterized by a few scattered foci of tubule regeneration. These regenerative tubules had increased numbers of more intensely stained basophilic cells. Basement membranes, both in glomeruli and around tubules, were slightly thickened. As nephropathy became more severe, tubule dilatation, proteinaceous casts, and interstitial fibrosis were evident. Severe nephropathy resulted in the formation of renal tubule cysts. The incidence of renal tubule cysts in 1,250 ppm males was significantly greater than that in the control group.

The increased severity of the nephropathy in 1,250 ppm males was associated with decreased survival after 80 weeks on study. Twenty-eight of 48 early deaths (58%) in this group, many moribund sacrificed, were attributed to nephropathy caused by benzophenone exposure. Because of the severe nephropathy, increases in several other findings usually associated with uremia were observed at multiple sites in male rats. These secondary findings included increased mineralization of blood vessels and basement membranes, including kidney cortex, heart, seminal vesicles, forestomach, glandular stomach, and lung, in addition to parathyroid gland hyperplasia and fibrous osteodystrophy in bone (Tables 9 and A5).

All Organs: Increased incidences of mononuclear cell leukemia occurred in exposed groups of females, and the difference from the control group was significant at 625 ppm (Tables 10, 11, and B3). Male rats exposed to 312 or 625 ppm also had significantly increased incidences of mononuclear cell leukemia, although that of 1,250 ppm males was slightly decreased (Tables 10, 11, and A3). The incidences in all exposed groups of females and 312 and 625 ppm males exceeded the range reported for historical controls from feed studies (Tables 10, B4b, and A4b).

The mononuclear cell leukemia in female and male rats in the control and dosed groups was classified according to the extent of involvement of the spleen, liver, lung, and other organs. Similar criteria have been used for previous NTP studies (NTP, 1986). In stage 1, the spleen was not enlarged or was only slightly enlarged with small numbers of neoplastic mononuclear cells in the red pulp; none or very few mononuclear cells were observed in the liver sinusoids. No identifiable neoplastic cells were observed in other organs. In stage 2, the spleen was moderately enlarged with moderate to large numbers of neoplastic mononuclear cells in the red pulp; architectural features, including lymphoid follicles and periarteriolar lymphocytic sheaths, remained intact. There was minimal to moderate involvement of the liver. Neoplastic mononuclear cells may have been evident in blood vessels in other organs, but the aggregates/masses of neoplastic cells were generally limited to the spleen and liver. In stage 3, there was advanced disease with multiple organ involvement. The spleen was usually markedly enlarged with an effacement of normal architectural features by accumulated neoplastic mononuclear cells. The liver was moderately to markedly enlarged and nodular; hepatic parenchyma showed variable degenerative changes associated with the accumulation of neoplastic cells. There were accumulations of neoplastic cells in other organs, including the lung, lymph nodes, kidney, brain, and adrenal gland. According to these criteria, the involvement of spleen, liver, and other organs in female rats increased with increased levels of benzophenone exposure (Table B1). The extent of involvement by leukemia in male rats decreased in exposed groups (Tables 10, 11, and A3).

Table 9
Incidences of Secondary Lesions Associated with Severe Nephropathy in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Blood Vessel[a]	50	50	50	50
Mineralization[b]	0	1 (1.0)[c]	1 (4.0)	11** (3.1)
Bone	50	50	50	50
Fibrous Osteodystrophy	0	0	1 (2.0)	4* (1.8)
Heart	50	50	50	50
Mineralization	0	0	2 (1.5)	6** (2.3)
Kidney	50	50	50	50
Cortex, Mineralization	0	1 (1.0)	4 (2.5)	14** (2.8)
Lung	50	49	50	50
Mineralization	0	0	2 (2.5)	10** (2.7)
Parathyroid Gland	49	45	48	49
Hyperplasia	2 (1.5)	1 (1.0)	19** (2.0)	32** (2.2)
Seminal Vesicle	50	50	50	49
Mineralization	0	0	0	4* (2.8)
Stomach, Forestomach	50	49	49	50
Mineralization	0	1 (1.0)	1 (2.0)	3 (2.7)
Stomach, Glandular	50	50	49	50
Mineralization	0	0	5* (2.6)	15** (2.7)

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table 10
Incidences of Mononuclear Cell Leukemia and Histiocytic Sarcoma in Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Males				
Mononuclear Cell Leukemia[a]				
Overall rate[b]	27/50 (54%)	41/50 (82%)	39/50 (78%)	24/50 (48%)
Adjusted rate[c]	55.8%	82.3%	81.2%	59.3%
Terminal rate[d]	6/22 (27%)	20/27 (74%)	25/31 (81%)	2/2 (100%)
First incidence (days)	425	344	494	487
Poly-3 test[e]	P=0.508	P=0.003	P=0.005	P=0.454
Females				
Mononuclear Cell Leukemia[f]				
Overall rate	19/50 (38%)	25/50 (50%)	30/50 (60%)	29/50 (58%)
Adjusted rate	42.3%	51.5%	61.3%	59.6%
Terminal rate	13/32 (41%)	19/38 (50%)	21/37 (57%)	20/34 (59%)
First incidence (days)	637	613	609	480
Poly-3 test	P=0.058	P=0.247	P=0.048	P=0.068
Histiocytic Sarcoma[g]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	2.1%	4.3%
Terminal rate	0/32 (0%)	0/38 (0%)	0/37 (0%)	0/34 (0%)
First incidence (days)	—[h]	—	528	480
Poly-3 test	P=0.074	—[i]	P=0.516	P=0.251

[a] Historical incidence in 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 231/460 (49.1% ± 11.9%), range 30%-68%; all routes 514/1,159 (43.1% ± 12.8%), range 22%-68%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[f] Historical incidence: feed 112/460 (24.6% ± 9.5%), range 12%-38%; all routes 330/1,209 (28.0% ± 11.2%), range 12%-52%
[g] Historical incidence: feed 0/460; all routes 1/1,209 (0.1% ± 0.4%), range 0%-2%
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed

Table 11
Incidences and Stages of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Males				
Number surviving at end of study	22	27	31	2
Number with grade 1 mononuclear cell leukemia	1	8	7	11
Number with grade 2 mononuclear cell leukemia	4	8	18	2
Number with grade 3 mononuclear cell leukemia	22	25	14	11
Total with mononuclear cell leukemia	27	41	39	24
Average staging grade	2.8	2.4*	2.2**	2.0**
Females				
Number surviving at end of study	32	38	37	34
Number with grade 1 mononuclear cell leukemia	10	13	11	10
Number with grade 2 mononuclear cell leukemia	6	4	7	8
Number with grade 3 mononuclear cell leukemia	3	8	12	11
Total with mononuclear cell leukemia	19	25	30	29
Average staging grade	1.6	1.8	2.0	2.0

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$

A few histiocytic sarcomas occurred in the 625 and 1,250 ppm groups of females (Table 10). This neoplasm has not been observed in historical feed study controls given NTP-2000 diet and has been observed in only one out of 1,209 historical controls for all routes (Tables 10 and B4b). Histiocytic sarcomas were observed in the lung and livers of all three affected rats. Histologically, the histiocytic sarcomas in these rats had several characteristic microscopic features. The neoplastic cells had typical histiocytic appearances with relatively abundant, pale eosinophilic cytoplasm. Their dark basophilic nuclei were oval to elongated with small or inconspicuous nucleoli. Variation in the size and shape of these neoplastic cells and high cytoplasmic-to-nuclear ratios were observed. Another characteristic histologic feature observed in two rats consisted of necrotic areas surrounded by rows of neoplastic cells. Prominent multi-nucleated giant cells were present in two animals. Fibrosis varied from minimal to extensive in one rat. Growth was both infiltrative and expansive and extended on pleural and peritoneal surfaces. Histologic features differed from animal to animal and from site to site in the same animal. Neoplastic histiocytic cells infiltrated the liver, diffusely expanding the hepatic parenchyma (Plate 1). In the lung, intravascular masses and perivascular infiltrates of neoplastic histiocytic cells were observed in all affected rats (Plate 2). Two of the three rats had moderate to marked accumulation of hyaline droplets in their kidneys, another finding consistent with histiocytic sarcomas.

Liver: The incidences of centrilobular hepatocellular hypertrophy in all exposed groups of males and females were significantly greater than those in the control groups (Tables 12, A5, and B5). This hepatocellular hypertrophy is consistent with the induction of P450 enzymes previously observed in the 14-week study (NTP, 2000). Incidences of cystic degeneration of hepatocytes and chronic active inflammation in 625 and 1,250 ppm males and bile duct hyperplasia in all exposed groups of females were significantly greater than those in the control groups. The incidences of chronic active inflammation in all exposed female groups were significantly decreased.

Thyroid Gland: The incidences of C-cell hyperplasia were significantly decreased in all exposed groups of males and females (Tables 12, A5, and B5). Increased thyroid gland C-cell hyperplasia is an age-associated

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Liver[a]	50	50	50	50
Hepatocyte, Centrilobular, Hypertrophy[b]	0	17** (1.3)[c]	31** (1.8)	19** (1.5)
Degeneration, Cystic	8 (1.3)	11 (1.0)	20** (1.3)	15* (1.2)
Inflammation, Chronic Active	22 (1.9)	21 (1.6)	35** (1.9)	33* (1.8)
Thyroid gland	50	50	50	50
C-Cell Hyperplasia	17 (2.0)	8* (2.0)	8* (2.1)	5* (1.4)
Female				
Liver	50	50	50	50
Hepatocyte, Centrilobular, Hypertrophy	0	27** (1.0)	30** (1.3)	33** (2.0)
Bile duct, Hyperplasia	10 (1.3)	35** (1.2)	39** (1.4)	40** (1.6)
Inflammation, Chronic Active	46 (1.5)	38* (1.5)	29** (1.3)	30** (1.4)
Thyroid gland	50	50	50	50
C-Cell Hyperplasia	34 (1.8)	11** (1.8)	13** (1.7)	8** (1.9)

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

change in rats (Boorman *et al.*, 1996). Therefore, this decrease appears to be treatment related.

Mammary Gland: Statistically significant decreases in the incidences of fibroadenoma (including multiple) occurred in females exposed to 625 or 1,250 ppm benzophenone (0 ppm, 27/50; 312 ppm, 24/50; 625 ppm, 15/50; 1,250 ppm, 7/50; Table B3). Multiple fibroadenomas were significantly decreased in the 1,250 ppm group (6/50; 4/50; 3/50; 0/50; Table B1). The incidence of fibroadenoma (including multiple) combined in the 1,250 ppm group is fewer than expected after adjusting for decreased body weight (14.7 expected, 7 observed) and is less than the historical control range from feed studies and from all routes combined [feed: 213/460 (44% ± 12%), range 28%-55%; all routes: 567/1,209 (46% ± 12%), range 28%-72%].

Skin: The incidences of keratoacanthoma were decreased in all exposed male groups, and the differences from the control group incidence were significant at 312 and 625 ppm (10/50; 3/50; 3/50; 3/50; Table A3). The incidence in the control group was the highest observed in historical controls in recent studies, whereas the exposed group incidences were within the historical ranges [feed: 34/460 (8% ± 6%), range 2%-20%; all routes: 69/1,159 (6% ± 4%), range 0%-20%]. Therefore, the decreased incidences were not considered to be related to benzophenone exposure.

MICE

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 13 and in the Kaplan-Meier survival curves (Figure 4). Survival of exposed groups of mice was similar to that of the control groups, except in 1,250 ppm females where there was decreased survival toward the end of the study. However, this decrease was not statistically significant.

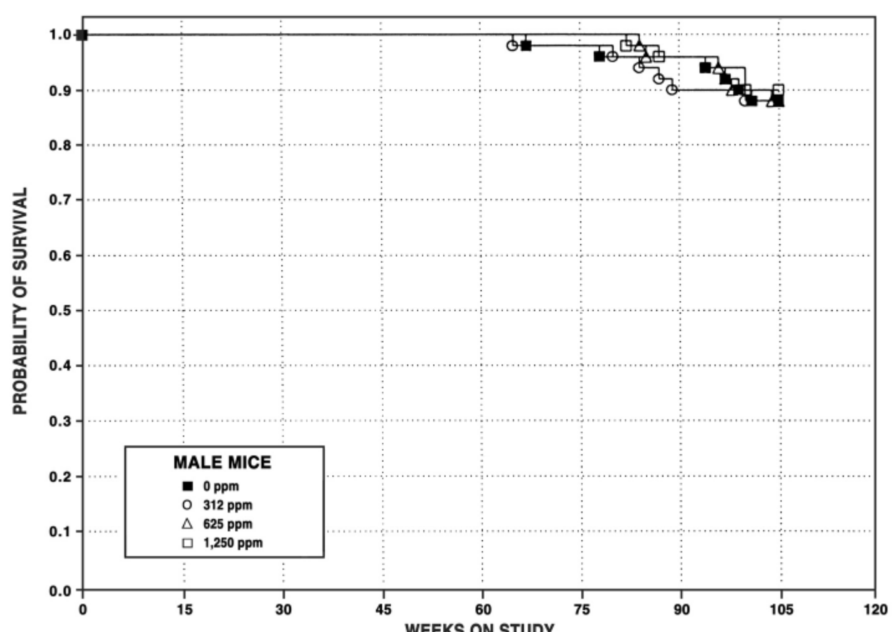
Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of males were similar to those of the controls throughout the study (Table 14 and Figure 5). Mean body weights of 1,250 ppm females were less than those of the controls after week 37; those of 625 ppm females were less during year 2 of the study; and those of 312 ppm females were less after week 86 (Table 15 and Figure 5). Feed consumption by exposed males and females was similar to that by the controls throughout the study (Tables G3

TABLE 13
Survival of Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	5	3	5	2
Natural deaths	1	3	1	3
Animals surviving to study termination	44	44	44	45
Percent probability of survival at end of study[a]	88	88	88	90
Mean survival (days)[b]	717	713	721	722
Survival analysis[c]	P=0.825N	P=1.000	P=1.000N	P=0.977N
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	0	1
Moribund	4	2	5	6
Natural deaths	6	6	4	12
Animals surviving to study termination	40	42	41	31
Percent probability of survival at end of study	80	84	82	63
Mean survival (days)	706	707	704	685
Survival analysis	P=0.032	P=0.813N	P=0.992N	P=0.107

a Kaplan-Meier determinations
b Mean of all deaths (uncensored, censored, and terminal sacrifice).
c The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.
d Censored from survival analyses



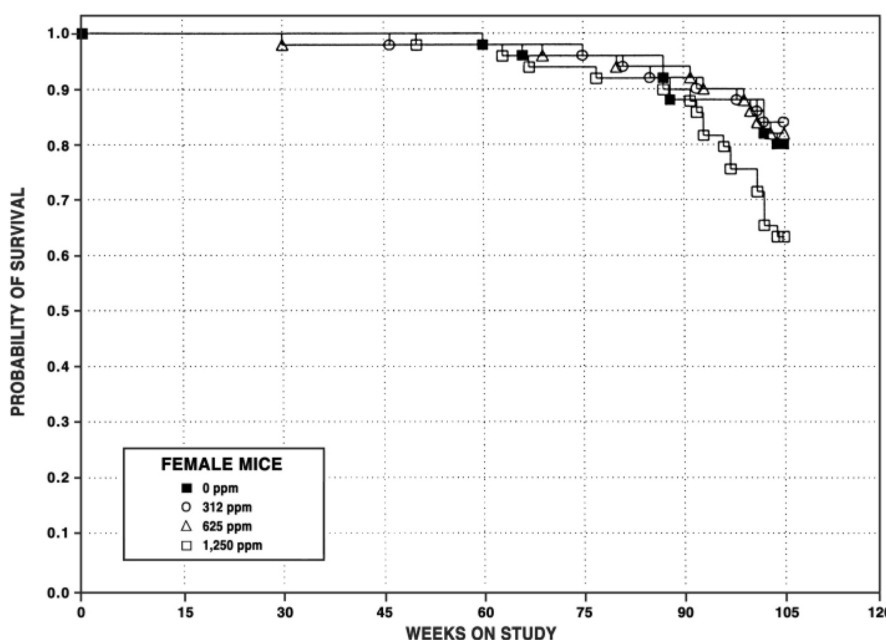


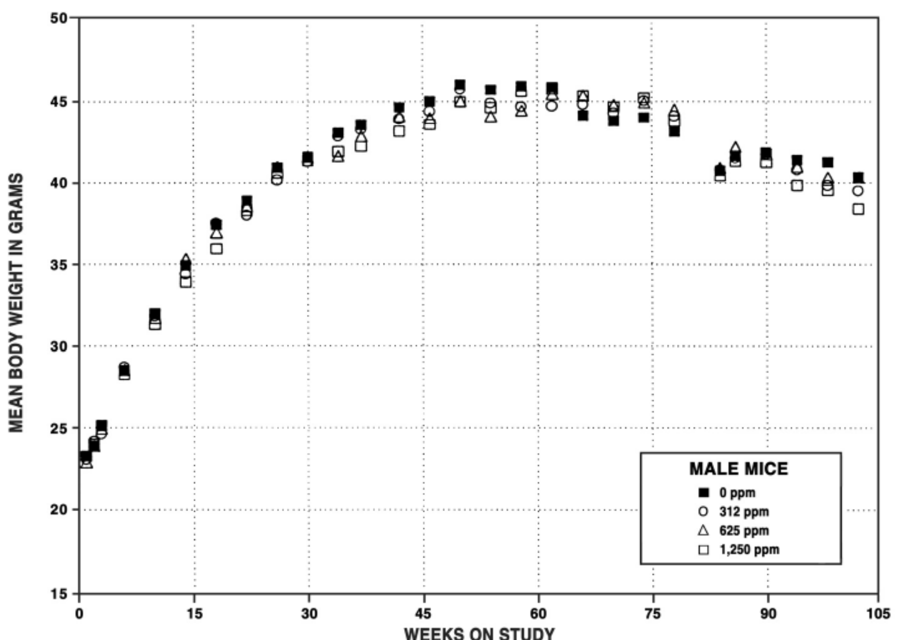
FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Benzophenone in Feed for 2 Years

Table 14
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Benzophenone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	312 ppm Av. Wt. (g)	312 ppm Wt. (% of controls)	312 ppm No. of Survivors	625 ppm Av. Wt. (g)	625 ppm Wt. (% of controls)	625 ppm No. of Survivors	1,250 ppm Av. Wt. (g)	1,250 ppm Wt. (% of controls)	1,250 ppm No. of Survivors
1	23.3	50	23.1	99	50	22.9	98	50	23.3	100	50
2	23.9	50	24.2	101	50	23.9	100	50	24.0	100	50
3	25.2	50	24.6	98	50	25.0	99	50	25.1	100	50
6	28.5	50	28.7	101	50	28.5	100	50	28.3	99	50
10	32.0	50	31.8	99	50	31.7	99	50	31.3	98	50
14	34.9	50	34.4	99	50	35.3	101	50	33.9	97	50
18	37.4	50	37.5	100	50	36.9	99	50	35.9	96	50
22	38.9	50	38.0	98	50	38.5	99	50	38.3	99	50
26	40.9	50	40.2	98	50	41.0	100	50	40.6	99	50
30	41.6	50	41.3	99	50	41.6	100	50	41.4	100	50
34	43.1	50	42.9	100	50	41.6	97	50	41.9	97	50
37	43.6	50	43.3	99	50	42.9	98	50	42.2	97	50
42	44.6	50	43.9	98	50	44.1	99	50	43.2	97	50
46	45.0	50	44.4	99	50	44.0	98	50	43.6	97	50
50	46.0	50	45.8	100	50	45.0	98	50	45.0	98	50
54	45.7	50	44.9	98	50	44.1	97	50	44.6	98	50
58	45.9	50	44.7	97	50	44.4	97	50	45.6	99	50
62	45.9	50	44.7	97	50	45.4	99	50	45.8	100	50
66	44.1	50	44.8	102	49	45.4	103	50	45.3	103	50
70	43.8	49	44.3	101	49	44.8	102	50	44.7	102	50
74	44.0	49	45.0	102	49	44.9	102	50	45.2	103	50
78	43.1	48	44.1	102	49	44.5	103	50	43.8	102	50
84	40.7	48	40.8	100	48	40.9	101	50	40.4	99	49
86	41.7	48	41.5	100	47	42.2	101	48	41.3	99	49
90	41.9	48	41.7	100	45	41.7	100	48	41.3	99	48
94	41.4	48	40.8	99	45	41.0	99	48	39.8	96	48
98	41.2	46	39.8	97	45	40.3	98	46	39.5	96	47
102	40.3	44	39.5	98	44	40.3	100	45	38.4	95	45
Mean for weeks											
1-13	26.6		26.5	100		26.4	99		26.4	99	
14-52	41.6		41.2	99		41.1	99		40.6	98	
53-102	43.1		42.8	99		43.1	100		42.7	99	

TABLE 15
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Benzophenone

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	50	18.6	99	50	18.6	99	50	18.5	98	50
2	19.1	50	19.5	102	50	18.6	97	50	19.5	102	50
6	22.5	50	22.4	100	50	22.7	101	50	22.8	101	50
10	25.0	50	25.7	103	50	24.0	96	50	25.7	103	50
14	27.2	50	28.4	104	50	26.9	99	50	28.0	103	50
18	29.5	50	30.8	104	50	30.3	103	50	30.3	103	50
22	32.8	50	33.7	103	50	33.3	102	50	32.2	98	50
26	33.2	50	35.1	106	50	33.6	101	50	33.0	99	50
30	35.3	50	36.6	104	50	35.5	101	50	34.4	98	50
34	36.5	50	38.0	104	50	36.9	101	49	35.1	96	50
37	38.3	50	39.7	104	50	38.0	99	49	36.2	95	50
42	39.8	50	40.6	102	50	39.3	99	49	37.2	94	50
46	41.2	50	42.0	102	49	40.0	97	49	37.2	90	50
50	41.8	50	42.9	103	49	41.1	98	49	37.6	90	50
54	43.3	50	42.8	99	49	41.4	96	49	37.2	86	49
58	43.7	50	43.5	100	49	40.2	92	49	37.8	87	48
62	44.6	49	43.5	98	49	41.4	93	49	37.9	85	48
66	44.4	48	43.1	97	49	41.4	93	49	38.5	87	47
70	44.4	48	43.3	98	49	41.7	94	48	38.9	88	46
74	45.6	48	44.2	97	49	42.2	93	48	39.4	86	46
78	46.2	48	43.8	95	48	41.5	90	48	39.5	86	45
84	43.5	48	41.4	95	47	39.5	91	47	37.4	86	45
86	43.9	48	42.0	96	46	41.0	93	47	37.8	86	45
90	44.4	44	41.1	93	46	40.6	91	47	38.4	87	44
94	44.4	44	41.1	93	45	41.1	93	45	38.6	87	40
98	45.1	44	41.3	92	45	41.4	92	45	38.4	85	37
102	43.5	44	40.4	93	43	40.2	92	42	37.2	86	35
Mean for weeks											
1-13	21.4		21.6	101		21.0	98		21.6	101	
14-52	35.6		36.8	103		35.5	100		34.1	96	
53-102	44.4		42.4	96		41.0	92		38.2	86	



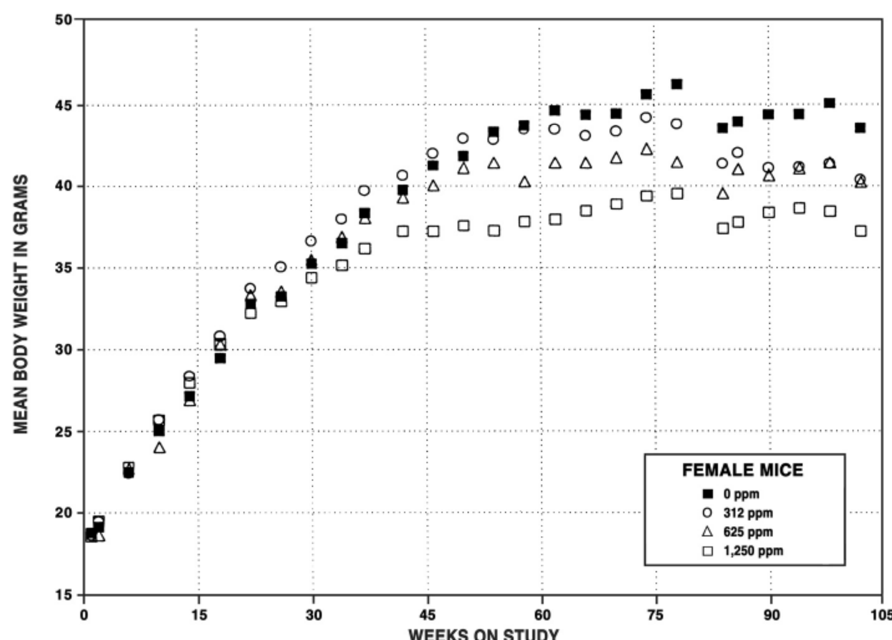


FIGURE 5
Growth Curves for Male and Female Mice Exposed to Benzophenone in Feed for 2 Years

and G4, respectively). Dietary concentrations of 312, 625, and 1,250 ppm resulted in average daily doses of approximately 40, 80, and 160 mg benzophenone/kg body weight to males and 35, 70, and 150 mg/kg to females. No clinical findings were attributed to benzophenone exposure.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of histiocytic sarcoma and neoplasms and/or nonneoplastic lesions of the liver, kidney, nose, spleen, and testes. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: There was a positive trend in the incidences of hepatocellular adenoma in male mice; the incidences in the 625 and 1,250 ppm groups were significantly greater than that in the controls and exceeded the historical control range from feed studies (Tables 16, C3, and C4). Statistically significant increases in the incidences of multiple hepatocellular adenomas occurred in all exposed male mice. However, the incidences of carcinomas did not increase. Hepatoblastomas were also observed in exposed males. The incidences of hepatocellular adenoma in 625 and 1,250 ppm female mice increased, but the differences from the controls were not significant (Tables 16 and D3). The incidence of liver tumors in mice, primarily consisting of hepatocellular adenomas in NTP studies, has been found to be positively associated with body weight (Haseman *et al.*, 1997). When adjusted for the decreased body weight of exposed female mice, there were more hepatocellular adenomas in the 625 ppm and 1,250 ppm groups than expected (0 ppm: 6.8 expected, 5 observed; 312 ppm: 7.0 expected, 4 observed; 625 ppm: 6.2 expected, 10 observed; 1,250 ppm: 4.3 expected, 8 observed). Exposed males and females had increased incidences of eosinophilic foci, and males had increases in clear and mixed cell foci (Tables 16, C5, and D5). However, only the increase in clear cell foci in 1,250 ppm males was significant.

Microscopically, hepatocellular foci, hepatocellular adenomas, and hepatocellular carcinomas represent a continuum and, in this study, had the typical appearance of these lesions reported in $B6C3F_1$ mice. Eosinophilic and basophilic foci were small to moderately large lesions composed of hepatocytes with eosinophilic or basophilic cytoplasm that generally were somewhat enlarged. The hepatocytes were arranged in normal hepatic cords that merged with the surrounding normal hepatocytes. Foci had little or no compression of the surrounding normal hepatocytes, although some degree of compression was present in some larger foci. Adenomas were discrete masses with distinct borders that caused compression of the surrounding normal hepatic parenchyma. Adenomas usually were composed of hepatocytes that appeared similar to those seen in eosinophilic foci, except that in adenomas, the normal lobular architecture was not apparent, and plates of neoplastic hepatocytes intersected the surrounding normal hepatocytes at sharp angles rather than merging with them as in foci. Carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical, but the major distinguishing features of carcinomas were the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly the neoplastic cells formed glandular structures or solid masses. Hepatoblastomas are malignant neoplasms that are presumed to be a primitive form of hepatocellular carcinoma. They were well-demarcated neoplastic masses independent of other hepatocellular tumors. The hepatoblastomas consisted of poorly differentiated, small, elongated, deeply basophilic cells with scant cytoplasm and hyperchromatic nuclei. The cells formed solid sheets, rosettes, and ribbons. They were often arranged around blood vessels. Some hepatoblastomas had large cystic spaces and necrotic areas. Three of the five mice had metastatic hepatoblastomas in the lungs.

Statistically significant increases in centrilobular hepatocyte hypertrophy were observed in all exposed groups of mice (Tables 16, C5, and D5). This hypertrophy was characterized by an increase in the size and staining intensity of the individual hepatocytes in centrilobular areas. These enlarged hepatocytes had a pale to brightly eosinophilic, finely granular cytoplasm. In males, the hypertrophied cells were admixed with numerous enlarged multinucleated hepatocytes having 5 to 20 hyperchromatic nuclei per cell. Most exposed males had multinucleated hepatocytes accompanied by

Table 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus[a]	2	7	7	12**
Eosinophilic Focus	5	8	11	10
Mixed Cell Focus	8	9	15	13
Hepatocyte, Centrilobular, Hypertrophy	0	44** (2.0)[b]	50** (2.0)	48** (3.0)
Hepatocyte, Multinucleated	0	41** (1.4)	47** (1.5)	48** (1.8)
Hepatocyte, Necrosis	1 (1.0)	6 (1.7)	8* (1.8)	8* (1.3)
Inflammation, Chronic Active	33 (1.0)	47** (1.1)	44** (1.2)	42* (1.1)
Hepatocyte, Degeneration, Cystic	0	0	5* (1.2)	30** (1.9)
Hepatocellular Adenoma, Multiple	2	8*	8*	12**
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	11/50 (22%)	15/50 (30%)	23/50 (46%)	23/50 (46%)
Adjusted rate[e]	22.9%	31.5%	46.9%	46.6%
Terminal rate[f]	10/44 (23%)	14/44 (32%)	21/44 (48%)	21/45 (47%)
First incidence (days)	703	606	585	568
Poly-3 test[g]	P=0.006	P=0.239	P=0.010	P=0.011
Hepatocellular Carcinoma	8	5	6	6
Hepatoblastoma[h]				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	2.1%	6.1%
Terminal rate	0/44 (0%)	1/44 (2%)	1/44 (2%)	2/45 (4%)
First incidence (days)	—[i]	730 (T)	730 (T)	606
Poly-3 test	P=0.057	P=0.497	P=0.502	P=0.123
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[j]				
Overall rate	18/50 (36%)	20/50 (40%)	25/50 (50%)	29/50 (58%)
Adjusted rate	37.0%	40.7%	50.9%	58.1%
Terminal rate	16/44 (36%)	16/44 (36%)	23/44 (52%)	25/45 (56%)
First incidence (days)	540	449	585	568
Poly-3 test	P=0.013	P=0.434	P=0.118	P=0.027

Table 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus	3	2	4	4
Eosinophilic Focus	2	2	7	7
Mixed Cell Focus	2	5	3	2
Hepatocyte, Centrilobular, Hypertrophy	0	29** (2.0)	44** (2.0)	37** (2.9)
Hepatocyte, Multinucleated	0	0	0	2 (1.0)
Hepatocyte, Necrosis	3 (2.0)	5 (2.0)	4 (1.5)	0
Inflammation, Chronic Active	44 (1.1)	40 (1.1)	41 (1.0)	36* (1.1)
Hepatocyte, Degeneration, Cystic	0	0	0	0
Hepatocellular Adenoma, Multiple	1	1	3	3
Hepatocellular Adenoma (includes multiple)[k]				
Overall rate	5/50 (10%)	4/50 (8%)	10/50 (20%)	8/50 (16%)
Adjusted rate	10.8%	8.5%	21.4%	18.1%
Terminal rate	5/40 (13%)	3/42 (7%)	10/41 (24%)	7/31 (23%)
First incidence (days)	729 (T)	680	729 (T)	435
Poly-3 test	P=0.109	P=0.494N	P=0.131	P=0.243
Hepatocellular Carcinoma	0	1	0	1
Hepatocellular Adenoma or Carcinoma[l]				
Overall rate	5/50 (10%)	5/50 (10%)	10/50 (20%)	9/50 (18%)
Adjusted rate	10.8%	10.7%	21.4%	20.3%
Terminal rate	5/40 (13%)	4/42 (10%)	10/41 (24%)	7/31 (23%)
First incidence (days)	729 (T)	680	729 (T)	435
Poly-3 test	P=0.081	P=0.624N	P=0.131	P=0.165

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with feed controls given NTP-2000 diet (mean ± standard deviation): 90/460 (20.0% ± 7.1%), range 12%-30%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
[h] Historical incidence: feed 1/460 (0.2% ± 0.6%), range 0%-2%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 145/460 (32.4% ± 9.1%), range 20%-47%
[k] Historical incidence: 40/457 (9.6% ± 2.4%), range 6%-12%
[l] Historical incidence: 53/457 (11.8% ± 3.1%), range 8%-16%

increases in the incidences of necrosis and chronic active inflammation. The 625 and 1,250 ppm male groups had significant increases in the incidences of cystic degeneration of hepatocytes. This lesion was characterized by multilocular cyst-like spaces within the hepatic parenchyma containing a pale, floccular, eosinophilic material. It is reported with a low incidence as a spontaneous finding in aged mice.

Histiocytic Sarcoma: In females, there was a positive trend in the incidences of histiocytic sarcoma (all organs); the incidence in 625 ppm females was significantly greater than that in the controls (Tables 17 and D3). Only two histiocytic sarcomas have been observed in historical feed study controls, and the incidence in the 625 ppm group exceeded the historical control range for all routes (Tables 17 and D4). In the current 2-year study, only females were affected, and the liver and lung were involved in all affected females. The histiocytic sarcomas were highly invasive in all three 1,250 ppm mice. Multiple organs throughout the body had neoplastic histiocytic lesions. Ovary, uterus, spleen, adrenal gland, kidney, urinary bladder, and multiple lymph nodes were affected in all three animals. Although multiple organs were involved in the five females of the 625 ppm group, fewer organs were affected. Histologically, cells that are characteristic of neoplastic histiocytes were large with relatively abundant, pale eosinophilic cytoplasm. Their nuclei were dark basophilic with round to oval shapes and inconspicuous nucleoli. Variation in the size and shape of some neoplastic cells and high cytoplasmic-to-nuclear ratios were observed. Occasional multinucleated giant cells were present (Plate 3). Fibrosis was scant. Growth was both infiltrative and expansive and extended on pleural and peritoneal surfaces. Metastatic neoplastic emboli were frequently present in blood vessels. Neoplastic histiocytic cells infiltrated the liver, expanded the hepatic sinusoids, and frequently formed nodular patterns or thick sheets that disrupted the hepatic parenchyma. In the lung, intravascular and perivascular infiltrates of neoplastic histiocytic cells were observed (Plate 4).

Kidney: Exposed female mice had significantly increased incidences of nephropathy accompanied by mineralization (Tables 18 and D5). The mineralization was characterized by basophilic mineral deposits in the cortical tubules and medullary collecting ducts. The severity of nephropathy was significantly increased in all exposed groups of male mice. Nephropathy was characterized by tubular degeneration, tubular regeneration, interstitial inflammation, dilatation of renal tubules, intratubular protein casts, and subcapsular regions of

TABLE 17
Incidences of Histiocytic Sarcoma in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Histiocytic Sarcoma[a]				
Overall rate[b]	0/50 (0%)	0/50 (0%)	5/50 (10%)	3/50 (6%)
Adjusted rate[c]	0.0%	0.0%	10.7%	6.9%
Terminal rate[d]	0/40 (0%)	0/42 (0%)	4/41 (10%)	2/31 (7%)
First incidence (days)	—[f]	—	718	651
Poly-3 test[e]	P=0.032	—[g]	P=0.031	P=0.108

[a] Historical incidence for 2-year studies with feed controls given NTP-2000 diet (mean ± standard deviation): 2/459 (0.3% ± 0.8%), range 0%-2%; all routes 18/1,258 (1.5% ± 2.2%), range 0%-8%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed

TABLE 18
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Kidney[a]	50	50	50	50
Nephropathy[b]	49 (1.2)[c]	48 (1.4)▲	50 (1.7)▲	50 (3.0)▲
Cortex, Cyst	4	8	12*	22**
Nose	50	50	50	50
Olfactory Epithelium, Metaplasia	0	2 (1.0)	2 (1.0)	24** (1.2)
Spleen	50	50	50	50
Lymphoid Follicle, Hyperplasia, Lymphoid	17 (2.1)	31** (2.5)	34** (2.0)	32** (2.2)
Testes	50	50	50	50
Mineralization	0	1 (1.0)	4 (1.0)	12** (1.1)
Female				
Kidney	50	50	50	50
Nephropathy	21 (1.2)	33** (1.1)	31* (1.5)	30* (1.7)▲
Mineralization	15 (1.0)	31** (1.0)	36** (1.1)	49** (1.5)
Nose	50	50	50	50
Olfactory Epithelium, Metaplasia	0	0	0	39** (1.7)
Spleen	50	50	50	50
Hematopoietic Cell Proliferation	16 (2.6)	35** (2.1)	32** (2.4)	27* (2.8)
Lymphoid Follicle, Hyperplasia, Lymphoid	24 (2.5)	36** (2.5)	37** (2.7)	22 (2.9)

* Significantly different ($P \le 0.05$) from the control group by the Poly-3 test
**$P \le 0.01$
▲ Significantly different ($P \le 0.05$) from the control group by the Mann-Whitney U test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

interstitial fibrosis scars. The nephropathy was accompanied by significantly increased incidences of cortex cysts in the 625 and 1,250 ppm groups.

Nose: The incidences of metaplasia of the olfactory epithelium were significantly increased in 1,250 ppm male and female groups (Tables 18, C5, and D5). The metaplasia was characterized by a replacement of normal olfactory epithelium by a single layer of ciliated columnar epithelium resembling normal respiratory epithelium. The metaplasia was focal to multifocal and involved the dorsal meatus, dorsal nasal septum, and ethmoid turbinates of levels II and III. Frequently, the metaplasia extended into underlying submucosal Bowman's glands.

Spleen: The incidences of hematopoietic cell proliferation in all exposed groups of female mice were significantly greater than that of the controls (Tables 18 and D5). Hematopoietic cell proliferation consisted of increased numbers of megakaryocytes and myeloid and erythroid precursors. Hyperplasia of lymphoid follicles was significantly increased in all exposed groups of males and in 312 and 625 ppm females (Tables 18, C5, and D5). Lymphoid follicular hyperplasia was characterized by white pulp lymphoid follicles enlarged from normal size to the point of follicular coalescence that is associated with malignant lymphoma.

Testes: The incidence of mineralization was significantly increased in 1,250 ppm males (Tables 18 and C5). The mineralization commonly occurred as basophilic deposits in the walls and lumen of small blood vessels and in the tunica. The mineralization was not associated with degeneration of the germinal epithelium.

Toxicokinetic Studies

Single-dose toxicokinetic studies were performed in male and female F344/N rats and B6C3F$_1$ mice (Appendix J). Plasma concentrations of the parent compound were determined following oral and intravenous administration of benzophenone. The plasma concentration of benzophenone versus time plots showed secondary maxima, apparently due to enterohepatic circulation. The data were analyzed by noncompartmental modeling and indicated no consistent sex-related or exposure-related effects in either species. In contrast, the plasma benzophenone concentrations taken during the 2-year study clearly showed a sex-related effect in rats. The area under the plasma concentration curve versus time plot was significantly higher for females at all but two exposure/AUC entries in Table J4. The dose based on food consumption (Tables G1 and G2), however, is similar for both sexes.

Genetic Toxicology

Benzophenone showed no evidence of mutagenicity *in vitro* or *in vivo*. Benzophenone (1 to 1,000 µg/plate) did not induce mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver metabolic activation enzymes (Table E1; Mortelmans *et al*., 1986). Intraperitoneal injections of 200 to 500 mg benzophenone per kg body weight (three injections at 24 hour intervals) did not induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) of male B6C3F$_1$ mice (Table E2). A small increase in the frequency of micronucleated PCEs was noted in the 400 mg/kg group, but the difference was not statistically significant. No increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood of male or female B6C3F$_1$ mice administered benzophenone for 14 weeks in feed over a concentration range of 1,250 to 20,000 ppm (Table E3). No significant alterations in the percentage of PCEs among total erythrocytes were noted in either micronucleus test, indicating no toxicity to the bone marrow from benzophenone treatment.

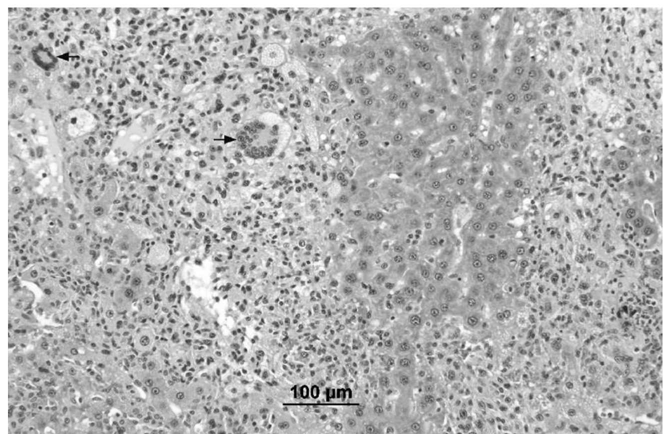


Plate 1
Histiocytic sarcoma in the liver of a female F344/N rat exposed to 625 ppm benzophenone in feed for 2 years. Note the hepatocytes surrounded by a massive infiltrate of neoplastic histiocytes with formation of multinucleated giant cells (arrows). H&E; 20×

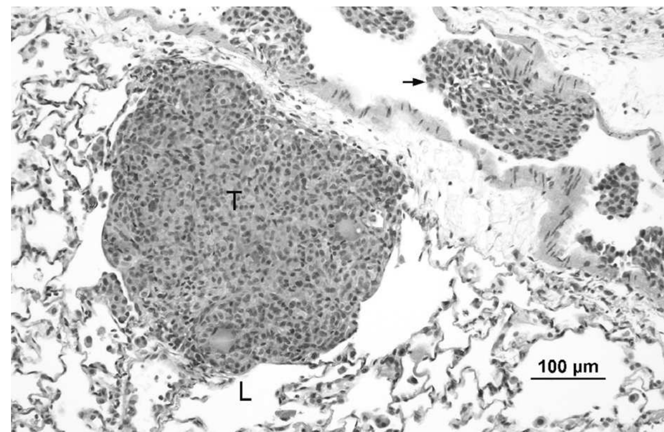


Plate 2
Metastatic histiocytic sarcoma (T) in the lung (L) of a female F344/N rat exposed to 625 ppm benzophenone in feed for 2 years. Note the multiple groups of intravascular neoplastic histiocytes (arrow). H&E; 20×

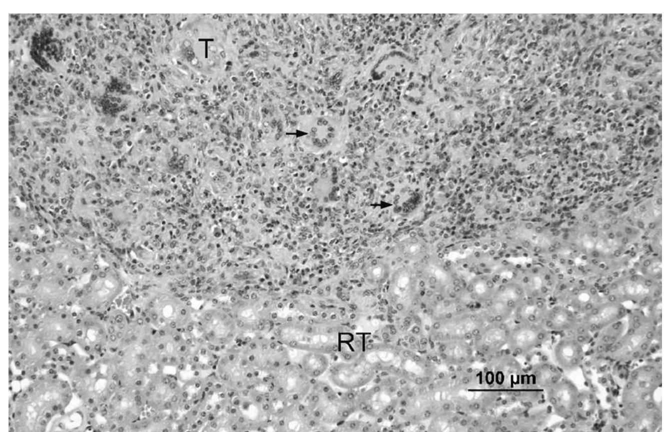


Plate 3
Histiocytic sarcoma (T) in the kidney of a female B6C3F$_1$ mouse exposed to 625 ppm benzophenone in feed for 2 years. Note the neoplastic histiocytes invading the adjacent renal tubule tissue (RT). Many multinucleated giant cells cells (arrows) are present. H&E; 20×

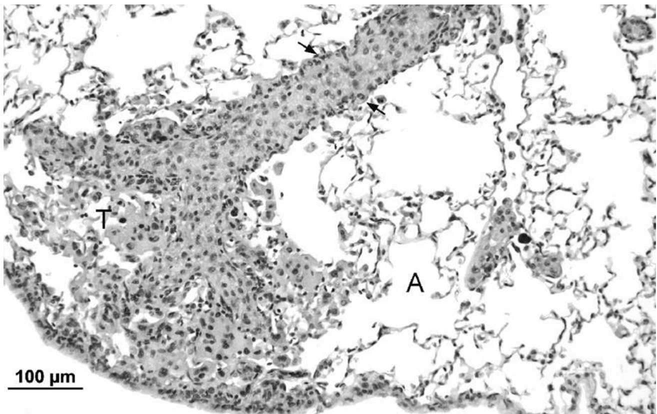


Plate 4
Metastatic histiocytic sarcoma (T) in the lung of a female B6C3F$_1$ mouse exposed to 1,250 ppm benzophenone in feed for 2 years. Note the intravascular neoplastic histiocytes (arrows) invading the adjacent alveoli (A). H&E; 20×

DISCUSSION AND CONCLUSIONS

Benzophenone is used to manufacture insecticides, agricultural chemicals, hypnotics, antihistamines, and other pharmaceuticals; as an ultraviolet curing agent in sunglasses and ink; as an additive in plastics, coatings, and adhesive formulations; and as a flavor ingredient. Concentrations of benzophenone in food products range from 0.57 ppm in nonalcoholic beverages to 3.27 ppm in frozen dairy products; it may also be an ingredient in baked goods, soft candy, gelatins, and puddings (NAS/NRC, 1979). Benzophenone was selected for toxicologic and carcinogenicity evaluations based on the potential for occupational and consumer exposure and the lack of chronic toxicity data. The National Toxicology Program previously performed 14-week toxicity studies on benzophenone and published the results in a separate report (NTP, 2000). The current 2-year studies were designed to evaluate and characterize the potential carcinogenicity of benzophenone in rats and mice. For the 2-year studies reported here, the highest exposure concentration selected was 1,250 ppm based on the 14-week studies that indicated this exposure level was minimally toxic across both species and sexes.

In the 14-week exposure to benzophenone at concentrations of 1,250, 2,500, 5,000, 10,000, or 20,000 ppm in rats and mice, the liver and kidney were identified as the primary target organs of benzophenone toxicity in rats (NTP, 2000). In mice, the liver was the major target of toxicity. In rats, liver changes were observed at exposure concentrations greater than or equal to 5,000 ppm, while in mice, microscopic changes in the liver were observed in all exposed groups. Gross (increased organ weights) and microscopic (hepatocellular hypertrophy) liver changes associated with benzophenone administration in males and females were accompanied by benzophenone-induced increases in the activity of pentoxyresorufin dealkylase, an enzyme activity linked to the cytochrome P450 2B isozyme. Liver hypertrophy (increases in cell size) is often attributed in part to induction of drug metabolizing enzymes. In rats, increased kidney weights were associated with a spectrum of renal changes in exposed male and female rats. One change found predominantly in 20,000 ppm animals, which died early, was papillary necrosis characterized by acute coagulative necrosis of the distal tips of the renal papillae. Unique lesions seen in rats were well-demarcated wedge-shaped areas of prominent tubule dilatation. In male rats, this change was present at exposure concentrations of 2,500 ppm and greater, while in females it occurred only at 10,000 and 20,000 ppm. Foci of tubule regeneration were increased in incidence and/or severity relative to the controls in exposed males and females.

In the current 2-year studies, there were no differences in survival of female rats or male mice exposed to benzophenone compared to controls. Survival was significantly reduced in 1,250 ppm male rats, most likely due to nephropathy. Female mice exposed to 1,250 ppm benzophenone tended to have decreased survival toward the end of the study, but the difference from the control group was not statistically significant.

The target organs of toxicity in the 2-year studies were liver, kidney, nose, and testes. Neoplastic responses occurred in the kidney, liver, and hematopoietic system.

In the 2-year rat study, exposed animals exhibited a positive trend in the incidences of renal tubule adenoma. The NTP has found that examination of the entire kidney, by step sectioning of residual tissues, enables a more precise evaluation of the potential chemical-related induction of renal proliferative lesions than observations made from single sections, particularly when the proliferative lesions are small and identified only by microscopic examination (Eustis *et al.*, 1994). For benzophenone, this extended evaluation of the male rat kidney showed significant increases in the incidences of renal tubule adenoma in 625 and 1,250 ppm males and increased incidences of hyperplasia in all exposed groups of males. Incidences of renal tubule hyperplasia in all exposed female groups were significantly greater than that of the control group when the single and step section evaluations were combined.

Within the NTP 2-year carcinogenicity studies, the kidney is the second most commonly affected site in male

rats for chemically associated site-specific neoplasms (NTP, 2004b). In the majority of the studies, the increases are primarily of adenomas, and in many instances there is a concurrent dose-related increase in the severity of chronic progressive nephropathy. Chronic nephropathy may influence the induction, development, or progression of renal neoplasms in several ways, including a reduction in target cell population and/or increased number of cells in the replicative cycle due to chronic inflammation and continued degeneration and necrosis, alterations in vascularity as a result of fibrosis, or other alterations in microenvironment. The pathogenesis of chemically induced renal tubule neoplasms has not been determined; however it appears to be complex with genotoxic and nongenotoxic modes (Barrett and Huff, 1991; Short, 1993; Hard, 1998). Data from retrospective reviews of NTP 2-year carcinogenesis studies suggest that an increased severity of nephropathy may contribute to overall tumor response (Seely *et al.*, 2002). However, any contribution appears to be marginal, and additional factors are likely involved.

In female rats, the incidence of mononuclear cell leukemia was marginally increased in the 625 ppm group. Male rats exposed to 312 or 625 ppm benzophenone exhibited significantly increased incidences of mononuclear cell leukemia. Significantly increased incidences were not observed in females exposed to 1,250 ppm, and the incidence in males exposed to 1,250 ppm was similar to the incidence in control males. Mononuclear cell leukemia is generally a late developing neoplasm with most observed in animals after 18 months on study. The incidence of mononuclear cell leukemia in males exposed to 1,250 ppm may have been somewhat higher had survival not been reduced in the last quarter of the study. The incidences of mononuclear cell leukemia in 312 and 625 ppm males and all exposed groups of females were outside the historical control ranges of 30% to 68% in male controls from 2-year NTP feed studies and 12% to 38% in control females; however, the incidence in the female control group was also outside the historical range. The data from this study of benzophenone were included in the historical control dataset, and the 38% incidence was the highest in the dataset. There is no obvious explanation for the higher incidence in the control group.

Mononuclear cell leukemia, a common neoplasm in F344/N rats, is generally thought to arise within the spleen. The spleen is the first and most commonly affected organ, followed by involvement of the liver. With progression, mononuclear cell leukemia becomes widespread and involves multiple organs. Earlier onset and wider distribution of mononuclear cell leukemia in exposed groups would indicate that the increased incidences of mononuclear cell leukemia were treatment related; however, there was no evidence that mononuclear cell leukemia occurred earlier in exposed groups than in control groups in this study. Assessment of the distribution of mononuclear cell leukemia in exposed and control males and females (Table 11) demonstrated lesser involvement of the spleen and liver in the 625 and 1,250 ppm male groups and greater involvement of the spleen and liver in the 625 and 1,250 ppm female groups when compared to the control groups. Although a hint of increased grade 3 mononuclear cell leukemia was observed in exposed females, there was no significant increase in the average severity grade in exposed versus control groups. The average severity grade was significantly decreased in males. Even though the incidences in exposed groups often exceeded the historical control ranges, because the incidences in the 1,250 ppm groups were not significantly increased and there was no evidence of early occurrence or wider distribution in exposed groups, the increased incidences were only considered equivocal evidence of carcinogenicity.

Benzophenone exposure resulted in a positive trend in the incidence of histiocytic sarcoma in female mice, and one 625 ppm and two 1,250 ppm female rats had histiocytic sarcomas. This neoplasm is rare; none have been observed in historical feed study control rats, and only two have been observed in feed study control mice given the NTP-2000 diet. In historical controls from all routes of exposure, histiocytic sarcoma was observed in one of 1,209 (0.08%) historical control rats and 18 of 1,258 historical control mice (1.4%). Histiocytic sarcomas are classified as hematopoietic tumors of the mononuclear phagocyte system based upon the morphology of the neoplastic cells and the presence of lysozyme, Mac-2, and mononuclear phagocyte antigens. The specific origin of the neoplastic histiocytic cells is undetermined. One or more cell populations may be involved. Bone marrow cells, tissue histiocytes, Kupffer's cells in the liver, and circulating macrophages have been suggested. Histiocytic sarcomas are slightly more common in female than male mice and in mice than rats (Frith *et al.*, 1993). Although the spontaneous incidence of this tumor is low in both mice and rats, the frequency varies widely among different strains of mice and rats. Histiocytic sarcomas are more common in Sprague-Dawley rats, with an overall incidence of 4.7%, than in the Fischer 344, used by the NTP, and Osborne-Mendel

strains. In mice exposed to benzophenone, the liver and lung were involved in all affected animals. In the 1,250 ppm female mice, the histiocytic sarcomas were highly invasive. Multiple organs throughout the body had neoplastic histiocytic lesions. All affected rats exposed to benzophenone had lung lesions. Only one rat in the 625 ppm group had organs affected throughout the body. Chemical-associated increases in the incidences of histiocytic sarcomas have not been seen in rats in NTP studies and are uncommon in mice. Increased incidences in mice occurred in studies of 1,3-butadiene (NTP, 1993), tetrafluoroethylene (NTP, 1996a), and phenolphthalein (NTP, 1996b). The increased incidences in the 625 and 1,250 ppm female groups and the increased invasiveness in the 1,250 ppm mice were considered related to benzophenone exposure and some evidence of carcinogenicity. The low incidence of this rare neoplasm in female rats was considered equivocal evidence of carcinogenic activity.

Female mice in all exposed groups had increased incidences of spleen hematopoietic cell proliferation. The proportions of these cells varied from animal to animal. Hematopoietic cell proliferation, also termed extramedullary hematopoiesis, is a common and normal phenomenon in the spleen of mice, to a greater degree in females than males. The incidence in the control female group in this study is consistent with previous NTP studies (Ward *et al.*, 1999). Increased hematopoietic cell proliferation has been associated with anemia and chronic inflammatory lesions. Evidence of an anemia with minimal severity was observed in rats and mice during the 14-week studies at higher doses than were used in the 2-year study (NTP, 2000).

Increases in the incidences of hepatocellular adenoma were observed in male and female mice. Hepatoblastomas were also observed in exposed males; however, the increased incidence was not statistically significant. Female mice showed more hepatocellular adenomas than expected in the 625 and 1,250 ppm groups when corrected for decreased body weight (0 ppm: 6.8 expected, 5 observed; 312 ppm: 7.0 expected, 4 observed; 625 ppm: 6.2 expected, 10 observed; 1,250 ppm: 4.3 expected, 8 observed) (Haseman *et al.*, 1997). Hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas represent a biological and morphological continuum in progression of proliferative lesions. Because the malignant potential of hepatoblastomas and hepatocellular carcinomas appears similar and hepatoblastomas are often observed within hepatocellular neoplasms (mostly carcinomas), it is appropriate to combine the incidences of hepatoblastoma with those of adenoma and carcinoma when interpreting the carcinogenic potential of a chemical. The combined incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma was significantly increased in 1,250 ppm males, and the incidences showed a positive trend. This was considered some evidence of carcinogenicity. The response in females was considered equivocal.

Benzophenone exposure significantly increased incidences of hepatocellular centrilobular hypertrophy in male and female rats and mice. The description of the centrilobular hypertrophy was in agreement with previous reports which also describe clumping of basophilic material in centrilobular hepatocytes (Burdock *et al.*, 1991). The hepatocellular enlargement observed in the current 2-year studies was probably accompanied by induction of cytochromes P450 as observed in the 14-week studies (NTP, 2000). The pattern of induction described in the 14-week studies was similar to that associated with exposure to phenobarbital, in that pentoxyresorufin dealkylase activity, and not that of ethoxyresorufin deethylase, was induced.

In the current study, the incidences of metaplasia of the olfactory epithelium were significantly increased in the 1,250 ppm male and female mice. This was a species-specific effect, as rats did not display similar lesions, possibly because of differences in the anatomy of the rat nasal cavity and potential lower relative exposures to benzophenone in rats. The metaplasia was focal to multifocal, primarily involved the dorsal meatus, dorsal nasal septum, and ethmoid turbinates, and was characterized by a replacement of normal olfactory epithelium by a single layer of ciliated columnar epithelium resembling normal respiratory epithelium. This metaplasia is considered the result of repair following earlier damage to the more sensitive olfactory epithelium. The submucosal (Bowman's) glands were also involved. The mechanism by which benzophenone caused this lesion is unknown; however, enzymatic metabolism of benzophenone in the olfactory epithelium, which has a high concentration of cytochrome P450, may be involved. Some compounds, such as phosphodiesterase inhibitors, that require metabolic activation by the cytochrome P450 enzyme system have been shown to cause olfactory epithelial injury, chronic hyperplastic/regenerative lesions, and olfactory neoplasms following oral or inhalation exposure in rodents (Pino *et al.*, 1999).

All exposed groups of male and female rats in the current studies displayed significantly decreased incidences of thyroid gland C-cell hyperplasia. The C-cells synthesize, store, and release the hormone calcitonin in response to physiologic alterations in serum calcium levels. As F344 rats age, there is a diffuse increase in C-cells. Thyroid gland C-cell hyperplasia is a common age-associated change in male and female rats in chronic NTP studies (Boorman *et al.*, 1996). Incidences of C-cell hyperplasia in control groups of both sexes are within expected values. The decreased incidences of thyroid gland C-cell hyperplasia were not related to the severity of nephropathy in males and appear to be treatment related. The possible relationship of calcium regulation by C-cells to benzophenone exposure is unknown.

Decreases in the incidences and multiplicities of mammary gland fibroadenoma were observed in female rats exposed to benzophenone. Fibroadenomas are the most common neoplasm of the mammary gland in female rats, occurring in 213/460 (46%, range 28% to 55%) NTP feed study control animals. The incidence of mammary gland tumors in NTP studies has been found to be positively associated with body weight. However, the decreased incidence of mammary gland tumors in this study could not be attributed to decreased body weights of exposed females, as the 1,250 ppm females had significantly lower incidences of this neoplasm after correcting for decreased body weight (Haseman *et al.*, 1997). Interestingly, benzophenone-based derivatives have shown impressive inhibitory activity of steroid sulfatase, an enzyme that regulates the formation of estrone and subsequent conversion to estradiol, and may be developed for therapeutic use in the treatment of hormone-dependent breast cancer (Hejaz *et al.*, 2004).

Plots of plasma concentration of benzophenone versus time in the single-dose toxicokinetic studies showed evidence of enterohepatic circulation. The Phase II metabolism of benzophenone has not been well characterized. Benzhydrol has been determined to be a metabolite (Nakagawa *et al.*, 2000). The metabolite participating in the recirculation is proposed to be the glucuronide of benzhydrol (Appendix J). This metabolite may be the labile glucuronide described earlier (Robinson, 1958; Robinson and Williams, 1957).

The higher plasma concentrations of benzophenone in female rats compared to males may depend on the sex-related differences in organic anion transporters (OAT) in the kidney (Buist and Klaassen, 2004). When the rate of elimination of parent or a metabolite is determined by the rate of renal clearance, elimination has been shown to be slower in female rats (Griffin *et al.*, 1997; Dill *et al.*, 1998). It is likely that benzhydrol glucuronide is a major urinary metabolite and is an OAT substrate. The enterohepatic circulation of this metabolite may mask the differences in renal clearance after a single dose, but as this process reaches "equilibrium," the difference in renal clearance becomes apparent.

Benzophenone showed no evidence of genotoxicity *in vitro* or *in vivo* in standard mutagenicity assays. Benzophenone was negative in *Salmonella typhimurium* gene mutation assays, with or without exogenous metabolic activation enzymes (Mortelmans *et al.*, 1986; Takemoto *et al.*, 2002), and no increases in micronucleated erythrocytes were noted in mice after acute or subchronic exposure to benzophenone. Interestingly, use of human recombinant P450 enzyme preparations, including P450 family 1 enzymes, in a *S. typhimurium umu* gene expression assay with benzophenone and two metabolites, benzhydrol and *p*-benzoylphenol, produced dose-related increases in gene expression (Takemoto *et al.*, 2002). This observation is intriguing because P450 1B1 is constitutively expressed in human skin cells, and benzophenone is an ingredient in some topical sunscreen preparations.

CONCLUSIONS

Under the conditions of these 2-year studies, there was *some evidence of carcinogenic activity** of benzophenone in male F344/N rats based on increased incidences of renal tubule adenoma; mononuclear cell leukemia in male F344/N rats may have been related to benzophenone exposure. There was *equivocal evidence of carcinogenic activity* of benzophenone in female F344/N rats based on the marginally increased incidences of mononuclear cell leukemia and histiocytic sarcoma. There was *some evidence of carcinogenic activity* of benzophenone in male B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was *some evidence of carcinogenic activity* of benzophenone in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcoma; the incidences of hepatocellular adenoma in female B6C3F$_1$ mice may have been related to benzophenone exposure.

Administration of benzophenone in feed resulted in increased incidences and/or severities of nonneoplastic lesions in the kidney and liver of male and female rats

and in the liver, kidney, nose, and spleen of male and female mice.

Decreased incidences of mammary gland fibroadenoma in female rats were related to benzophenone exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF BENZOPHENONE

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Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	25	12	16	44
Natural deaths	3	11	3	4
Survivors				
Terminal sacrifice	22	27	31	2
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Leiomyoma			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma			1 (2%)	
Hepatocellular adenoma	2 (4%)		1 (2%)	
Mesentery	(9)	(12)	(8)	(4)
Oral mucosa	(8)	(8)	(5)	(7)
Gingival, squamous cell papilloma		1 (13%)		
Pharyngeal, squamous cell papilloma				1 (14%)
Pancreas	(50)	(50)	(50)	(50)
Mixed tumor benign	1 (2%)			
Salivary glands	(49)	(50)	(50)	(49)
Schwannoma malignant	1 (2%)			
Stomach, forestomach	(50)	(49)	(49)	(50)
Stomach, glandular	(50)	(50)	(49)	(50)
Tongue	(1)	(1)		(1)
Squamous cell papilloma	1 (100%)	1 (100%)		1 (100%)
Tooth	(3)	(5)	(2)	(3)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Chordoma, metastatic, bone	1 (2%)			
Schwannoma malignant	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma malignant			2 (4%)	1 (2%)
Pheochromocytoma benign	8 (16%)	5 (10%)	6 (12%)	3 (6%)
Bilateral, pheochromocytoma benign			2 (4%)	

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	1 (2%)
Parathyroid gland	(49)	(45)	(48)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	10 (20%)	6 (12%)	12 (24%)	5 (10%)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma			3 (6%)	
C-cell, adenoma	10 (20%)	6 (12%)	6 (12%)	4 (8%)
C-cell, carcinoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Follicular cell, adenoma	2 (4%)	2 (4%)	3 (6%)	3 (6%)
General Body System				
Peritoneum	(1)		(1)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma		3 (6%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)	3 (6%)	3 (6%)	1 (2%)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(49)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	43 (86%)	41 (82%)	40 (80%)	42 (84%)
Interstitial cell, adenoma	2 (4%)	5 (10%)	6 (12%)	6 (12%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Lymph node	(19)	(20)	(16)	(19)
Lymph node, mandibular	(5)	(1)	(2)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Chordoma, metastatic, bone	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(48)	(48)	(48)
Chordoma, metastatic, bone	1 (2%)			
Integumentary System				
Mammary gland	(50)	(48)	(50)	(50)
Fibroadenoma	2 (4%)	2 (4%)		1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		3 (6%)	1 (2%)	
Fibrous histiocytoma	1 (2%)			
Keratoacanthoma	10 (20%)	3 (6%)	3 (6%)	3 (6%)
Osteosarcoma	1 (2%)	2 (4%)		
Schwannoma malignant				1 (2%)
Squamous cell papilloma	1 (2%)	2 (4%)		
Trichoepithelioma			1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	3 (6%)	3 (6%)	2 (4%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)			
Osteosarcoma			1 (2%)	
Skeletal muscle	(2)	(2)	(1)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	1 (2%)		
Alveolar/bronchiolar carcinoma			1 (2%)	2 (4%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Carcinoma, metastatic, adrenal cortex		1 (2%)		
Chordoma, metastatic, bone	1 (2%)			
Osteosarcoma, metastatic, skin		2 (4%)		
Pheochromocytoma malignant, metastatic, adrenal medulla			2 (4%)	1 (2%)
Mediastinum, myxosarcoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Pelvis, transitional epithelium, carcinoma				1 (2%)
Renal tubule, adenoma	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Renal tubule, carcinoma		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	27 (54%)	41 (82%)	39 (78%)	24 (48%)
Mesothelioma malignant	2 (4%)	3 (6%)	2 (4%)	

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	50	50
Total primary neoplasms	139	139	143	109
Total animals with benign neoplasms	49	47	49	50
Total benign neoplasms	103	86	92	77
Total animals with malignant neoplasms	33	42	40	26
Total malignant neoplasms	36	53	51	32
Total animals with metastatic neoplasms	2	5	4	1
Total metastatic neoplasms	9	12	6	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

	4	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7
Number of Days on Study	2	9	0	4	5	8	8	3	3	3	4	6	6	7	7	8	8	8	9	9	9	0	0	0	0
	5	5	6	9	3	2	4	2	4	8	5	2	6	6	6	0	0	1	0	3	4	4	4	9	9
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carcass ID Number	2	2	1	1	1	3	3	0	4	2	1	3	3	3	5	0	2	4	4	3	2	2	2	1	2
	6	3	7	4	9	3	7	8	4	1	2	9	2	0	0	9	8	5	9	5	5	2	9	3	4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma														X											
Mesentery		+		+																			+		
Mesothelioma malignant, metastatic, epididymis				X																					
Oral mucosa									+			+			+				+			+	+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis				X																					
Mixed tumor benign																									
Salivary glands	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Squamous cell papilloma																									
Tooth										+				+											
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma, metastatic, bone						X																			
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																			X				X	X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X								
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X						X				X			X	X	X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma										X								X	X				X		X
C-cell, carcinoma																									
Follicular cell, adenoma									X																

+: Tissue examined microscopically — M: Missing tissue — X: Lesion present
A: Autolysis precludes examination — I: Insufficient tissue — Blank: Not examined

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	715	725	726	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
Carcass ID Number	047	020	003	001	002	007	010	015	016	018	027	031	034	036	038	040	041	042	043	048	004	005	006	011	046	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																								X		2
Mesentery		+							+						+		+			+	+					9
Mesothelioma malignant, metastatic, epididymis																										1
Oral mucosa	+															+										8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Mixed tumor benign									X																	1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma malignant																							X			1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue											+															1
Squamous cell papilloma											X															1
Tooth											+															3
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma, metastatic, bone																										1
Schwannoma malignant									X																	1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign			X				X													X				X	X	8
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X																	X								10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma										X		X					X	X		X						10
C-cell, carcinoma							X																			1
Follicular cell, adenoma																				X						2

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	4 2 5	4 9 5	5 0 6	5 4 9	5 5 3	5 8 2	5 8 4	6 3 2	6 3 4	6 3 8	6 4 5	6 6 2	6 6 6	6 7 6	6 7 6	6 8 0	6 8 0	6 8 1	6 9 0	6 9 3	6 9 4	7 0 4	7 0 4	7 0 9	7 0 9
Carcass ID Number	0 2 6	0 2 3	0 1 7	0 1 4	0 1 9	0 3 3	0 3 7	0 0 8	0 4 4	0 2 1	0 1 2	0 3 9	0 3 2	0 3 0	0 5 0	0 0 9	0 2 8	0 4 5	0 4 9	0 3 5	0 2 5	0 2 2	0 2 9	0 1 3	0 2 4
General Body System																									
Peritoneum				+																					
Mesothelioma malignant, metastatic, epididymis				X																					
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis				X																					
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis				X																					
Bilateral, interstitial cell, adenoma				X	X	X		X	X	X	X	X	X	X	X	X		X	X	X		X	X		X
Interstitial cell, adenoma		X	X																						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+	+		+		+	+	+	+		+	+	+		+	+		+			+	
Lymph node, mandibular	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	+	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma, metastatic, bone						X																			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma, metastatic, bone						X																			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																		X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Keratoacanthoma							X									X					X	X			
Osteosarcoma																									
Squamous cell papilloma																								X	
Subcutaneous tissue, fibroma																X					X				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma						X																			
Skeletal muscle	+	+																							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	715	725	726	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
Carcass ID Number	047	020	003	001	002	007	010	015	016	018	027	031	034	036	038	040	041	042	043	048	004	005	006	011	046	Total Tissues/ Tumors
General Body System																										
Peritoneum																										1
Mesothelioma malignant, metastatic, epididymis																										1
Genital System																										
Coagulating gland																		+								1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																							X			1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Interstitial cell, adenoma																										2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+		+			+																		+	+	19
Lymph node, mandibular	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	5
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma, metastatic, bone																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Chordoma, metastatic, bone																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma					X																					2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma		X																								1
Keratoacanthoma	X	X			X								X	X				X								10
Osteosarcoma								X																		1
Squamous cell papilloma																										1
Subcutaneous tissue, fibroma							X												X					X		5
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma																										1
Skeletal muscle																										2

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	425	495	506	549	553	582	584	632	634	638	645	662	666	676	676	680	680	681	690	693	694	704	704	709	709
Carcass ID Number	026	023	017	014	019	033	037	008	044	021	012	039	032	030	050	009	028	045	049	035	025	022	029	013	024
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																								+	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X						X						
Chordoma, metastatic, bone						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X		X		X	X	X	X	X	X		X	X	X	X	X	X		X		X	X	
Mesothelioma malignant				X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	715	725	726	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
Carcass ID Number	047	020	003	001	002	007	010	015	016	018	027	031	034	036	038	040	041	042	043	048	004	005	006	011	046	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X													X	4
Chordoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X					X					X	X				X				X		X		27
Mesothelioma malignant																								X		2

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	344	373	484	499	526	535	543	590	609	610	624	641	653	657	673	698	698	702	704	709	710	715	715	729	729
Carcass ID Number	096	054	085	061	069	066	070	079	072	053	091	088	100	095	086	064	067	099	075	097	093	052	059	051	057
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery					+			+			+			+											
Mesothelioma malignant, metastatic, epididymis								X																	
Oral mucosa					+												+								
Gingival, squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis								X																	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																									
Tongue																									
Squamous cell papilloma																									
Tooth	+				+						+			+	+										
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign															X		X								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X										X	X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							X		
C-cell, carcinoma																									
Follicular cell, adenoma																				X		X			
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	
Carcass ID Number	060	065	068	073	076	077	078	080	081	082	083	087	089	090	094	098	055	056	058	062	063	071	074	084	092	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery				+							+			+			+		+	+	+				+	12
Mesothelioma malignant, metastatic, epididymis				X																						2
Oral mucosa		+				+	+			+		+						+								8
Gingival, squamous cell papilloma												X														1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis				X																						1
Tongue															+											1
Squamous cell papilloma															X											1
Tooth																										5
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma				X																						1
Carcinoma														X												1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign												X				X	X									5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma													X				X			X						6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X										X			X	X			X		6
C-cell, carcinoma				X					X																	2
Follicular cell, adenoma																										2
General Body System																										
None																										

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	344	373	484	499	526	535	543	590	609	610	624	641	653	657	673	698	698	702	704	709	710	715	715	729	729
Carcass ID Number	096	054	085	061	069	066	070	079	072	053	091	088	100	095	086	064	067	099	075	097	093	052	059	051	057
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																				X					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis								X																	
Bilateral, interstitial cell, adenoma							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma			X		X																				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+		+	+		+		+	+	+		+	+		+			+		
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis								X																	
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Fibroadenoma														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma									X																
Keratoacanthoma								X				X								X					
Osteosarcoma																					X				
Squamous cell papilloma																									
Subcutaneous tissue, fibroma																			X			X	X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+													+							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	
Carcass ID Number	060	065	068	073	076	077	078	080	081	082	083	087	089	090	094	098	055	056	058	062	063	071	074	084	092	Total Tissues/ Tumors
Genital System																										
Coagulating gland																								+		1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X				X					3
Carcinoma												X							X							3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis				X																						1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis				X																						2
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	41
Interstitial cell, adenoma			X						X	X																5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node			+		+												+		+							20
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Fibroadenoma														X												2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma					X													X								3
Keratoacanthoma																										3
Osteosarcoma													X													2
Squamous cell papilloma			X	X																						2
Subcutaneous tissue, fibroma																										3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	344	373	484	499	526	535	543	590	609	610	624	641	653	657	673	698	698	702	704	709	710	715	715	729	729
Carcass ID Number	096	054	085	061	069	066	070	079	072	053	091	088	100	095	086	064	067	099	075	097	093	052	059	051	057
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Carcinoma, metastatic, thyroid gland																									
Carcinoma, metastatic, adrenal cortex																									
Osteosarcoma, metastatic, skin																					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									X
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X		X		X
Mesothelioma malignant								X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	
Carcass ID Number	060	065	068	073	076	077	078	080	081	082	083	087	089	090	094	098	055	056	058	062	063	071	074	084	092	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, thyroid gland				X																						1
Carcinoma, metastatic, adrenal cortex														X												1
Osteosarcoma, metastatic, skin													X													2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Renal tubule, carcinoma															X											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X			41
Mesothelioma malignant				X							X															3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	486	494	499	543	543	590	613	624	669	687	690	691	705	708	708	708	708	711	726	729	729	729	729	729	729
Carcass ID Number	141	122	126	108	140	116	135	129	101	104	150	103	111	119	124	134	138	146	118	105	109	110	113	114	115
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma																									
Mesentery				+									+				+	+							
Oral mucosa										+								+							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Tooth						+						+													
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign																X	X								
Bilateral, pheochromocytoma benign																						X			
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X					X			X	X	X	X							X				X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma												X													
C-cell, adenoma														X		X					X	X			
C-cell, carcinoma																									
Follicular cell, adenoma							X													X					
General Body System																									
Peritoneum													+												
Mesothelioma malignant, metastatic, epididymis													X												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	117	120	121	125	127	131	132	136	137	147	148	149	102	106	107	112	123	128	130	133	139	142	143	144	145	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																				X						1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma	X																									1
Hepatocellular adenoma																						X				1
Mesentery										+		+							+			+				8
Oral mucosa				+		+										+										5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																										2
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant	X								X																	2
Pheochromocytoma benign				X								X		X							X					6
Bilateral, pheochromocytoma benign						X																				2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																X										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma				X			X										X								X	12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma			X									X														3
C-cell, adenoma										X						X										6
C-cell, carcinoma												X								X						2
Follicular cell, adenoma														X												3
General Body System																										
Peritoneum																										1
Mesothelioma malignant, metastatic, epididymis																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	4 8 6	4 9 4	4 9 9	5 4 3	5 4 3	5 9 0	6 1 3	6 2 4	6 6 9	6 8 7	6 9 0	6 9 1	7 0 5	7 0 8	7 0 8	7 0 8	7 0 8	7 1 1	7 2 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 4 1	1 2 2	1 2 6	1 0 8	1 4 0	1 1 6	1 3 5	1 2 9	1 0 1	1 0 4	1 5 0	1 0 3	1 1 1	1 1 9	1 2 4	1 3 4	1 3 8	1 4 6	1 1 8	1 0 5	1 0 9	1 1 0	1 1 3	1 1 4	1 1 5
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Carcinoma				X			X																		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis								X					X												
Bilateral, interstitial cell, adenoma				X	X		X		X	X	X	X			X	X	X		X	X	X	X	X	X	
Interstitial cell, adenoma	X					X								X				X							X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+	+				+			+	+	+		+							
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	+	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal medulla																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																						X			
Keratoacanthoma									X																
Trichoepithelioma																									
Subcutaneous tissue, fibroma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Skeletal muscle						+																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma										X															
Pheochromocytoma malignant, metastatic, adrenal medulla																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	117	120	121	125	127	131	132	136	137	147	148	149	102	106	107	112	123	128	130	133	139	142	143	144	145	Total Tissues/ Tumors
Genital System																										
Coagulating gland												+														1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																	X									3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										2
Bilateral, interstitial cell, adenoma	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	40
Interstitial cell, adenoma						X																				6
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node				+	+		+					+	+			+		+							+	16
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal medulla									X																	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Keratoacanthoma			X										X													3
Trichoepithelioma							X																			1
Subcutaneous tissue, fibroma																							X	X		3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma		X																								1
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Pheochromocytoma malignant, metastatic, adrenal medulla	X								X																	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

	4	4	4	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7
Number of Days on Study	8	9	9	4	4	9	1	2	6	8	9	9	0	0	0	0	0	1	2	2	2	2	2	2	2
	6	4	9	3	3	0	3	4	9	7	0	1	5	8	8	8	8	1	6	9	9	9	9	9	9
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Carcass ID Number	4	2	2	0	4	1	3	2	0	0	5	0	1	1	2	3	3	4	1	0	0	1	1	1	1
	1	2	6	8	0	6	5	9	1	4	0	3	1	9	4	4	8	6	8	5	9	0	3	4	5
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland								+											+						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma										X															
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X	X			X	X	X	X	X	X	X	X	X		X	X	X	X	X	
Mesothelioma malignant								X					X												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	
	9	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Total
Carcass ID Number	1	2	2	2	2	3	3	3	3	4	4	4	0	0	0	1	2	2	3	3	3	4	4	4	4	Tissues/
	7	0	1	5	7	1	2	6	7	7	8	9	2	6	7	2	3	8	0	3	9	2	3	4	5	Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lacrimal gland																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																				X						2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X			X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X		X	39
Mesothelioma malignant																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	487	488	507	529	529	529	537	537	540	549	555	569	569	582	593	593	601	604	604	613	617	621	621	624	624
Carcass ID Number	197	155	173	160	176	188	166	168	200	152	181	162	198	189	158	182	190	161	175	191	192	153	156	174	180
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery																									
Oral mucosa	+			+	+											+									
Pharyngeal, squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																+									
Squamous cell papilloma																X									
Tooth																				+					
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant			X																						
Pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma													X					X						X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																						X			X
C-cell, carcinoma																									
Follicular cell, adenoma	X						X							X											
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	632	634	634	634	635	637	639	641	641	653	662	669	675	680	681	691	694	708	708	708	708	708	726	729	729	
Carcass ID Number	171	163	172	194	159	164	177	167	198	178	196	195	186	187	170	151	154	157	165	184	185	193	183	169	179	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery				+											+	+			+							4
Oral mucosa											+				+		+									7
Pharyngeal, squamous cell papilloma											X															1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Tooth																+								+		3
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign											X											X				3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X														1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X																		X					5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma												X						X								4
C-cell, carcinoma																				X						1
Follicular cell, adenoma																										3
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	487	488	507	529	529	529	537	537	540	549	555	569	569	582	593	593	601	604	604	613	617	621	621	624	624
Carcass ID Number	197	155	173	160	176	188	166	168	200	152	181	162	198	189	158	182	190	161	175	191	192	153	156	174	180
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Adenoma																									
Carcinoma												X													
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X	X	X	X	X		X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma						X						X													
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+				+		+	+		+	+					+	+	+					
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma				X																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma								X																	
Schwannoma malignant														X											
Subcutaneous tissue, fibroma											X												X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Pheochromocytoma malignant, metastatic, adrenal medulla			X																						
Mediastinum, myxosarcoma					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	632	634	634	634	635	637	639	641	641	653	662	669	675	680	681	691	694	708	708	708	708	708	726	729	729	
Carcass ID Number	171	163	172	194	159	164	177	167	198	178	196	195	186	187	170	151	154	157	165	184	185	193	183	169	179	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																							X			1
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X		X	42
Interstitial cell, adenoma			X										X							X				X		6
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+		+	+		+				+								+			+	+			19
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma									X									X								3
Schwannoma malignant																										1
Subcutaneous tissue, fibroma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma						X																X				2
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Mediastinum, myxosarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	487	488	507	529	529	529	537	537	540	549	555	569	569	582	593	593	601	604	604	613	617	621	621	624	624
Carcass ID Number	197	155	173	160	176	188	166	168	200	152	181	162	198	189	158	182	190	161	175	191	192	153	156	174	180
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pelvis, transitional epithelium, carcinoma					X																				
Renal tubule, adenoma							X																		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X	X					X		X	X			X		X	X		X					X

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	632	634	634	634	635	637	639	641	641	653	662	669	675	680	681	691	694	708	708	708	708	708	726	729	729	
Carcass ID Number	171	163	172	194	159	164	177	167	199	178	196	195	186	187	170	151	154	157	165	184	185	193	183	169	179	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pelvis, transitional epithelium, carcinoma																										1
Renal tubule, adenoma	X																X	X								4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X	X	X	X						X	X						X	X	X	X	X	X	24

Table A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	5/49 (10%)	8/50 (16%)	3/50 (6%)
Adjusted rate[b]	18.9%	12.1%	18.0%	9.2%
Terminal rate[c]	4/22 (18%)	3/27 (11%)	6/31 (19%)	0/2 (0%)
First incidence (days)	690	673	708	624
Poly-3 test[d]	P=0.235N	P=0.290N	P=0.568N	P=0.199N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	5/49 (10%)	10/50 (20%)	4/50 (8%)
Adjusted rate	18.9%	12.1%	22.5%	12.0%
Terminal rate	4/22 (18%)	3/27 (11%)	8/31 (26%)	0/2 (0%)
First incidence (days)	690	673	708	507
Poly-3 test	P=0.405N	P=0.290N	P=0.439	P=0.310N
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.4%	2.4%	4.5%	12.1%
Terminal rate	0/22 (0%)	1/27 (4%)	1/31 (3%)	0/2 (0%)
First incidence (days)	709	729 (T)	687	537
Poly-3 test	P=0.046	P=0.758	P=0.519	P=0.114
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	1/50 (2%)	1/50 (2%)	5/50 (10%)	4/50 (8%)
Adjusted rate	2.4%	2.4%	11.2%	12.1%
Terminal rate	0/22 (0%)	1/27 (4%)	4/31 (13%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	624
Poly-3 test	P=0.034	P=0.757	P=0.114	P=0.113
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	2/50 (4%)	2/50 (4%)	7/50 (14%)	8/50 (16%)
Adjusted rate	4.7%	4.8%	15.6%	23.3%
Terminal rate	0/22 (0%)	2/27 (7%)	5/31 (16%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	537
Poly-3 test	P=0.004	P=0.688	P=0.093	P=0.017
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.4%	4.8%	4.5%	12.1%
Terminal rate	0/22 (0%)	2/27 (7%)	1/31 (3%)	0/2 (0%)
First incidence (days)	709	729 (T)	687	537
Poly-3 test	P=0.073	P=0.495	P=0.519	P=0.114
Kidney (Renal Tubule): Adenoma or Carcinoma (Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	4/50 (8%)
Adjusted rate	2.4%	4.8%	11.2%	12.1%
Terminal rate	0/22 (0%)	2/27 (7%)	4/31 (13%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	624
Poly-3 test	P=0.052	P=0.494	P=0.114	P=0.113
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	2/50 (4%)	3/50 (6%)	7/50 (14%)	8/50 (16%)
Adjusted rate	4.7%	7.2%	15.6%	23.3%
Terminal rate	0/22 (0%)	3/27 (11%)	5/31 (16%)	0/2 (0%)
First incidence (days)	680	729 (T)	590	537
Poly-3 test	P=0.006	P=0.491	P=0.093	P=0.017

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	1/49 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	9.4%	2.4%	0.0%	0.0%
Terminal rate	2/22 (9%)	0/27 (0%)	0/31 (0%)	0/2 (0%)
First incidence (days)	666	704	—[e]	—
Poly-3 test	P=0.017N	P=0.188N	P=0.055N	P=0.105N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	1/49 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	9.4%	2.4%	2.3%	6.2%
Terminal rate	2/22 (9%)	0/27 (0%)	0/31 (0%)	0/2 (0%)
First incidence (days)	666	704	687	637
Poly-3 test	P=0.332N	P=0.188N	P=0.164N	P=0.467N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	10/50 (20%)	6/50 (12%)	12/50 (24%)	5/50 (10%)
Adjusted rate	23.0%	14.2%	26.2%	14.8%
Terminal rate	1/22 (5%)	3/27 (11%)	6/31 (19%)	0/2 (0%)
First incidence (days)	584	535	499	569
Poly-3 test	P=0.384N	P=0.219N	P=0.456	P=0.272N
Preputial Gland: Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/49 (2%)
Adjusted rate	0.0%	7.1%	2.3%	3.2%
Terminal rate	0/22 (0%)	2/27 (7%)	1/31 (3%)	0/2 (0%)
First incidence (days)	—	543	729 (T)	726
Poly-3 test	P=0.461	P=0.118	P=0.510	P=0.442
Preputial Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	1/49 (2%)
Adjusted rate	2.4%	7.2%	6.6%	3.1%
Terminal rate	1/22 (5%)	2/27 (7%)	1/31 (3%)	0/2 (0%)
First incidence (days)	729 (T)	709	543	569
Poly-3 test	P=0.529	P=0.301	P=0.332	P=0.696
Preputial Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	6/50 (12%)	4/50 (8%)	2/49 (4%)
Adjusted rate	2.4%	14.2%	8.8%	6.3%
Terminal rate	1/22 (5%)	4/27 (15%)	2/31 (7%)	0/2 (0%)
First incidence (days)	729 (T)	543	543	569
Poly-3 test	P=0.440	P=0.055	P=0.201	P=0.405
Skin: Keratoacanthoma				
Overall rate	10/50 (20%)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate	23.2%	7.1%	6.7%	9.1%
Terminal rate	4/22 (18%)	0/27 (0%)	2/31 (7%)	0/2 (0%)
First incidence (days)	584	590	669	537
Poly-3 test	P=0.047N	P=0.035N	P=0.028N	P=0.093N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	11/50 (22%)	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate	25.5%	11.8%	6.7%	9.1%
Terminal rate	4/22 (18%)	2/27 (7%)	2/31 (7%)	0/2 (0%)
First incidence (days)	584	590	669	537
Poly-3 test	P=0.021N	P=0.086N	P=0.015N	P=0.061N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Skin: Basal Cell Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	2.3%	0.0%
Terminal rate	0/22 (0%)	2/27 (7%)	1/31 (3%)	0/2 (0%)
First incidence (days)	—	609	729 (T)	—[f]
Poly-3 test	P=0.454N	P=0.118	P=0.510	—
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	4.5%	0.0%
Terminal rate	0/22 (0%)	2/27 (7%)	2/31 (7%)	0/2 (0%)
First incidence (days)	—	609	729 (T)	—
Poly-3 test	P=0.534N	P=0.118	P=0.249	—
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma				
Overall rate	11/50 (22%)	8/50 (16%)	5/50 (10%)	3/50 (6%)
Adjusted rate	25.5%	18.7%	11.2%	9.1%
Terminal rate	4/22 (18%)	4/27 (15%)	4/31 (13%)	0/2 (0%)
First incidence (days)	584	590	669	537
Poly-3 test	P=0.027N	P=0.307N	P=0.070N	P=0.061N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	11.8%	7.2%	6.8%	6.1%
Terminal rate	3/22 (14%)	0/27 (0%)	2/31 (7%)	0/2 (0%)
First incidence (days)	680	704	687	555
Poly-3 test	P=0.245N	P=0.364N	P=0.330N	P=0.329N
Skin (Subcutaneous Tissue): Fibroma or Fibrous Histiocytoma				
Overall rate	6/50 (12%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	14.2%	7.2%	6.8%	6.1%
Terminal rate	3/22 (14%)	0/27 (0%)	2/31 (7%)	0/2 (0%)
First incidence (days)	680	704	687	555
Poly-3 test	P=0.158N	P=0.248N	P=0.218N	P=0.228N
Testes: Adenoma				
Overall rate	45/50 (90%)	46/50 (92%)	46/50 (92%)	48/50 (96%)
Adjusted rate	93.2%	98.0%	95.5%	98.1%
Terminal rate	22/22 (100%)	27/27 (100%)	31/31 (100%)	2/2 (100%)
First incidence (days)	495	484	486	487
Poly-3 test	P=0.172	P=0.219	P=0.473	P=0.203
Thyroid Gland (C-cell): Adenoma				
Overall rate	10/50 (20%)	6/50 (12%)	9/50 (18%)	4/50 (8%)
Adjusted rate	23.3%	14.4%	20.2%	12.1%
Terminal rate	5/22 (23%)	5/27 (19%)	6/31 (19%)	0/2 (0%)
First incidence (days)	638	715	691	621
Poly-3 test	P=0.197N	P=0.221N	P=0.462N	P=0.171N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	8/50 (16%)	10/50 (20%)	5/50 (10%)
Adjusted rate	25.6%	19.2%	22.4%	15.1%
Terminal rate	6/22 (27%)	7/27 (26%)	7/31 (23%)	0/2 (0%)
First incidence (days)	638	715	691	621
Poly-3 test	P=0.205N	P=0.327N	P=0.459N	P=0.201N

Table A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	4.7%	4.8%	6.7%	8.9%
Terminal rate	1/22 (5%)	0/27 (0%)	2/31 (7%)	0/2 (0%)
First incidence (days)	634	709	613	487
Poly-3 test	P=0.272	P=0.688	P=0.524	P=0.398
All Organs: Mononuclear Cell Leukemia				
Overall rate	27/50 (54%)	41/50 (82%)	39/50 (78%)	24/50 (48%)
Adjusted rate	55.8%	82.3%	81.2%	59.3%
Terminal rate	6/22 (27%)	20/27 (74%)	25/31 (81%)	2/2 (100%)
First incidence (days)	425	344	494	487
Poly-3 test	P=0.508	P=0.003	P=0.005	P=0.454
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.7%	7.1%	4.5%	0.0%
Terminal rate	1/22 (5%)	2/27 (7%)	0/31 (0%)	0/2 (0%)
First incidence (days)	549	590	624	—
Poly-3 test	P=0.203N	P=0.493	P=0.677N	P=0.306N
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	47/50 (94%)	49/50 (98%)	50/50 (100%)
Adjusted rate	99.6%	98.8%	99.4%	100.0%
Terminal rate	22/22 (100%)	27/27 (100%)	31/31 (100%)	2/2 (100%)
First incidence (days)	495	484	486	487
Poly-3 test	P=0.655	P=0.932N	P=0.991N	P=1.000
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	42/50 (84%)	40/50 (80%)	26/50 (52%)
Adjusted rate	66.7%	84.3%	82.7%	62.5%
Terminal rate	9/22 (41%)	21/27 (78%)	25/31 (81%)	2/2 (100%)
First incidence (days)	425	344	494	487
Poly-3 test	P=0.275N	P=0.033	P=0.053	P=0.417N
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	22/22 (100%)	27/27 (100%)	31/31 (100%)	2/2 (100%)
First incidence (days)	425	344	486	487
Poly-3 test	—	—	—	—

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, lung, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4a
Historical Incidence of Renal Tubule Adenoma in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	1/50
Citral	0/100
p,p′-Dichlorodiphenyl sulfone	0/50
trans-Cinnamaldehyde	0/100
2-Methylimidazole	0/49
o-Nitrotoluene	0/60
p-Nitrotoluene	0/50
Step sections	
Stoddard solvent IIC	
Single	0/50
Step section	3/50
Total	3/50
Propylene glycol mono-*t*-butyl ether	
Single	1/50
Step section	0/50
Total	1/50
Overall Total	4/100
Overall Historical Incidence: Feed Studies	
Total (%)	1/459 (0.2%)
Mean ± standard deviation	0.3% ± 0.8%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	5/1,152 (0.4%)
Mean ± standard deviation	0.5% ± 0.9%
Range	0%-2%

[a] Data as of April 19, 2004

TABLE A4b
Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	27/50
Citral	68/100
p,p'-Dichlorodiphenyl sulfone	27/50
trans-Cinnamaldehyde	40/100
2-Methylimidazole	15/50
o-Nitrotoluene	30/60
p-Nitrotoluene	24/50
Overall Historical Incidence: Feed Studies	
Total (%)	231/460 (50.2%)
Mean ± standard deviation	49.1% ± 11.9%
Range	30%-68%
Overall Historical Incidence: All Routes	
Total (%)	514/1,159 (44.4%)
Mean ± standard deviation	43.1% ± 12.8%
Range	22%-68%

[a] Data as of April 19, 2004

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	25	12	16	44
Natural deaths	3	11	3	4
Survivors				
Terminal sacrifice	22	27	31	2
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Erosion			1 (2%)	
Inflammation			1 (2%)	
Parasite metazoan	1 (2%)	1 (2%)	1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	6 (12%)	6 (12%)	8 (16%)	5 (10%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Erosion	1 (2%)		1 (2%)	1 (2%)
Inflammation				4 (8%)
Mineralization				2 (4%)
Ulcer				1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Basophilic focus	24 (48%)	25 (50%)	32 (64%)	19 (38%)
Clear cell focus	19 (38%)	19 (38%)	18 (36%)	
Congestion	1 (2%)			
Degeneration, cystic	8 (16%)	11 (22%)	20 (40%)	15 (30%)
Eosinophilic focus	5 (10%)	14 (28%)	10 (20%)	9 (18%)
Fatty change, diffuse	22 (44%)	20 (40%)	22 (44%)	15 (30%)
Hematopoietic cell proliferation	4 (8%)	1 (2%)	2 (4%)	4 (8%)
Hepatodiaphragmatic nodule	2 (4%)	6 (12%)	1 (2%)	1 (2%)
Infarct				1 (2%)
Inflammation, chronic active	22 (44%)	21 (42%)	35 (70%)	33 (66%)
Inflammation, granulomatous	1 (2%)			2 (4%)
Mineralization				1 (2%)
Mixed cell focus	9 (18%)	17 (34%)	22 (44%)	4 (8%)
Necrosis	19 (38%)	19 (38%)	9 (18%)	14 (28%)
Bile duct, cyst		1 (2%)		
Bile duct, hyperplasia	49 (98%)	46 (92%)	48 (96%)	50 (100%)
Hepatocyte, multinucleated	1 (2%)		1 (2%)	
Hepatocyte, centrilobular, hypertrophy		17 (34%)	31 (62%)	19 (38%)
Hepatocyte, centrilobular, necrosis				1 (2%)
Vein, inflammation				1 (2%)
Mesentery	(9)	(12)	(8)	(4)
Inflammation	1 (11%)		1 (13%)	1 (25%)
Fat, necrosis	7 (78%)	7 (58%)	7 (88%)	3 (75%)
Oral mucosa	(8)	(8)	(5)	(7)
Gingival, hyperplasia			1 (20%)	
Gingival, inflammation	8 (100%)	6 (75%)	5 (100%)	5 (71%)
Pharyngeal, inflammation				1 (14%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus		1 (2%)		
Cyst				3 (6%)
Inflammation	14 (28%)	16 (32%)	6 (12%)	9 (18%)
Metaplasia, hepatocyte				1 (2%)
Acinus, atrophy	20 (40%)	21 (42%)	22 (44%)	10 (20%)
Acinus, hyperplasia	1 (2%)			
Salivary glands	(49)	(50)	(50)	(49)
Atrophy	1 (2%)	1 (2%)	3 (6%)	
Inflammation		2 (4%)		
Stomach, forestomach	(50)	(49)	(49)	(50)
Autolysis		1 (2%)		
Hyperplasia, squamous	7 (14%)	4 (8%)	10 (20%)	17 (34%)
Inflammation	8 (16%)	6 (12%)	8 (16%)	12 (24%)
Mineralization		1 (2%)	1 (2%)	3 (6%)
Ulcer	4 (8%)	1 (2%)	8 (16%)	8 (16%)
Stomach, glandular	(50)	(50)	(49)	(50)
Erosion	5 (10%)	2 (4%)	3 (6%)	3 (6%)
Hyperplasia			1 (2%)	
Inflammation	5 (10%)	5 (10%)	4 (8%)	7 (14%)
Mineralization			5 (10%)	15 (30%)
Ulcer	1 (2%)		1 (2%)	4 (8%)
Tooth	(3)	(5)	(2)	(3)
Inflammation	1 (33%)		1 (50%)	1 (33%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Mineralization		1 (2%)	1 (2%)	11 (22%)
Pulmonary artery, pulmonary vein, hypertrophy			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	48 (96%)	48 (96%)	47 (94%)	49 (98%)
Mineralization			2 (4%)	6 (12%)
Aorta, tunic, media, mineralization		1 (2%)		
Atrium, thrombosis	3 (6%)	5 (10%)	2 (4%)	1 (2%)
Endocardium, hyperplasia		2 (4%)		
Valve, thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Angiectasis	4 (8%)	4 (8%)	2 (4%)	1 (2%)
Atrophy		1 (2%)		
Cytoplasmic alteration	1 (2%)			
Degeneration, cystic	1 (2%)			
Hematopoietic cell proliferation	5 (10%)		2 (4%)	
Hyperplasia	1 (2%)	4 (8%)	11 (22%)	4 (8%)
Hypertrophy	8 (16%)	2 (4%)	3 (6%)	3 (6%)
Necrosis	1 (2%)	4 (8%)		1 (2%)
Vacuolization cytoplasmic	27 (54%)	27 (55%)	31 (62%)	27 (54%)
Adrenal medulla	(50)	(49)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia	6 (12%)	8 (16%)	11 (22%)	5 (10%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System (continued)				
Parathyroid gland	(49)	(45)	(48)	(49)
Hyperplasia	2 (4%)	1 (2%)	19 (40%)	32 (65%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	10 (20%)	11 (22%)	3 (6%)
Cyst	6 (12%)	3 (6%)	1 (2%)	5 (10%)
Hyperplasia	16 (32%)	21 (42%)	12 (24%)	13 (26%)
Inflammation		1 (2%)		1 (2%)
Pigmentation, hemosiderin		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)			
Ultimobranchial cyst			2 (4%)	1 (2%)
C-cell, hyperplasia	17 (34%)	8 (16%)	8 (16%)	5 (10%)
Follicle, cyst		1 (2%)		
Follicle, degeneration	1 (2%)	2 (4%)		
Follicular cell, hyperplasia		1 (2%)		1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)		1 (2%)	2 (4%)
Inflammation	3 (6%)		4 (8%)	1 (2%)
Preputial gland	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Inflammation	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Duct, cyst			1 (2%)	1 (2%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	7 (14%)	8 (16%)	4 (8%)
Hyperplasia	2 (4%)	3 (6%)	4 (8%)	
Inflammation	18 (36%)	17 (34%)	26 (52%)	26 (52%)
Seminal vesicle	(50)	(50)	(50)	(49)
Atrophy	3 (6%)	7 (14%)	8 (16%)	4 (8%)
Inflammation	1 (2%)		1 (2%)	1 (2%)
Mineralization				4 (8%)
Testes	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		1 (2%)
Necrosis		2 (4%)		
Germinal epithelium, degeneration	6 (12%)	5 (10%)	6 (12%)	7 (14%)
Interstitial cell, hyperplasia	15 (30%)	12 (24%)	14 (28%)	22 (44%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hyperplasia, megakaryocyte		1 (2%)		
Inflammation, granulomatous			1 (2%)	2 (4%)
Myelofibrosis		1 (2%)	2 (4%)	2 (4%)
Necrosis		1 (2%)		
Erythroid cell, hyperplasia	13 (26%)	3 (6%)	10 (20%)	10 (20%)
Myeloid cell, hyperplasia	21 (42%)	27 (55%)	20 (40%)	14 (28%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Hematopoietic System (continued)				
Lymph node	(19)	(20)	(16)	(19)
Deep cervical, ectasia	2 (11%)			1 (5%)
Deep cervical, infiltration cellular, plasma cell	1 (5%)	2 (10%)		2 (11%)
Mediastinal, ectasia	3 (16%)		4 (25%)	3 (16%)
Mediastinal, hemorrhage	1 (5%)		1 (6%)	2 (11%)
Mediastinal, hyperplasia			1 (6%)	2 (11%)
Mediastinal, infiltration cellular, plasma cell		1 (5%)	2 (13%)	3 (16%)
Mediastinal, infiltration cellular, histiocyte	2 (11%)			
Mediastinal, inflammation			1 (6%)	
Pancreatic, ectasia			2 (13%)	2 (11%)
Pancreatic, hemorrhage			1 (6%)	1 (5%)
Pancreatic, infiltration cellular, histiocyte	1 (5%)	1 (5%)	2 (13%)	
Pancreatic, inflammation			3 (19%)	1 (5%)
Renal, ectasia				1 (5%)
Renal, infiltration cellular, histiocyte				1 (5%)
Lymph node, mandibular	(5)	(1)	(2)	
Ectasia	1 (20%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			6 (12%)
Ectasia	4 (8%)	2 (4%)	5 (10%)	4 (8%)
Infiltration cellular, histiocyte	47 (94%)	41 (82%)	48 (96%)	46 (92%)
Inflammation	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)		
Autolysis		1 (2%)		
Fibrosis	1 (2%)		3 (6%)	1 (2%)
Hematopoietic cell proliferation	45 (90%)	42 (84%)	45 (90%)	41 (82%)
Hemorrhage			1 (2%)	1 (2%)
Hyperplasia, lymphoid	6 (12%)	2 (4%)	9 (18%)	4 (8%)
Inflammation, granulomatous	1 (2%)	3 (6%)	7 (14%)	1 (2%)
Necrosis	1 (2%)	2 (4%)		1 (2%)
Pigmentation, hemosiderin	39 (78%)	43 (86%)	44 (88%)	43 (86%)
Lymphoid follicle, atrophy	1 (2%)		1 (2%)	2 (4%)
Thymus	(48)	(48)	(48)	(48)
Atrophy	44 (92%)	42 (88%)	47 (98%)	46 (96%)
Ectopic parathyroid gland		1 (2%)		4 (8%)
Integumentary System				
Mammary gland	(50)	(48)	(50)	(50)
Fibrosis	1 (2%)			
Galactocele	2 (4%)		1 (2%)	
Inflammation	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation	2 (4%)			
Subcutaneous tissue, inflammation		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy			1 (2%)	4 (8%)
Osteosclerosis			1 (2%)	
Skeletal muscle	(2)	(2)	(1)	
Degeneration	1 (50%)	1 (50%)	1 (100%)	
Inflammation	1 (50%)			

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	5 (10%)	5 (10%)	2 (4%)	5 (10%)
Necrosis	2 (4%)	4 (8%)		
Peripheral nerve	(1)			
Infiltration cellular, mononuclear cell	1 (100%)			
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Inflammation, acute	2 (4%)	2 (4%)	5 (10%)	7 (14%)
Inflammation, granulomatous	2 (4%)	4 (8%)	5 (10%)	2 (4%)
Metaplasia, squamous				1 (2%)
Mineralization			2 (4%)	10 (20%)
Necrosis	1 (2%)			
Thrombosis		1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia	16 (32%)	5 (10%)	13 (26%)	7 (14%)
Alveolus, infiltration cellular, histiocyte	46 (92%)	47 (96%)	49 (98%)	50 (100%)
Bronchiole, hyperplasia	1 (2%)	3 (6%)		
Interstitium, fibrosis		2 (4%)		
Nose	(50)	(50)	(50)	(50)
Inflammation	7 (14%)	7 (14%)	8 (16%)	9 (18%)
Thrombosis	1 (2%)	2 (4%)	1 (2%)	
Olfactory epithelium, degeneration	1 (2%)			
Olfactory epithelium, metaplasia	1 (2%)		1 (2%)	
Respiratory epithelium, hyperplasia	1 (2%)		1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	1 (2%)	8 (16%)	3 (6%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Anterior chamber, inflammation				1 (2%)
Ciliary body, inflammation			1 (2%)	
Cornea, inflammation				1 (2%)
Iris, fibrosis	1 (2%)		1 (2%)	
Iris, inflammation	1 (2%)			1 (2%)
Lens, cataract	2 (4%)		1 (2%)	
Lens, mineralization	1 (2%)			
Retina, atrophy	1 (2%)			
Retina, degeneration	5 (10%)	2 (4%)	5 (10%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	1 (2%)
Infiltration cellular, lymphoid	9 (18%)	4 (8%)	9 (18%)	3 (6%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	4 (8%)	1 (2%)	4 (8%)	2 (4%)
Hyperplasia, oncocytic			1 (2%)	
Infarct	1 (2%)	3 (6%)		
Nephropathy	50 (100%)	45 (90%)	50 (100%)	50 (100%)
Artery, inflammation				1 (2%)
Cortex, mineralization		1 (2%)	4 (8%)	14 (28%)
Medulla, mineralization	47 (94%)	49 (98%)	47 (94%)	49 (98%)
Papilla, transitional epithelium, hyperplasia				1 (2%)
Pelvis, inflammation			1 (2%)	1 (2%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	11 (22%)	29 (58%)	34 (68%)
Renal tubule, cyst			1 (2%)	9 (18%)
Renal tubule, hyperplasia	1 (2%)	5 (10%)	20 (40%)	23 (46%)
Renal tubule, hyperplasia, oncocytic			1 (2%)	
Renal tubule, pigmentation	1 (2%)	1 (2%)	2 (4%)	
Urinary bladder	(50)	(50)	(50)	(50)
Calculus gross observation		1 (2%)	1 (2%)	
Inflammation	3 (6%)		2 (4%)	2 (4%)
Muscularis, degeneration	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF BENZOPHENONE

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Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	10	9	13
Natural deaths	2	2	4	3
Survivors				
Terminal sacrifice	32	38	37	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Schwannoma malignant			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma		1 (2%)		
Hepatocellular adenoma			1 (2%)	
Histiocytic sarcoma			1 (2%)	2 (4%)
Mesentery	(12)	(18)	(10)	(6)
Histiocytic sarcoma				1 (17%)
Oral mucosa	(4)	(8)	(4)	(4)
Gingival, squamous cell carcinoma		1 (13%)		
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Schwannoma malignant				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Tooth			(3)	(3)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Endocardium, schwannoma malignant	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		2 (4%)	2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma			2 (4%)	1 (2%)

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System (continued)				
Parathyroid gland	(47)	(48)	(44)	(48)
Adenoma			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	17 (34%)	17 (34%)	11 (22%)	13 (26%)
Thyroid gland	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Bilateral, C-cell, carcinoma			1 (2%)	
C-cell, adenoma	4 (8%)	6 (12%)	3 (6%)	6 (12%)
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma				1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)		5 (10%)	6 (12%)
Carcinoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Histiocytic sarcoma			1 (2%)	
Squamous cell papilloma	1 (2%)			
Bilateral, adenoma	1 (2%)	1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign				1 (2%)
Histiocytic sarcoma			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	6 (12%)	9 (18%)	10 (20%)	10 (20%)
Cervix, histiocytic sarcoma				1 (2%)
Cervix, schwannoma malignant				1 (2%)
Vagina		(1)	(1)	
Schwannoma malignant		1 (100%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Lymph node	(8)	(8)	(11)	(12)
Deep cervical, carcinoma, metastatic, thyroid gland			1 (9%)	
Lymph node, mandibular		(1)	(1)	(1)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(49)	(50)	(50)	(50)
Thymus	(50)	(49)	(46)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Thymoma malignant		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	3 (6%)	
Carcinoma		1 (2%)	1 (2%)	
Fibroadenoma	21 (42%)	20 (40%)	12 (24%)	7 (14%)
Fibroadenoma, multiple	6 (12%)	4 (8%)	3 (6%)	
Histiocytic sarcoma			1 (2%)	1 (2%)

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Keratoacanthoma	3 (6%)		2 (4%)	
Lipoma		1 (2%)		
Squamous cell papilloma				1 (2%)
Subcutaneous tissue, fibroma	3 (6%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(3)	(1)
Histiocytic sarcoma			1 (33%)	1 (100%)
Rhabdomyosarcoma			1 (33%)	
Sarcoma			1 (33%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)		1 (2%)	
Carcinoma, metastatic, thyroid gland			1 (2%)	1 (2%)
Histiocytic sarcoma			1 (2%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Histiocytic sarcoma				1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Iris, melanoma benign	1 (2%)			
Zymbal's gland			(1)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Renal tubule, adenoma, multiple			2 (4%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	2 (4%)
Leukemia mononuclear	19 (38%)	25 (50%)	30 (60%)	29 (58%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	46	45	44
Total primary neoplasms	93	95	97	85
Total animals with benign neoplasms	42	40	37	33
Total benign neoplasms	71	62	59	48
Total animals with malignant neoplasms	20	29	32	32
Total malignant neoplasms	22	33	38	37
Total animals with metastatic neoplasms			1	1
Total metastatic neoplasms			4	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	288	358	430	528	543	613	624	634	637	673	697	708	708	724	724	724	724	725	729	729	729	729	729	729	730
Carcass ID Number	244	245	249	213	243	206	210	211	218	223	237	219	224	229	230	234	247	220	204	205	209	221	222	241	201
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery										+		+		+	+		+		+						
Oral mucosa		+		+											+									+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocardium, schwannoma malignant																		X							
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X						X					X		X	X	X			X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma														X											X
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X									X					
Carcinoma														X											
Squamous cell papilloma																							X		
Bilateral, adenoma																								X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																			X						

+: Tissue examined microscopically M: Missing tissue X: Lesion present
A: Autolysis precludes examination I: Insufficient tissue Blank: Not examined

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	202	203	207	208	212	215	217	225	231	232	235	236	238	239	240	214	216	226	227	228	233	242	246	248	250	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery				+						+	+		+								+	+				12
Oral mucosa																										4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocardium, schwannoma malignant																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X				X				X	X	X							X				X	17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma								X											X							4
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Carcinoma																										1
Squamous cell papilloma																										1
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal								X			X					X								X	X	6

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	288	358	430	528	543	613	624	634	637	673	697	708	708	724	724	724	724	725	729	729	729	729	729	729	730
Carcass ID Number	244	245	249	213	243	206	210	211	218	223	237	219	224	229	230	234	247	220	204	205	209	221	222	241	201
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+				+			+	+	+	+			+	+								
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X																								
Fibroadenoma				X				X	X	X			X						X	X		X		X	
Fibroadenoma, multiple						X						X						X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma								X							X										
Subcutaneous tissue, fibroma															X					X					
Subcutaneous tissue, fibrosarcoma																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iris, melanoma benign					X																				
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X	X	X	X		X				X	X		X	X		X	

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	202	203	207	208	212	215	217	225	231	232	235	236	238	239	240	214	216	226	227	228	233	242	246	248	250	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										8
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								2
Fibroadenoma		X	X	X	X		X	X		X			X		X						X			X	X	21
Fibroadenoma, multiple						X										X				X						6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																				X						3
Subcutaneous tissue, fibroma																					X					3
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X			X									X				X			4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Iris, melanoma benign																										1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X					X	X		X	X						X	X		X	X						19

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	609	613	613	633	653	690	693	697	697	710	725	725	729	729	729	729	730	730	730	730	730	730	730	730	730
Carcass ID Number	288	264	297	256	262	295	271	272	279	287	259	296	258	285	293	300	251	261	265	267	268	269	270	273	275
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Mesentery						+	+	+	+	+			+	+	+			+	+						+
Oral mucosa	+					+								+					+						
Gingival, squamous cell carcinoma						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign												X													
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X	X	X			X		X			X			X		X			X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X				X		X								
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X		X							X			X						X		X	
Vagina	+																								
Schwannoma malignant	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	278	282	286	291	292	294	299	252	253	254	255	257	260	263	266	274	276	277	280	281	283	284	289	290	298	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																X										1
Mesentery	+	+	+						+	+			+									+				18
Oral mucosa		+	+	+								+														8
Gingival, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign							X																			2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma										X		X			X		X					X		X	X	17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma									X												X			X		6
C-cell, carcinoma											X															1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																				X	X					2
Bilateral, adenoma																									X	1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal							X					X						X								9
Vagina																										1
Schwannoma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	609	613	613	633	653	690	693	697	697	710	725	725	729	729	729	729	730	730	730	730	730	730	730	730	730
Carcass ID Number	288	264	297	256	262	295	271	272	279	287	259	296	258	285	293	300	251	261	265	267	268	269	270	273	275
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+			+	+				+	+							+				+			+
Lymph node, mandibular	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Thymoma malignant																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Carcinoma													X												
Fibroadenoma								X	X	X		X	X			X		X		X	X	X	X		
Fibroadenoma, multiple							X				X						X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear							+																		
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X			X	X	X			X		X			X			X	X			X	X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	278	282	286	291	292	294	299	252	253	254	255	257	260	263	266	274	276	277	280	281	283	284	289	290	298	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										8
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma malignant	X																									1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X														2
Carcinoma																										1
Fibroadenoma	X			X	X							X		X		X			X	X					X	20
Fibroadenoma, multiple							X																			4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle								+																		1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X	X		X	X		X	X						X			X		X	X	X	X	X	25

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	528	609	633	644	662	679	690	699	701	704	709	709	728	729	729	729	729	729	729	729	730	730	730	730	730
Carcass ID Number	301	332	329	310	305	330	345	318	349	338	307	335	340	311	313	321	323	327	331	350	302	304	306	308	314
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant							X																		
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																			X						
Histiocytic sarcoma	X																								
Mesentery																						+	+		+
Oral mucosa		+				+						+	+												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Tooth																						+			
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign									X																
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X			X													
Parathyroid gland	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X					X							X	X				X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Bilateral, C-cell, carcinoma																									
C-cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Carcass ID Number	315	316	317	324	328	333	336	337	342	343	344	347	303	309	312	319	320	322	325	326	334	339	341	346	348	Total Tissues/ Tumors
Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Histiocytic sarcoma																										1
Mesentery				+							+					+		+		+	+		+			10
Oral mucosa																										4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Tooth										+											+					3
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign												X														2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	44
Adenoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma				X						X				X		X										11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Bilateral, C-cell, carcinoma												X														1
C-cell, adenoma							X									X							X			3
General Body System																										
None																										

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	528	609	633	644	662	679	690	699	701	704	709	709	728	729	729	729	729	729	729	729	730	730	730	730	730
Carcass ID Number	301	332	329	310	305	330	345	318	349	338	307	335	340	311	313	321	323	327	331	350	302	304	306	308	314
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Carcinoma												X													
Histiocytic sarcoma	X																								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X							X											X	X			
Vagina																			+						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymph node		+	+	+	+					+	+		+						+			+			
Deep cervical, carcinoma, metastatic, thyroid gland																									
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Histiocytic sarcoma	X																								
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X					X														
Carcinoma											X														
Fibroadenoma		X									X			X				X	X	X	X				
Fibroadenoma, multiple					X																				
Histiocytic sarcoma	X																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Keratoacanthoma									X																
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+								+					+											
Histiocytic sarcoma	X																								
Rhabdomyosarcoma														X											
Sarcoma									X																
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	315	316	317	324	328	333	336	337	342	343	344	347	303	309	312	319	320	322	325	326	334	339	341	346	348	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X				X									X				X						5
Carcinoma																				X						2
Histiocytic sarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal		X							X	X		X			X					X						10
Vagina																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node				+								+														11
Deep cervical, carcinoma, metastatic, thyroid gland												X														1
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	46
Carcinoma, metastatic, thyroid gland												X														1
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X									3
Carcinoma																										1
Fibroadenoma				X		X				X													X		X	12
Fibroadenoma, multiple	X																	X								3
Histiocytic sarcoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Keratoacanthoma								X																		2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Histiocytic sarcoma																										1
Rhabdomyosarcoma																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	528	609	633	644	662	679	690	699	701	704	709	709	728	729	729	729	729	729	729	729	730	730	730	730	730
Carcass ID Number	301	332	329	310	305	330	345	318	349	338	307	335	340	311	313	321	323	327	331	350	302	304	306	308	314
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, thyroid gland																									
Histiocytic sarcoma	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Special Senses System																									
Ear											+			+											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland			+																						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma, multiple									X			X													
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Leukemia mononuclear		X	X	X	X			X	X		X	X	X	X		X		X	X		X				X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	315	316	317	324	328	333	336	337	342	343	344	347	303	309	312	319	320	322	325	326	334	339	341	346	348	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X																									1
Carcinoma, metastatic, thyroid gland												X														1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma											X															1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland												X														1
Special Senses System																										
Ear																					+					3
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma, multiple																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear		X	X	X			X		X	X		X			X		X	X		X	X	X	X		X	30

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	480	528	581	624	638	653	659	679	679	681	686	687	701	709	714	725	729	729	729	729	729	729	729	729	729
Carcass ID Number	385	369	374	388	351	396	381	382	402	372	361	367	362	375	376	393	353	358	359	364	373	380	389	391	399
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																				
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X		X																						
Mesentery			+																						
Histiocytic sarcoma			X																						
Oral mucosa				+								+			+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth				+								+													
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X						X	X						X	X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma	X														X	X								X	
C-cell, carcinoma																									
Follicular cell, adenoma																							X		
General Body System																									
None																									

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	356	363	366	373	383	384	386	387	390	352	354	355	357	360	365	368	370	371	378	379	392	394	395	397	398	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Mesentery							+				+	+				+	+									6
Histiocytic sarcoma																										1
Oral mucosa	+																									4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant	X																									1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth								+																		3
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X																									1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X						X					X				X	X						X	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma		X												X												6
C-cell, carcinoma									X																	1
Follicular cell, adenoma																										1
General Body System																										
None																										

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	480	528	581	624	638	653	659	679	679	681	686	687	701	704	714	725	729	729	729	729	729	729	729	729	729
Carcass ID Number	385	369	374	388	351	396	381	382	400	372	361	367	372	375	376	393	353	358	359	364	373	380	389	391	399
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X		X								X											
Carcinoma																									X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																								X	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X					X							X			X			X	
Cervix, histiocytic sarcoma	X																								
Cervix, schwannoma malignant		X																							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+		+	+	+		+		+		+	+	+									
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma											X	X	X	X											X
Histiocytic sarcoma			X																						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Histiocytic sarcoma			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Histiocytic sarcoma	X		X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland																									
Carcinoma																									

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 5 6	3 6 3	3 6 6	3 7 7	3 8 3	3 8 4	3 8 6	3 8 7	3 9 0	3 5 2	3 5 4	3 5 5	3 5 7	3 6 0	3 6 5	3 6 8	3 7 0	3 7 1	3 7 8	3 7 9	3 9 2	3 9 4	3 9 5	3 9 7	3 9 8	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X			X														X					6
Carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal										X							X	X			X				X	10
Cervix, histiocytic sarcoma																										1
Cervix, schwannoma malignant																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+										+							+						12
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma								X			X															7
Histiocytic sarcoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma					X																					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Histiocytic sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland									X																	1
Histiocytic sarcoma																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland										+																1
Carcinoma										X																1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	480	528	581	624	638	653	659	679	679	681	686	687	701	709	714	725	729	729	729	729	729	729	729	729	729
Carcass ID Number	385	369	374	388	351	396	381	382	400	372	361	367	362	375	376	393	353	358	359	364	373	380	389	391	399
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Renal tubule, adenoma, multiple									X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X		X																						
Leukemia mononuclear	X	X		X	X	X		X		X		X			X			X	X		X	X	X	X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Benzophenone: 1,250 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
Carcass ID Number	5	6	6	7	8	8	8	8	9	5	5	5	5	6	6	6	7	7	7	7	9	9	9	9	9	Tissues/
	6	3	6	7	3	4	6	7	0	2	4	5	7	0	5	8	0	1	8	9	2	4	5	7	8	Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Renal tubule, adenoma, multiple																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Leukemia mononuclear		X	X		X			X	X	X	X		X			X	X			X	X	X			X	29

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Clitoral Gland: Adenoma				
Overall rate[a]	3/50 (6%)	1/50 (2%)	5/50 (10%)	6/50 (12%)
Adjusted rate[b]	6.8%	2.1%	10.6%	12.9%
Terminal rate[c]	2/32 (6%)	1/38 (3%)	5/37 (14%)	3/34 (9%)
First incidence (days)	697	729 (T)	729 (T)	624
Poly-3 test[d]	P=0.083	P=0.280N	P=0.393	P=0.267
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	6/50 (12%)	7/50 (14%)
Adjusted rate	9.0%	6.3%	12.7%	15.1%
Terminal rate	2/32 (6%)	3/38 (8%)	5/37 (14%)	4/34 (12%)
First incidence (days)	697	729 (T)	709	624
Poly-3 test	P=0.142	P=0.462N	P=0.412	P=0.290
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.8%	0.0%	4.2%	2.2%
Terminal rate	1/32 (3%)	0/38 (0%)	0/37 (0%)	0/34 (0%)
First incidence (days)	708	—[e]	701	679
Poly-3 test	P=0.343N	P=0.106N	P=0.468N	P=0.292N
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.8%	0.0%	4.2%	2.2%
Terminal rate	1/32 (3%)	0/38 (0%)	0/37 (0%)	0/34 (0%)
First incidence (days)	708	—	701	679
Poly-3 test	P=0.343N	P=0.106N	P=0.468N	P=0.292N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	9.1%	0.0%	2.1%	0.0%
Terminal rate	4/32 (13%)	0/38 (0%)	1/37 (3%)	0/34 (0%)
First incidence (days)	729 (T)	—	729 (T)	—
Poly-3 test	P=0.040N	P=0.051N	P=0.159N	P=0.056N
Mammary Gland: Fibroadenoma				
Overall rate	27/50 (54%)	24/50 (48%)	15/50 (30%)	7/50 (14%)
Adjusted rate	58.4%	50.0%	31.3%	15.1%
Terminal rate	19/32 (59%)	18/38 (47%)	12/37 (32%)	3/34 (9%)
First incidence (days)	528	693	609	686
Poly-3 test	P<0.001N	P=0.269N	P=0.006N	P<0.001N
Mammary Gland: Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.4%	4.2%	6.3%	0.0%
Terminal rate	1/32 (3%)	1/38 (3%)	1/37 (3%)	0/34 (0%)
First incidence (days)	288	633	679	—
Poly-3 test	P=0.199N	P=0.672N	P=0.524	P=0.234N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	28/50 (56%)	25/50 (50%)	17/50 (34%)	7/50 (14%)
Adjusted rate	59.4%	51.7%	35.3%	15.1%
Terminal rate	19/32 (59%)	18/38 (47%)	13/37 (35%)	3/34 (9%)
First incidence (days)	288	633	609	686
Poly-3 test	P<0.001N	P=0.291N	P=0.013N	P<0.001N

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Mammary Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.4%	6.3%	6.3%	0.0%
Terminal rate	1/32 (3%)	2/38 (5%)	1/37 (3%)	0/34 (0%)
First incidence (days)	288	633	679	—
Poly-3 test	P=0.162N	P=0.527	P=0.524	P=0.234N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	28/50 (56%)	25/50 (50%)	17/50 (34%)	7/50 (14%)
Adjusted rate	59.4%	51.7%	35.3%	15.1%
Terminal rate	19/32 (59%)	18/38 (47%)	13/37 (35%)	3/34 (9%)
First incidence (days)	288	633	609	686
Poly-3 test	P<0.001N	P=0.291N	P=0.013N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	17/50 (34%)	17/50 (34%)	11/50 (22%)	13/50 (26%)
Adjusted rate	37.2%	34.8%	23.0%	28.1%
Terminal rate	12/32 (38%)	11/38 (29%)	8/37 (22%)	10/34 (29%)
First incidence (days)	430	613	644	659
Poly-3 test	P=0.157N	P=0.488N	P=0.100N	P=0.239N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.7%	0.0%	4.2%	0.0%
Terminal rate	1/32 (3%)	0/38 (0%)	1/37 (3%)	0/34 (0%)
First incidence (days)	634	—	701	—
Poly-3 test	P=0.128N	P=0.107N	P=0.472N	P=0.114N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.7%	0.0%	4.2%	2.2%
Terminal rate	1/32 (3%)	0/38 (0%)	1/37 (3%)	1/34 (3%)
First incidence (days)	634	—	701	729 (T)
Poly-3 test	P=0.344N	P=0.107N	P=0.472N	P=0.295N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.8%	0.0%	0.0%	0.0%
Terminal rate	2/32 (6%)	0/38 (0%)	0/37 (0%)	0/34 (0%)
First incidence (days)	724	—	—	—
Poly-3 test	P=0.048N	P=0.106N	P=0.107N	P=0.112N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	9.1%	0.0%	0.0%	0.0%
Terminal rate	2/32 (6%)	0/38 (0%)	0/37 (0%)	0/34 (0%)
First incidence (days)	724	—	—	—
Poly-3 test	P=0.020N	P=0.052N	P=0.052N	P=0.056N
Thyroid Gland (C-cell): Adenoma				
Overall rate	4/50 (8%)	6/50 (12%)	3/50 (6%)	6/50 (12%)
Adjusted rate	9.1%	12.6%	6.4%	12.9%
Terminal rate	3/32 (9%)	5/38 (13%)	3/37 (8%)	3/34 (9%)
First incidence (days)	724	725	729 (T)	480
Poly-3 test	P=0.415	P=0.417	P=0.463N	P=0.403

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	4/50 (8%)	7/50 (14%)
Adjusted rate	9.1%	14.7%	8.5%	15.0%
Terminal rate	3/32 (9%)	6/38 (16%)	4/37 (11%)	4/34 (12%)
First incidence (days)	724	725	729 (T)	480
Poly-3 test	P=0.325	P=0.305	P=0.606N	P=0.292
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	9/50 (18%)	10/50 (20%)	10/50 (20%)
Adjusted rate	13.6%	18.8%	21.0%	21.6%
Terminal rate	6/32 (19%)	7/38 (18%)	8/37 (22%)	8/34 (24%)
First incidence (days)	729 (T)	633	633	653
Poly-3 test	P=0.218	P=0.350	P=0.257	P=0.234
All Organs: Mononuclear Cell Leukemia				
Overall rate	19/50 (38%)	25/50 (50%)	30/50 (60%)	29/50 (58%)
Adjusted rate	42.3%	51.5%	61.3%	59.6%
Terminal rate	13/32 (41%)	19/38 (50%)	21/37 (57%)	20/34 (59%)
First incidence (days)	637	613	609	480
Poly-3 test	P=0.058	P=0.247	P=0.048	P=0.068
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	40/50 (80%)	37/50 (74%)	33/50 (66%)
Adjusted rate	86.2%	81.3%	75.4%	68.5%
Terminal rate	27/32 (84%)	30/38 (79%)	28/37 (76%)	22/34 (65%)
First incidence (days)	288	613	609	480
Poly-3 test	P=0.016N	P=0.351N	P=0.130N	P=0.028N
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	29/50 (58%)	32/50 (64%)	32/50 (64%)
Adjusted rate	44.5%	59.2%	64.4%	65.1%
Terminal rate	13/32 (41%)	22/38 (58%)	21/37 (57%)	22/34 (65%)
First incidence (days)	637	609	528	480
Poly-3 test	P=0.041	P=0.109	P=0.039	P=0.033
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	46/50 (92%)	45/50 (90%)	44/50 (88%)
Adjusted rate	94.4%	92.0%	90.0%	88.0%
Terminal rate	30/32 (94%)	34/38 (90%)	32/37 (87%)	28/34 (82%)
First incidence (days)	288	609	528	480
Poly-3 test	P=0.167N	P=0.473N	P=0.330N	P=0.221N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, kidney, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

Table B4a
Historical Incidence of Renal Tubule Adenoma in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	0/50
Citral	1/100
p,p′-Dichlorodiphenyl sulfone	0/50
trans-Cinnamaldehyde	0/100
2-Methylimidazole	0/50
o-Nitrotoluene	0/60
p-Nitrotoluene	0/50
Overall Historical Incidence: Feed Studies	
Total (%)	1/460 (0.2%)
Mean ± standard deviation	0.1% ± 0.4%
Range	0%-1%
Overall Historical Incidence: All Routes	
Total (%)	1/1,205 (0.1%)
Mean ± standard deviation	0.1% ± 0.2%
Range	0%-1%

[a] Data as of April 19, 2004

TABLE B4b
Historical Incidence of Mononuclear Cell Leukemia and Histiocytic Sarcoma in Untreated Female F344/N Rats[a]

Study	Incidence in Controls	
	Mononuclear Cell Leukemia	**Histiocytic Sarcoma**
Historical Incidence in Feed Controls Given NTP-2000 Diet		
Benzophenone	19/50	0/50
Citral	24/100	0/100
p,p′-Dichlorodiphenyl sulfone	8/50	0/50
trans-Cinnamaldehyde	21/100	0/100
2-Methylimidazole	6/50	0/50
o-Nitrotoluene	21/60	0/60
p-Nitrotoluene	13/50	0/50
Overall Historical Incidence: Feed Studies		
Total	112/460 (24.4%)	0/460
Mean ± standard deviation	24.6% ± 9.5%	
Range	12%-38%	
Overall Historical Incidence: All Routes		
Total (%)	330/1,209 (27.3%)	1/1,209 (0.1%)
Mean ± standard deviation	28.0% ± 11.2%	0.1% ± 0.4%
Range	12%-52%	0%-2%

[a] Data as of April 19, 2004

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	10	9	13
Natural deaths	2	2	4	3
Survivors				
Terminal sacrifice	32	38	37	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Periesophageal tissue, necrosis, fatty		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Metaplasia, mucous	1 (2%)			
Parasite metazoan	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	8 (16%)	8 (16%)	14 (28%)	6 (12%)
Ulcer		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Mineralization			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	2 (4%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)	5 (10%)	1 (2%)
Basophilic focus	48 (96%)	48 (96%)	41 (82%)	43 (86%)
Clear cell focus	15 (30%)	13 (26%)	10 (20%)	10 (20%)
Degeneration, cystic	1 (2%)		3 (6%)	4 (8%)
Eosinophilic focus		6 (12%)	6 (12%)	9 (18%)
Fatty change, diffuse	11 (22%)	8 (16%)	9 (18%)	20 (40%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	1 (2%)	6 (12%)	4 (8%)	2 (4%)
Hepatodiaphragmatic nodule	5 (10%)	6 (12%)	6 (12%)	6 (12%)
Inflammation, chronic active	46 (92%)	38 (76%)	29 (58%)	30 (60%)
Mixed cell focus	10 (20%)	11 (22%)	5 (10%)	5 (10%)
Necrosis	12 (24%)	6 (12%)	11 (22%)	12 (24%)
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	10 (20%)	35 (70%)	39 (78%)	40 (80%)
Hepatocyte, centrilobular, hypertrophy		27 (54%)	30 (60%)	33 (66%)
Oval cell, hyperplasia				3 (6%)
Mesentery	(12)	(18)	(10)	(6)
Fat, necrosis	11 (92%)	18 (100%)	10 (100%)	4 (67%)
Oral mucosa	(4)	(8)	(4)	(4)
Hyperplasia, squamous				1 (25%)
Gingival, inflammation	4 (100%)	7 (88%)	4 (100%)	4 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus			1 (2%)	1 (2%)
Inflammation	12 (24%)	3 (6%)	2 (4%)	6 (12%)
Acinus, atrophy	17 (34%)	10 (20%)	11 (22%)	12 (24%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	2 (4%)		1 (2%)	1 (2%)
Basophilic focus	1 (2%)		1 (2%)	
Inflammation			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Inflammation	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Ulcer		1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion		1 (2%)	1 (2%)	1 (2%)
Inflammation			2 (4%)	3 (6%)
Mineralization		2 (4%)	2 (4%)	
Ulcer	1 (2%)			1 (2%)
Epithelium, atrophy			1 (2%)	
Tooth			(3)	(3)
Inflammation			3 (100%)	2 (67%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Mineralization				1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	46 (92%)	48 (96%)	46 (92%)
Atrium, thrombosis		1 (2%)		1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	42 (84%)	36 (72%)	18 (36%)	24 (48%)
Atrophy	1 (2%)			1 (2%)
Degeneration, cystic	3 (6%)	2 (4%)	2 (4%)	
Hematopoietic cell proliferation	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Hyperplasia	6 (12%)	10 (20%)	6 (12%)	6 (12%)
Hypertrophy	7 (14%)	5 (10%)	5 (10%)	8 (16%)
Karyomegaly	1 (2%)			
Necrosis	5 (10%)	7 (14%)	5 (10%)	2 (4%)
Vacuolization cytoplasmic	14 (28%)	16 (32%)	15 (30%)	20 (40%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	2 (4%)	
Necrosis	2 (4%)			
Vacuolization cytoplasmic	1 (2%)			
Parathyroid gland	(47)	(48)	(44)	(48)
Hyperplasia		1 (2%)		1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	30 (60%)	30 (60%)	23 (46%)	23 (46%)
Cyst	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Hyperplasia	23 (46%)	24 (48%)	20 (40%)	18 (36%)
Inflammation	1 (2%)			

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	34 (68%)	11 (22%)	13 (26%)	8 (16%)
Follicle, cyst	1 (2%)		1 (2%)	1 (2%)
Follicle, hyperplasia		2 (4%)		
Follicular cell, hyperplasia			1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst	3 (6%)	2 (4%)	1 (2%)	
Hyperplasia	6 (12%)	1 (2%)	2 (4%)	
Hyperplasia, squamous				1 (2%)
Inflammation	2 (4%)	2 (4%)	2 (4%)	
Metaplasia, squamous	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Cyst	6 (12%)	13 (26%)	10 (20%)	9 (18%)
Interstitial cell, hyperplasia		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Angiectasis, focal				1 (2%)
Inflammation				1 (2%)
Cervix, hypertrophy				1 (2%)
Endometrium, hyperplasia, cystic	12 (24%)	20 (40%)	20 (40%)	20 (40%)
Endometrium, hyperplasia, adenomatous	1 (2%)	1 (2%)		1 (2%)
Serosa, inflammation, granulomatous		2 (4%)		
Vagina		(1)	(1)	
Inflammation			1 (100%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Myelofibrosis	2 (4%)		2 (4%)	6 (12%)
Necrosis			1 (2%)	1 (2%)
Erythroid cell, hyperplasia	8 (16%)	6 (12%)	7 (14%)	2 (4%)
Myeloid cell, hyperplasia	11 (22%)	13 (26%)	20 (40%)	13 (26%)
Lymph node	(8)	(8)	(11)	(12)
Axillary, right, ectasia			1 (9%)	
Axillary, right, infiltration cellular, plasma cell			1 (9%)	
Deep cervical, ectasia	1 (13%)			
Deep cervical, hemorrhage	1 (13%)			
Deep cervical, infiltration cellular, plasma cell	3 (38%)			2 (17%)
Deep cervical, infiltration cellular, histiocyte	3 (38%)			
Mediastinal, hemorrhage				2 (17%)
Mediastinal, infiltration cellular, plasma cell	2 (25%)	1 (13%)	4 (36%)	2 (17%)
Mediastinal, infiltration cellular, histiocyte			1 (9%)	1 (8%)
Pancreatic, hemorrhage		2 (25%)		
Pancreatic, infiltration cellular, histiocyte		3 (38%)		1 (8%)
Pancreatic, pigmentation, hemosiderin		2 (25%)		
Renal, infiltration cellular, histiocyte		1 (13%)		1 (8%)
Renal, pigmentation				1 (8%)

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Infiltration cellular, plasma cell		1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, histiocyte	50 (100%)	49 (98%)	48 (96%)	49 (98%)
Spleen	(49)	(50)	(50)	(50)
Accessory spleen			1 (2%)	1 (2%)
Autolysis			2 (4%)	
Fibrosis	1 (2%)			1 (2%)
Hematopoietic cell proliferation	47 (96%)	45 (90%)	40 (80%)	45 (90%)
Hyperplasia, lymphoid		1 (2%)	1 (2%)	2 (4%)
Infarct	1 (2%)			
Inflammation, granulomatous	1 (2%)	1 (2%)		
Pigmentation, hemosiderin	48 (98%)	50 (100%)	49 (98%)	49 (98%)
Lymphoid follicle, atrophy		5 (10%)		
Red pulp, hyperplasia				1 (2%)
Thymus	(50)	(49)	(46)	(50)
Atrophy	49 (98%)	48 (98%)	44 (96%)	49 (98%)
Cyst				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Galactocele	7 (14%)	3 (6%)	2 (4%)	2 (4%)
Hyperplasia	3 (6%)	4 (8%)	1 (2%)	3 (6%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	1 (2%)
Sebaceous gland, hyperplasia				1 (2%)
Subcutaneous tissue, necrosis			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy				2 (4%)
Skeletal muscle	(1)	(1)	(3)	(1)
Inflammation	1 (100%)	1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis			1 (2%)	
Hemorrhage	2 (4%)	1 (2%)	2 (4%)	
Hydrocephalus	1 (2%)			
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Mineralization	1 (2%)			
Necrosis		1 (2%)	1 (2%)	1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation, acute		2 (4%)	1 (2%)	
Inflammation, granulomatous	12 (24%)	5 (10%)	9 (18%)	7 (14%)
Metaplasia, squamous			1 (2%)	
Alveolar epithelium, hyperplasia	18 (36%)	18 (36%)	13 (26%)	15 (30%)
Alveolus, infiltration cellular, histiocyte	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Interstitium, fibrosis			1 (2%)	

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Thrombosis			2 (4%)	
Trachea	(50)	(50)	(50)	(50)
Inflammation		4 (8%)	2 (4%)	1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Lens, cataract	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Optic nerve, fibrosis				1 (2%)
Retina, degeneration	2 (4%)	2 (4%)	4 (8%)	4 (8%)
Retina, dysplasia		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	14 (28%)	10 (20%)	6 (12%)	9 (18%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet			2 (4%)	1 (2%)
Fibrosis	1 (2%)			
Infarct		1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic active	1 (2%)			
Nephropathy	47 (94%)	49 (98%)	48 (96%)	49 (98%)
Cortex, inflammation				1 (2%)
Cortex, mineralization	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Medulla, fibrosis	1 (2%)			
Medulla, mineralization	43 (86%)	49 (98%)	49 (98%)	46 (92%)
Pelvis, inflammation	1 (2%)		4 (8%)	2 (4%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	2 (4%)	2 (4%)	4 (8%)
Renal tubule, cyst				1 (2%)
Renal tubule, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Renal tubule, pigmentation				1 (2%)
Renal tubule, vacuolization cytoplasmic		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	1 (2%)	
Inflammation	1 (2%)	1 (2%)		

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF BENZOPHENONE

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Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	3	5	2
Natural deaths	1	3	1	3
Survivors				
Terminal sacrifice	44	44	44	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(49)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Polyp adenomatous			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatoblastoma		1 (2%)	1 (2%)	3 (6%)
Hepatocellular carcinoma	6 (12%)	5 (10%)	4 (8%)	6 (12%)
Hepatocellular carcinoma, multiple	2 (4%)		2 (4%)	
Hepatocellular adenoma	9 (18%)	7 (14%)	15 (30%)	11 (22%)
Hepatocellular adenoma, multiple	2 (4%)	8 (16%)	8 (16%)	12 (24%)
Ito cell tumor benign			1 (2%)	
Mesentery		(2)	(8)	(5)
Fat, hemangiosarcoma, metastatic, spleen		1 (50%)		
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma	1 (2%)	2 (4%)	3 (6%)	
Squamous cell papilloma, multiple		1 (2%)		
Tongue		(1)		
Squamous cell carcinoma		1 (100%)		
Tooth	(20)	(31)	(33)	(21)
Odontoma	1 (5%)			1 (5%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Hemangiosarcoma	1 (2%)			
Hemangiosarcoma, metastatic, liver		1 (2%)		

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Prostate	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, skeletal muscle	1 (2%)			
Schwannoma malignant, metastatic, skin	1 (2%)			
Lymph node	(1)	(1)	(3)	(5)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)			
Lymph node, mandibular	(48)	(50)	(49)	(47)
Lymph node, mesenteric	(49)	(50)	(49)	(48)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Thymus	(46)	(48)	(46)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, histiocytic sarcoma	1 (2%)			
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Schwannoma malignant, metastatic, skin	1 (2%)			
Skeletal muscle	(1)	(1)	(2)	
Hemangiosarcoma	1 (100%)			
Hemangiosarcoma, metastatic, spleen		1 (100%)		
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (50%)	
Nervous System				
None				

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	9 (18%)	4 (8%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)		1 (2%)	
Carcinoma, metastatic, harderian gland	1 (2%)	1 (2%)		
Hepatoblastoma, metastatic, liver		1 (2%)		2 (4%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Mediastinum, alveolus, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Special Senses System				
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	6 (12%)	4 (8%)	4 (8%)
Carcinoma	1 (2%)	1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	2 (4%)		2 (4%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	38	30	34	35
Total primary neoplasms	52	46	52	52
Total animals with benign neoplasms	27	23	30	27
Total benign neoplasms	34	36	40	39
Total animals with malignant neoplasms	17	10	11	13
Total malignant neoplasms	18	10	12	13
Total animals with metastatic neoplasms	6	5	3	3
Total metastatic neoplasms	11	7	3	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	463	540	652	679	690	703	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731
Carcass ID Number	046	044	004	019	038	003	002	009	012	013	014	015	016	017	018	033	035	047	048	049	001	005	010	020	021
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma		X							X				X			X					X				
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma						X								X							X	X			
Hepatocellular adenoma, multiple								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																X									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth		+							+							+		+	+	+					+
Odontoma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Hemangiosarcoma				X																					
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

+: Tissue examined microscopically M: Missing tissue X: Lesion present
A: Autolysis precludes examination I: Insufficient tissue Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 2 2	0 2 3	0 2 4	0 2 5	0 2 7	0 2 8	0 2 9	0 4 0	0 4 1	0 4 3	0 5 0	0 0 6	0 0 7	0 0 8	0 1 1	0 2 6	0 3 0	0 3 1	0 3 2	0 3 4	0 3 6	0 3 7	0 3 9	0 4 2	0 4 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma										X																6
Hepatocellular carcinoma, multiple						X																X				2
Hepatocellular adenoma		X			X									X										X	X	9
Hepatocellular adenoma, multiple							X																			2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+		+		+	+			+	+	+				+		+	+			+		+	+		20
Odontoma																								X		1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma					X																					1
General Body System																										
None																										

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	463	540	652	679	690	703	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731
Carcass ID Number	046	044	004	019	038	003	002	009	012	013	014	015	016	017	018	033	035	047	048	049	001	005	010	020	021
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, skeletal muscle			X																						
Schwannoma malignant, metastatic, skin	X																								
Lymph node				+																					
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Lymph node, mandibular	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma																									X
Subcutaneous tissue, schwannoma, malignant	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin	X																								
Skeletal muscle			+																						
Hemangiosarcoma			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve			+																						
Spinal cord			+																						

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	022	023	024	025	027	028	029	040	041	043	050	006	007	008	011	026	030	031	032	034	036	037	039	042	045	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, skeletal muscle																										1
Schwannoma malignant, metastatic, skin																										1
Lymph node																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																	X									1
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	46
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, schwannoma, malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma malignant, metastatic, skin																										1
Skeletal muscle																										1
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	463	540	652	679	690	703	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731
Carcass ID Number	046	044	004	019	038	003	002	009	012	013	014	015	016	017	018	033	035	047	048	049	001	005	010	020	021
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X			X					X						X			X		X	
Alveolar/bronchiolar adenoma, multiple											X						X								
Alveolar/bronchiolar carcinoma				X																					
Carcinoma, metastatic, harderian gland						X																			
Hepatocellular carcinoma, metastatic, liver		X							X																
Mediastinum, alveolus, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X		X												X			X					
Carcinoma						X																			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									X
Lymphoma malignant					X																				

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	022	023	024	025	027	028	029	040	041	043	050	006	007	008	011	026	030	031	032	034	036	037	039	042	045	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X			X	X						X								10
Alveolar/bronchiolar adenoma, multiple					X															X						4
Alveolar/bronchiolar carcinoma									X																	2
Carcinoma, metastatic, harderian gland																										1
Hepatocellular carcinoma, metastatic, liver																										2
Mediastinum, alveolus, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X		X											6
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant																					X					2

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	449	545	585	606	620	695	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	091	064	059	076	067	092	055	056	057	060	061	068	069	072	078	085	087	088	100	051	052	053	063	071	073
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																				X					
Hemangiosarcoma, metastatic, spleen					X																				
Hepatoblastoma																									
Hepatocellular carcinoma	X	X				X																			X
Hepatocellular adenoma				X			X																	X	
Hepatocellular adenoma, multiple													X	X		X				X					
Mesentery					+																				
Fat, hemangiosarcoma, metastatic, spleen					X																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma													X												
Squamous cell papilloma, multiple												X													
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																			+						
Squamous cell carcinoma																			X						
Tooth	+	+		+		+	+	+		+		+		+		+		+			+	+	+		+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver																				X					
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	M	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	074	077	081	084	096	097	098	099	054	058	062	065	066	070	075	079	080	082	083	086	089	090	093	094	095	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hemangiosarcoma, metastatic, spleen																										1
Hepatoblastoma												X														1
Hepatocellular carcinoma		X																								5
Hepatocellular adenoma	X		X																X						X	7
Hepatocellular adenoma, multiple								X				X					X						X			8
Mesentery												+														2
Fat, hemangiosarcoma, metastatic, spleen																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma													X													2
Squamous cell papilloma, multiple																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell carcinoma																										1
Tooth	+	+				+	+	+	+			+	+		+	+	+	+		+	+	+			+	31
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	M	+	M	+	M	M	+	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	38
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	449	554	585	606	620	695	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	091	064	059	076	067	092	055	056	057	060	061	068	069	072	078	085	087	088	100	051	052	053	063	071	073
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node															+										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+																				
Hemangiosarcoma, metastatic, spleen					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X	X		X						X												
Alveolar/bronchiolar adenoma, multiple																X									
Carcinoma, metastatic, harderian gland								X																	
Hepatoblastoma, metastatic, liver																									
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X										X						X					
Carcinoma								X																	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
Carcass ID Number	7	7	8	8	9	9	9	9	5	5	6	6	6	7	7	7	8	8	8	8	8	9	9	9	9	Tissues/
	4	7	1	4	6	7	8	9	4	8	2	5	6	0	5	9	0	2	3	6	9	0	3	4	5	Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																	X									1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Hemangiosarcoma, metastatic, spleen																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																									+	1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X					X	X			X								X								9
Alveolar/bronchiolar adenoma, multiple													X													2
Carcinoma, metastatic, harderian gland																										1
Hepatoblastoma, metastatic, liver												X														1
Hepatocellular carcinoma, metastatic, liver		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X			X												X							6
Carcinoma																										1

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	449	554	585	606	620	695	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	091	064	059	076	067	092	055	056	057	060	061	068	069	072	078	085	087	088	100	051	052	053	063	071	073
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	074	077	081	084	096	097	098	099	054	058	062	065	066	070	075	079	080	082	083	086	089	090	093	094	095	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	585	589	671	679	683	722	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	119	124	118	142	139	150	101	106	109	110	120	125	141	149	103	105	108	111	112	113	116	122	123	126	129
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous						X																			
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma																									
Hepatocellular carcinoma				X															X						
Hepatocellular carcinoma, multiple																		X							
Hepatocellular adenoma	X								X	X		X		X		X			X			X			
Hepatocellular adenoma, multiple				X				X												X					
Ito cell tumor benign																			X						
Mesentery	+			+	+																+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma					X																				
Squamous cell papilloma														X											
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+	+	+	+			+	+	+	+	+				+		+	+	+	+		+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma												X													
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Total
Carcass ID Number	3	3	3	4	4	4	4	0	0	0	1	1	1	2	2	2	3	3	3	3	3	3	4	4	4	Tissues/
	2	5	7	0	4	7	8	2	4	7	4	5	7	1	7	8	0	1	3	4	6	8	3	5	6	Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma	X																									1
Hepatocellular carcinoma	X										X															4
Hepatocellular carcinoma, multiple																				X						2
Hepatocellular adenoma								X									X	X	X				X	X	X	15
Hepatocellular adenoma, multiple	X	X					X				X					X										8
Ito cell tumor benign																										1
Mesentery	+						+									+							+			8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Squamous cell papilloma											X						X									3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+	+	+		+		+	+			+	+	+					+	+		+		+	+	33
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																1
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	585	589	671	679	683	722	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	119	124	118	142	139	150	101	106	109	110	120	125	141	149	103	105	108	111	112	113	116	122	123	126	129
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+		+																				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																							X		
Thymus	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+																				
Squamous cell carcinoma, metastatic, stomach, forestomach					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X																			
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma								X																	
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X					X																			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	1 3 2	1 3 5	1 3 7	1 4 0	1 4 4	1 4 7	1 4 8	1 0 2	1 0 4	1 0 7	1 1 4	1 1 5	1 1 7	1 2 1	1 2 7	1 2 8	1 3 0	1 3 1	1 3 3	1 3 4	1 3 6	1 3 8	1 4 3	1 4 5	1 4 6	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																		+								3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle					+																					2
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																					+					1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X								X												X		4
Alveolar/bronchiolar adenoma, multiple										X			X													2
Alveolar/bronchiolar carcinoma																										1
Hepatocellular carcinoma, metastatic, liver											X									X						2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X							X					4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant								X										X								2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	568	606	656	695	700	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	153	157	180	174	192	163	166	171	176	179	188	196	197	198	151	152	154	160	162	165	169	170	177	181	184
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma		X														X									
Hepatocellular carcinoma			X	X																		X			
Hepatocellular adenoma	X		X					X	X	X							X								
Hepatocellular adenoma, multiple											X	X	X	X				X	X				X		
Mesentery	+				+																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+		+	+					+	+	+				+					+				+	+
Odontoma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	M	+	M	+	M	+	+	M	+	+	M	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma								X																	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	185	187	190	193	195	199	200	155	156	158	159	161	164	167	168	172	173	175	178	182	183	186	189	191	194	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																							X			1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma																				X						3
Hepatocellular carcinoma															X		X								X	6
Hepatocellular adenoma	X	X			X										X				X							11
Hepatocellular adenoma, multiple						X												X		X			X	X		12
Mesentery		+								+		+														5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+			+	+					+	+				+		+		+		+			+	21
Odontoma		X																								1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M	+	M	+	M	+	+	M	+	+	36
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma															X											1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	5 6 8	6 0 6	6 5 6	6 9 5	7 0 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	1 5 3	1 5 7	1 8 0	1 7 4	1 9 2	1 6 3	1 6 6	1 7 1	1 7 6	1 7 9	1 8 8	1 9 6	1 9 7	1 9 8	1 5 1	1 5 2	1 5 4	1 6 0	1 6 2	1 6 5	1 6 9	1 7 0	1 7 7	1 8 1	1 8 4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+																				
Lymph node, mandibular	+	+	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X			X		X		X					X							
Alveolar/bronchiolar adenoma, multiple												X													
Hepatoblastoma, metastatic, liver		X														X									
Hepatocellular carcinoma, metastatic, liver			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X									X				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter																					+				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant					X																				

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	185	187	190	193	195	199	200	155	156	158	159	161	164	167	168	172	173	175	178	182	183	186	189	191	194	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+					+					+												+		5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	47
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X				X	X																			8
Alveolar/bronchiolar adenoma, multiple																										1
Hepatoblastoma, metastatic, liver																										2
Hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X										X							4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant		X										X														3

Table C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	6/50 (12%)	4/50 (8%)	4/50 (8%)
Adjusted rate[b]	12.4%	12.6%	8.2%	8.3%
Terminal rate[c]	4/44 (9%)	5/44 (11%)	2/44 (5%)	4/45 (9%)
First incidence (days)	652	606	585	730 (T)
Poly-3 test[d]	P=0.258N	P=0.610	P=0.363N	P=0.368N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	7/50 (14%)	4/50 (8%)	4/50 (8%)
Adjusted rate	14.4%	14.7%	8.2%	8.3%
Terminal rate	4/44 (9%)	6/44 (14%)	2/44 (5%)	4/45 (9%)
First incidence (days)	652	606	585	730 (T)
Poly-3 test	P=0.158N	P=0.600	P=0.257N	P=0.261N
Liver: Hepatocellular Adenoma				
Overall rate	11/50 (22%)	15/50 (30%)	23/50 (46%)	23/50 (46%)
Adjusted rate	22.9%	31.5%	46.9%	46.6%
Terminal rate	10/44 (23%)	14/44 (32%)	21/44 (48%)	21/45 (47%)
First incidence (days)	703	606	585	568
Poly-3 test	P=0.006	P=0.239	P=0.010	P=0.011
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	5/50 (10%)	6/50 (12%)	6/50 (12%)
Adjusted rate	16.5%	10.3%	12.3%	12.3%
Terminal rate	7/44 (16%)	2/44 (5%)	5/44 (11%)	4/45 (9%)
First incidence (days)	540	449	679	656
Poly-3 test	P=0.396N	P=0.274N	P=0.386N	P=0.381N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	18/50 (36%)	20/50 (40%)	25/50 (50%)	27/50 (54%)
Adjusted rate	37.0%	40.7%	50.9%	54.6%
Terminal rate	16/44 (36%)	16/44 (36%)	23/44 (52%)	24/45 (53%)
First incidence (days)	540	449	585	568
Poly-3 test	P=0.034	P=0.434	P=0.118	P=0.060
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	2.1%	6.1%
Terminal rate	0/44 (0%)	1/44 (2%)	1/44 (2%)	2/45 (4%)
First incidence (days)	—[e]	730 (T)	730 (T)	606
Poly-3 test	P=0.057	P=0.497	P=0.502	P=0.123
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	8/50 (16%)	6/50 (12%)	6/50 (12%)	9/50 (18%)
Adjusted rate	16.5%	12.3%	12.3%	18.2%
Terminal rate	7/44 (16%)	3/44 (7%)	5/44 (11%)	6/45 (13%)
First incidence (days)	540	449	679	606
Poly-3 test	P=0.393	P=0.384N	P=0.386N	P=0.515
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	18/50 (36%)	20/50 (40%)	25/50 (50%)	29/50 (58%)
Adjusted rate	37.0%	40.7%	50.9%	58.1%
Terminal rate	16/44 (36%)	16/44 (36%)	23/44 (52%)	25/45 (56%)
First incidence (days)	540	449	585	568
Poly-3 test	P=0.013	P=0.434	P=0.118	P=0.027

Table C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	14/50 (28%)	11/50 (22%)	6/50 (12%)	9/50 (18%)
Adjusted rate	29.1%	22.9%	12.4%	18.6%
Terminal rate	13/44 (30%)	9/44 (21%)	5/44 (11%)	9/45 (20%)
First incidence (days)	690	606	722	730 (T)
Poly-3 test	P=0.110N	P=0.322N	P=0.036N	P=0.163N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	11/50 (22%)	7/50 (14%)	9/50 (18%)
Adjusted rate	33.2%	22.9%	14.5%	18.6%
Terminal rate	14/44 (32%)	9/44 (21%)	6/44 (14%)	9/45 (20%)
First incidence (days)	679	606	722	730 (T)
Poly-3 test	P=0.057N	P=0.186N	P=0.025N	P=0.078N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.1%	6.4%	6.2%	0.0%
Terminal rate	1/44 (2%)	3/44 (7%)	3/44 (7%)	0/45 (0%)
First incidence (days)	730 (T)	730 (T)	730 (T)	—
Poly-3 test	P=0.272N	P=0.300	P=0.309	P=0.498N
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.1%	6.4%	8.2%	0.0%
Terminal rate	1/44 (2%)	3/44 (7%)	3/44 (7%)	0/45 (0%)
First incidence (days)	730 (T)	730 (T)	683	—
Poly-3 test	P=0.304N	P=0.300	P=0.183	P=0.498N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.2%	4.2%	2.1%	0.0%
Terminal rate	1/44 (2%)	1/44 (2%)	1/44 (2%)	0/45 (0%)
First incidence (days)	652	620	730 (T)	—
Poly-3 test	P=0.061N	P=0.507N	P=0.306N	P=0.119N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.2%	0.0%	4.1%	6.2%
Terminal rate	1/44 (2%)	0/44 (0%)	2/44 (5%)	2/45 (4%)
First incidence (days)	690	—	730 (T)	700
Poly-3 test	P=0.245	P=0.241N	P=0.691N	P=0.505
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	23/50 (46%)	30/50 (60%)	27/50 (54%)
Adjusted rate	55.7%	47.9%	61.1%	54.8%
Terminal rate	24/44 (55%)	21/44 (48%)	27/44 (61%)	25/45 (56%)
First incidence (days)	652	606	585	568
Poly-3 test	P=0.446	P=0.285N	P=0.370	P=0.543N
All Organs: Malignant Neoplasms				
Overall rate	17/50 (34%)	10/50 (20%)	11/50 (22%)	13/50 (26%)
Adjusted rate	34.0%	20.4%	22.6%	26.3%
Terminal rate	11/44 (25%)	6/44 (14%)	9/44 (21%)	9/45 (20%)
First incidence (days)	463	449	679	606
Poly-3 test	P=0.326N	P=0.097N	P=0.150N	P=0.269N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	38/50 (76%)	30/50 (60%)	34/50 (68%)	35/50 (70%)
Adjusted rate	76.0%	60.6%	69.0%	70.0%
Terminal rate	32/44 (73%)	25/44 (57%)	30/44 (68%)	30/45 (67%)
First incidence (days)	463	449	585	568
Poly-3 test	P=0.463N	P=0.074N	P=0.288N	P=0.327N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE C4
Historical Incidence of Liver Neoplasms in Untreated Male $B6C3F_1$ Mice[a]

Study	Incidence in Controls: Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence in Feed Controls Given NTP-2000 Diet				
Benzophenone	11/50	8/50	0/50	18/50
Citral	20/100	13/100	0/100	28/100
p p′-Dichlorodiphenyl sulfone	6/50	9/50	0/50	15/50
trans-Cinnamaldehyde	14/100	13/100	0/100	26/100
2-Methylimidazole	7/50	4/50	0/50	10/50
o-Nitrotoluene	18/60	12/60	1/60	28/60
p-Nitrotoluene	14/50	8/50	0/50	20/50
Overall Historical Incidence: Feed Studies				
Total (%)	90/460 (19.6%)	67/460 (14.6%)	1/460 (0.2%)	145/460 (31.5%)
Mean ± standard deviation	20% ± 7.1%	14.9% ± 3.9%	0.2% ± 0.6%	32.4% ± 9.1%
Range	12%-30%	8%-20%	0%-2%	20%-47%
Overall Historical Incidence: All Routes				
Total (%)	398/1,257 (31.7%)	275/1,257 (21.9%)	22/1,257 (1.8%)	607/1,257 (48.3%)
Mean ± standard deviation	33.2% ± 12.1%	22.9% ± 9.4%	1.9% ± 3.4%	50.4% ± 16.1%
Range	12%-63%	8%-46%	0%-13%	20%-85%

[a] Data as of April 19, 2004

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	3	5	2
Natural deaths	1	3	1	3
Survivors				
Terminal sacrifice	44	44	44	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(49)	(49)	(49)
Cyst			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Basophilic focus	4 (8%)	2 (4%)	5 (10%)	
Clear cell focus	2 (4%)	7 (14%)	7 (14%)	12 (24%)
Eosinophilic focus	5 (10%)	8 (16%)	11 (22%)	10 (20%)
Fatty change			1 (2%)	
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	2 (4%)	1 (2%)		
Infarct		2 (4%)		1 (2%)
Infiltration cellular, lymphoid	6 (12%)	10 (20%)		4 (8%)
Inflammation, chronic active	33 (66%)	47 (94%)	44 (88%)	42 (84%)
Mixed cell focus	8 (16%)	9 (18%)	15 (30%)	13 (26%)
Tension lipidosis	1 (2%)	1 (2%)		1 (2%)
Bile duct, cyst		3 (6%)		1 (2%)
Hepatocyte, degeneration, cystic			5 (10%)	30 (60%)
Hepatocyte, multinucleated		41 (82%)	47 (94%)	48 (96%)
Hepatocyte, necrosis	1 (2%)	6 (12%)	8 (16%)	8 (16%)
Hepatocyte, vacuolization cytoplasmic	44 (88%)	45 (90%)	46 (92%)	44 (88%)
Hepatocyte, centrilobular, hypertrophy		44 (88%)	50 (100%)	48 (96%)
Oval cell, hyperplasia			1 (2%)	
Mesentery		(2)	(8)	(5)
Fat, fibrosis		1 (50%)	5 (63%)	1 (20%)
Fat, inflammation, chronic active		1 (50%)	5 (63%)	2 (40%)
Fat, mineralization		1 (50%)	3 (38%)	
Fat, necrosis		1 (50%)	5 (63%)	
Fat, pigmentation		1 (50%)		
Pancreas	(50)	(50)	(50)	(50)
Cyst			1 (2%)	2 (4%)
Cytoplasmic alteration	1 (2%)			
Infiltration cellular, lymphoid	14 (28%)	11 (22%)	8 (16%)	8 (16%)
Acinus, atrophy		1 (2%)	1 (2%)	1 (2%)
Acinus, cytoplasmic alteration			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Infiltration cellular, lymphoid	31 (62%)	40 (80%)	40 (80%)	28 (56%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation, chronic active	1 (2%)	2 (4%)		
Epithelium, cyst		1 (2%)		
Epithelium, hyperkeratosis	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Epithelium, hyperplasia, squamous	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Epithelium, ulcer	1 (2%)	1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Glands, ectasia	7 (14%)	13 (26%)	18 (36%)	5 (10%)
Glands, mineralization			1 (2%)	1 (2%)
Tooth	(20)	(31)	(33)	(21)
Inflammation, chronic active	1 (5%)		3 (9%)	
Malformation	3 (15%)	1 (3%)	3 (9%)	
Gingiva, inflammation, chronic active	18 (90%)	30 (97%)	32 (97%)	20 (95%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hyperplasia, atypical				1 (2%)
Inflammation, chronic active	3 (6%)	5 (10%)	3 (6%)	3 (6%)
Mineralization	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Degeneration, fatty			2 (4%)	
Hyperplasia	7 (14%)	10 (20%)	9 (18%)	6 (12%)
Hypertrophy	26 (52%)	22 (44%)	24 (48%)	18 (36%)
Inflammation, chronic active			1 (2%)	
Necrosis		1 (2%)		
Subcapsular, hyperplasia	50 (100%)	46 (92%)	46 (92%)	47 (94%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		
Pituitary gland	(48)	(49)	(50)	(50)
Pars distalis, cyst	7 (15%)	5 (10%)	5 (10%)	4 (8%)
Pars distalis, hyperplasia	1 (2%)	1 (2%)		
Pars nervosa, cyst		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid			1 (2%)	1 (2%)
Inflammation, chronic active	2 (4%)			
Follicle, cyst	2 (4%)			1 (2%)
Follicle, degeneration	12 (24%)	9 (18%)	6 (12%)	6 (12%)
Follicular cell, hyperplasia			1 (2%)	1 (2%)
General Body System				
None				

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Degeneration		1 (2%)		
Granuloma sperm	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, lymphoid	32 (64%)	27 (54%)	30 (60%)	31 (62%)
Mineralization			4 (8%)	
Spermatocele			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Inflammation, chronic active	21 (42%)	23 (46%)	25 (50%)	32 (64%)
Duct, ectasia	20 (40%)	15 (30%)	15 (30%)	28 (56%)
Prostate	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	32 (64%)	43 (86%)	46 (92%)	28 (56%)
Inflammation, chronic active	1 (2%)			
Artery, inflammation, chronic active		1 (2%)		1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Artery, inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Mineralization		1 (2%)	4 (8%)	12 (24%)
Germinal epithelium, degeneration		3 (6%)	1 (2%)	2 (4%)
Rete testes, cyst		1 (2%)		
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Myeloid cell, hyperplasia			1 (2%)	
Lymph node	(1)	(1)	(3)	(5)
Deep cervical, hyperplasia, lymphoid			1 (33%)	
Mediastinal, hyperplasia, lymphoid		1 (100%)	1 (33%)	
Mediastinal, infiltration cellular, histiocyte	1 (100%)			
Mediastinal, inflammation, granulomatous				1 (20%)
Lymph node, mandibular	(48)	(50)	(49)	(47)
Hyperplasia, lymphoid	5 (10%)	1 (2%)	6 (12%)	5 (11%)
Lymph node, mesenteric	(49)	(50)	(49)	(48)
Hyperplasia, lymphoid	2 (4%)	2 (4%)		2 (4%)
Infiltration cellular, histiocyte	1 (2%)			
Inflammation, granulomatous				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Hematopoietic cell proliferation	11 (22%)	7 (14%)	9 (18%)	14 (28%)
Lymphoid follicle, hyperplasia, lymphoid	17 (34%)	31 (62%)	34 (68%)	32 (64%)
Thymus	(46)	(48)	(46)	(47)
Atrophy	12 (26%)	9 (19%)	6 (13%)	5 (11%)
Cyst	16 (35%)	25 (52%)	20 (43%)	20 (43%)
Ectopic parathyroid gland	2 (4%)			
Ectopic thyroid			1 (2%)	
Infiltration cellular, histiocyte	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Inflammation, granulomatous	1 (2%)			
Mineralization				1 (2%)

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Musculoskeletal System				
None				
Nervous System				
Spinal cord	(1)	(1)	(1)	
Degeneration	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	32 (64%)	38 (76%)	47 (94%)	36 (72%)
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Metaplasia, osseous			1 (2%)	
Necrosis	1 (2%)			
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	6 (12%)	7 (14%)	8 (16%)	4 (8%)
Alveolus, infiltration cellular, histiocyte	7 (14%)	1 (2%)	1 (2%)	3 (6%)
Mediastinum, inflammation, suppurative			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, chronic active	2 (4%)		1 (2%)	2 (4%)
Inflammation, focal, suppurative				1 (2%)
Inflammation, suppurative			1 (2%)	
Nasolacrimal duct, inflammation, chronic active			2 (4%)	1 (2%)
Olfactory epithelium, degeneration	1 (2%)			
Olfactory epithelium, metaplasia		2 (4%)	2 (4%)	24 (48%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Anterior chamber, iris, inflammation, suppurative		1 (2%)		
Cornea, inflammation, chronic active		1 (2%)	2 (4%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Infiltration cellular, lymphoid	37 (74%)	33 (66%)	24 (48%)	29 (58%)
Inflammation, chronic active	1 (2%)		1 (2%)	
Mineralization				1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Infiltration cellular, lymphoid	44 (88%)	44 (88%)	45 (90%)	45 (90%)
Inflammation, chronic active		1 (2%)	2 (4%)	
Metaplasia, osseous	4 (8%)	2 (4%)	1 (2%)	8 (16%)
Mineralization	49 (98%)	50 (100%)	50 (100%)	50 (100%)
Necrosis			1 (2%)	
Nephropathy	49 (98%)	48 (96%)	50 (100%)	50 (100%)
Artery, inflammation, chronic active	1 (2%)	1 (2%)		
Cortex, cyst	4 (8%)	8 (16%)	12 (24%)	22 (44%)
Renal tubule, pigmentation				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	24 (48%)	24 (48%)	32 (64%)	23 (46%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF BENZOPHENONE

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	4	2	5	6
Natural deaths	6	6	4	12
Survivors				
Terminal sacrifice	40	42	41	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(50)	(50)	(50)
Histiocytic sarcoma				2 (4%)
Intestine large, colon	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, skeletal muscle				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Polyp adenomatous	1 (2%)		3 (6%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	1 (2%)	
Histiocytic sarcoma				1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hemangiosarcoma			1 (2%)	1 (2%)
Hepatocellular carcinoma		1 (2%)		1 (2%)
Hepatocellular adenoma	4 (8%)	3 (6%)	7 (14%)	5 (10%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Histiocytic sarcoma			5 (10%)	3 (6%)
Mast cell tumor malignant			1 (2%)	
Sarcoma stromal, metastatic, uterus	1 (2%)			
Mesentery	(6)	(9)	(11)	(9)
Fat, fibrosarcoma, metastatic, skin				1 (11%)
Fat, fibrous histiocytoma, metastatic, skin				1 (11%)
Fat, hemangiosarcoma, metastatic, skeletal muscle				1 (11%)
Fat, histiocytic sarcoma				2 (22%)
Fat, sarcoma stromal, metastatic, uterus	1 (17%)			
Oral mucosa		(2)		
Gingival, mast cell tumor malignant		1 (50%)		
Pharyngeal, squamous cell papilloma		1 (50%)		
Pancreas	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Histiocytic sarcoma				2 (4%)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	1 (2%)		2 (4%)	1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Histiocytic sarcoma				1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Aorta, fibrous histiocytoma, metastatic, skin				1 (2%)
Aorta, histiocytic sarcoma				1 (2%)
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Hemangiosarcoma, metastatic, liver				1 (2%)
Histiocytic sarcoma				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, skin				1 (2%)
Histiocytic sarcoma				3 (6%)
Subcapsular, adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma benign	1 (2%)	1 (2%)		2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Parathyroid gland	(42)	(41)	(40)	(40)
Pituitary gland	(50)	(50)	(49)	(47)
Pars distalis, adenoma	2 (4%)		2 (4%)	3 (6%)
Pars distalis, histiocytic sarcoma				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
C-cell, carcinoma				1 (2%)
Follicular cell, adenoma				1 (2%)
Follicular cell, carcinoma		1 (2%)	2 (4%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Ovary	(49)	(50)	(50)	(50)
Cystadenoma	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Histiocytic sarcoma			2 (4%)	3 (6%)
Bilateral, granulosa cell tumor malignant			1 (2%)	
Bilateral, tubulostromal adenoma	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Genital System (continued)				
Uterus	(49)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, skeletal muscle				1 (2%)
Histiocytic sarcoma			1 (2%)	3 (6%)
Leiomyoma		1 (2%)		
Polyp stromal	3 (6%)			
Polyp stromal, multiple	1 (2%)			
Sarcoma stromal	1 (2%)			
Bilateral, polyp stromal		1 (2%)		
Endometrium, adenoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		1 (2%)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	2 (4%)
Mast cell tumor malignant			1 (2%)	
Lymph node	(8)	(12)	(11)	(15)
Deep cervical, carcinoma, metastatic, thyroid gland		1 (8%)		
Inguinal, histiocytic sarcoma				1 (7%)
Lumbar, histiocytic sarcoma			1 (9%)	2 (13%)
Mediastinal, carcinoma, metastatic, thyroid gland				1 (7%)
Mediastinal, fibrous histiocytoma, metastatic, skin				1 (7%)
Mediastinal, hemangiosarcoma, metastatic, skeletal muscle				1 (7%)
Mediastinal, histiocytic sarcoma				3 (20%)
Pancreatic, fibrous histiocytoma, metastatic, skin				1 (7%)
Pancreatic, histiocytic sarcoma				1 (7%)
Renal, fibrous histiocytoma, metastatic, skin				1 (7%)
Renal, histiocytic sarcoma			1 (9%)	2 (13%)
Lymph node, mandibular	(50)	(50)	(50)	(49)
Histiocytic sarcoma			3 (6%)	2 (4%)
Mast cell tumor malignant, metastatic, oral mucosa		1 (2%)		
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hemangiosarcoma, metastatic, skeletal muscle				1 (2%)
Histiocytic sarcoma			2 (4%)	3 (6%)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hemangioma		1 (2%)		
Hemangiosarcoma, metastatic, bone marrow	1 (2%)			
Histiocytic sarcoma			2 (4%)	3 (6%)
Thymus	(46)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Mast cell tumor malignant			1 (2%)	
Mast cell tumor malignant, metastatic, oral mucosa		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Histiocytic sarcoma				1 (2%)

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)			
Squamous cell carcinoma		1 (2%)		
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, fibrosarcoma	1 (2%)		4 (8%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma				1 (2%)
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, histiocytic sarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)	1 (2%)		
Subcutaneous tissue, schwannoma malignant, multiple		1 (2%)		
Subcutaneous tissue, trichoepithelioma				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Skeletal muscle	(2)	(4)	(2)	(4)
Hemangiosarcoma				1 (25%)
Histiocytic sarcoma				1 (25%)
Sarcoma stromal, metastatic, uterus	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		5 (10%)	2 (4%)	2 (4%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)			
Carcinoma, metastatic, thyroid gland		1 (2%)		1 (2%)
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Fibrosarcoma, metastatic, skin			1 (2%)	1 (2%)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Granulosa cell tumor malignant, metastatic, ovary			1 (2%)	1 (2%)
Hemangiosarcoma, metastatic, liver				1 (2%)
Hemangiosarcoma, metastatic, skeletal muscle				1 (2%)
Histiocytic sarcoma			5 (10%)	3 (6%)
Osteosarcoma, metastatic, skin	1 (2%)			
Mediastinum, fibrosarcoma, metastatic, skin				1 (2%)
Nose	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Mast cell tumor malignant			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Carcinoma				1 (2%)
Histiocytic sarcoma				1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Histiocytic sarcoma			3 (6%)	3 (6%)
Urinary bladder	(50)	(49)	(50)	(50)
Histiocytic sarcoma				3 (6%)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			5 (10%)	3 (6%)
Lymphoma malignant	8 (16%)	9 (18%)	5 (10%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	25	28	32	30
Total primary neoplasms	34	39	49	45
Total animals with benign neoplasms	15	17	19	19
Total benign neoplasms	18	23	25	24
Total animals with malignant neoplasms	16	13	18	20
Total malignant neoplasms	16	16	24	21
Total animals with metastatic neoplasms	3	2	3	6
Total metastatic neoplasms	10	7	3	31
Total animals with malignant neoplasms of uncertain primary site			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	414	456	606	608	610	613	708	709	709	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	227	211	204	249	221	223	238	216	246	226	201	203	205	206	207	210	214	215	217	224	225	228	229	230	231
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus								X																	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple																									
Sarcoma stromal, metastatic, uterus								X																	
Mesentery				+		+		+														+			
Fat, sarcoma stromal, metastatic, uterus								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus								X																	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Squamous cell papilloma																		X							
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+									+	+	+	+	+	+		+	+			+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 3 4	2 3 5	2 3 9	2 4 1	2 4 2	2 4 3	2 4 8	2 0 2	2 0 8	2 0 9	2 1 2	2 1 9	2 2 0	2 4 0	2 4 4	2 4 5	2 4 7	2 5 0	2 1 3	2 1 8	2 2 2	2 3 2	2 3 3	2 3 6	2 3 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal, metastatic, uterus																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																				X						1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma					X		X								X		X									4
Hepatocellular adenoma, multiple			X																							1
Sarcoma stromal, metastatic, uterus																										1
Mesentery									+				+													6
Fat, sarcoma stromal, metastatic, uterus																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal, metastatic, uterus																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																X										1
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth				+				+		+			+	+		+	+				+				+	21
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcapsular, adenoma														X												1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	M	+	+	+	+	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma										X					X											2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	414	456	606	608	610	613	708	709	709	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	227	211	204	249	221	223	238	216	246	226	201	203	205	206	207	210	214	215	217	224	225	228	229	230	231
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma							X																		
Bilateral, tubulostromal adenoma																									
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																X									
Polyp stromal, multiple																									
Sarcoma stromal								X																	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Lymph node	+		+	+		+		+														+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus								X																	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, bone marrow							X																		
Thymus	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Subcutaneous tissue, fibrosarcoma									X																
Subcutaneous tissue, schwannoma, malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus								X																	
Skeletal muscle						+		+																	
Sarcoma stromal, metastatic, uterus								X																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve							+																		
Spinal cord							+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, multiple					X																				
Osteosarcoma, metastatic, skin																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	234	235	239	241	242	243	248	202	208	209	212	219	220	240	244	245	247	250	213	218	222	232	233	236	237	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma																										1
Bilateral, tubulostromal adenoma								X																		1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal								X											X							3
Polyp stromal, multiple																								X		1
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node										+			+													8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal, metastatic, uterus																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, bone marrow																										1
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma						X																				1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																			X							1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, schwannoma, malignant														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal, metastatic, uterus																										1
Skeletal muscle																										2
Sarcoma stromal, metastatic, uterus																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, multiple																										1
Osteosarcoma, metastatic, skin																			X							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	414	456	606	608	610	613	708	709	709	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	227	211	204	249	221	223	238	216	246	226	201	203	205	206	207	210	214	215	217	224	225	228	229	230	231
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus								X																	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant	X					X				X								X		X		X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 0 ppm

Number of Days on Study	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	234	235	239	241	242	243	248	202	208	209	212	219	220	240	244	245	247	250	213	218	222	232	233	236	237	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X													1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal, metastatic, uterus																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant										X			X													8

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	316	521	561	595	641	680	703	709	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	264	273	266	251	299	292	259	295	252	255	257	258	262	265	267	269	270	271	275	278	281	285	287	289	296
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X								
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland			X																						
Hepatocellular carcinoma																			X						
Hepatocellular adenoma						X										X									
Hepatocellular adenoma, multiple																		X							
Mesentery						+	+										+					+			
Oral mucosa										+															
Gingival, mast cell tumor malignant																									
Pharyngeal, squamous cell papilloma										X															
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+	+		+	+			+	+	+	+	+	+			+				+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma			X																						
General Body System																									
None																									

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	298	304	254	256	260	261	263	268	274	277	279	282	283	284	288	291	293	294	297	253	272	276	280	286	290	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1
Hepatocellular carcinoma																										1
Hepatocellular adenoma		X																								3
Hepatocellular adenoma, multiple																										1
Mesentery						+		+					+									+			+	9
Oral mucosa																									+	2
Gingival, mast cell tumor malignant																									X	1
Pharyngeal, squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+	+	+	+	+	+	+	+		+		+				+		+	+	+				+	29
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign			X																							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	M	M	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, carcinoma																										1
General Body System																										
None																										

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	316	522	561	595	641	680	703	709	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	264	273	266	251	299	292	259	295	252	255	257	258	262	265	267	269	270	271	275	278	281	285	287	289	296
Genital System																									
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																							X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma									X																
Bilateral, polyp stromal																									
Endometrium, adenoma																					X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland			X																						
Lymph node			+			+	+				+	+					+		+			+			+
Deep cervical, carcinoma, metastatic, thyroid gland			X																						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant, metastatic, oral mucosa																									
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X				
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant, metastatic, oral mucosa																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma					X																				
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, schwannoma, malignant		X																							
Subcutaneous tissue, schwannoma malignant, multiple																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle							+	+										+							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X																			X					X
Carcinoma, metastatic, thyroid gland			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland			X																						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 9 8	3 0 0	2 5 4	2 5 6	2 6 0	2 6 1	2 6 3	2 6 8	2 7 4	2 7 7	2 7 9	2 8 2	2 8 3	2 8 4	2 8 8	2 9 1	2 9 3	2 9 4	2 9 7	2 5 3	2 7 2	2 7 6	2 8 0	2 8 6	2 9 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma		X																X								3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																										1
Bilateral, polyp stromal											X															1
Endometrium, adenoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1
Lymph node													+					+					+			12
Deep cervical, carcinoma, metastatic, thyroid gland																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant, metastatic, oral mucosa																									X	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant, metastatic, oral mucosa																									X	1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Subcutaneous tissue, fibroma																					X					1
Subcutaneous tissue, hemangioma											X															1
Subcutaneous tissue, schwannoma, malignant																										1
Subcutaneous tissue, schwannoma malignant, multiple																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+																								4
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X																X					5
Carcinoma, metastatic, thyroid gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	316	522	561	595	641	680	703	709	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	264	273	266	251	299	292	259	295	252	255	257	258	262	265	267	269	270	271	275	278	281	285	287	289	296
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant		X				X	X				X											X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 312 ppm

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	298	300	254	256	260	261	263	268	274	277	279	282	283	284	288	291	293	294	297	253	272	276	280	286	290	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X	X																						3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant				X				X														X			X	9

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	204	478	555	634	645	690	700	704	718	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	332	318	321	335	329	345	306	328	336	301	302	304	307	313	314	316	319	320	324	339	341	343	346	350	303
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																					X				
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple												X													
Histiocytic sarcoma									X																
Mast cell tumor malignant		X																							
Mesentery				+				+		+							+	+							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+		+	+		+	+	+		+		+		+				+	+		+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	M	M	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																								X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma																	X	X							
General Body System																									
None																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
Carcass ID Number	0	0	0	1	1	1	1	1	2	2	2	3	3	3	3	4	4	4	4	4	4	2	2	3	3	Tissues/
	5	8	9	0	1	2	5	7	5	6	7	3	4	7	8	0	2	4	7	8	9	2	3	0	1	Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																							X		X	3
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																			X							1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular adenoma					X								X	X		X	X			X		X				7
Hepatocellular adenoma, multiple																			X		X					3
Histiocytic sarcoma											X		X	X		X										5
Mast cell tumor malignant																										1
Mesentery							+								+		+			+		+		+		11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma														X												1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																			X							2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth			+	+		+		+			+		+		+		+			+			+	+	+	24
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	49
Pars distalis, adenoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, carcinoma																										2
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	204	478	555	634	645	690	700	704	718	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	332	318	321	335	329	345	306	328	336	301	302	304	307	313	314	316	319	320	324	339	341	343	346	350	303
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma												X													
Histiocytic sarcoma																									
Bilateral, granulosa cell tumor, malignant																						X			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Mast cell tumor malignant		X																							
Lymph node		+			+			+	+			+						+							
Lumbar, histiocytic sarcoma																									
Renal, histiocytic sarcoma									X																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Mast cell tumor malignant		X																							
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma	X							X										X				X			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																				+					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	305	308	309	310	311	312	315	317	325	326	327	333	334	337	338	340	342	344	347	348	349	322	323	330	331	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																X							X			3
Histiocytic sarcoma														X		X										2
Bilateral, granulosa cell tumor, malignant																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													1
Mast cell tumor malignant																										1
Lymph node	+					+	+						+			+										11
Lumbar, histiocytic sarcoma													X													1
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X	X		X										3
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma													X			X										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X			X										2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													1
Mast cell tumor malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X																							1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										4
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle												+														2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	204	478	555	634	645	690	700	704	718	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	332	318	321	335	329	345	306	328	336	301	302	304	307	313	314	316	319	320	324	339	341	343	346	350	303
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, uncertain primary site																									
Fibrosarcoma, metastatic, skin																		X							
Granulosa cell tumor malignant, metastatic, ovary																						X			
Histiocytic sarcoma									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Mast cell tumor malignant		X																							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X											X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Lymphoma malignant					X									X											

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 625 ppm

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	0	0	0	1	1	1	1	1	2	2	2	3	3	3	3	4	4	4	4	4	4	2	2	3	3	Tissues/
	5	8	9	0	1	2	5	7	5	6	7	3	4	7	8	0	2	4	7	8	9	2	3	0	1	Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																			X					X		2
Carcinoma, metastatic, uncertain primary site																								X		1
Fibrosarcoma, metastatic, skin																										1
Granulosa cell tumor, malignant, metastatic, ovary																										1
Histiocytic sarcoma											X		X	X		X										5
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Mast cell tumor malignant																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X	X												3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma											X		X	X		X										5
Lymphoma malignant	X					X										X										5

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	344	400	435	466	536	606	637	638	645	651	672	675	677	704	705	711	712	714	723	729	729	729	729	729	729
Carcass ID Number	384	397	363	366	353	391	395	380	373	389	352	388	392	383	365	400	375	371	356	372	376	377	378	381	386
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, skeletal muscle													X												
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin															X										
Hemangiosarcoma						X																			
Hepatocellular carcinoma																	X								
Hepatocellular adenoma			X																			X			
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma										X															
Mesentery						+		+		+		+	+	+	+		+								
Fat, fibrosarcoma, metastatic, skin														X											
Fat, fibrous histiocytoma, metastatic, skin															X										
Fat, hemangiosarcoma, metastatic, skeletal muscle													X												
Fat, histiocytic sarcoma										X															
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin														X											
Fibrous histiocytoma, metastatic, skin															X										
Histiocytic sarcoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																							X		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin														X											
Fibrous histiocytoma, metastatic, skin															X										
Histiocytic sarcoma																									
Tooth				+				+			+	+	+		+	+				+				+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Aorta, fibrous histiocytoma, metastatic, skin															X										
Aorta, histiocytic sarcoma																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver						X																			
Histiocytic sarcoma																									

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	387	390	394	398	351	354	355	357	359	360	361	367	369	370	374	379	382	396	399	358	362	364	368	385	393	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X								X																2
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, skeletal muscle																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma										X																1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Hemangiosarcoma																										1
Hepatocellular carcinoma																										1
Hepatocellular adenoma												X				X									X	5
Hepatocellular adenoma, multiple				X									X							X						3
Histiocytic sarcoma		X								X																3
Mesentery		+																								9
Fat, fibrosarcoma, metastatic, skin																										1
Fat, fibrous histiocytoma, metastatic, skin																										1
Fat, hemangiosarcoma, metastatic, skeletal muscle																										1
Fat, histiocytic sarcoma		X																								2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Fibrous histiocytoma, metastatic, skin																										1
Histiocytic sarcoma		X								X																2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Fibrous histiocytoma, metastatic, skin																										1
Histiocytic sarcoma		X																								1
Tooth		+		+		+	+	+						+	+				+		+			+	+	20
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Aorta, fibrous histiocytoma, metastatic, skin																										1
Aorta, histiocytic sarcoma										X																1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																										1
Histiocytic sarcoma		X																								1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	344	400	435	466	536	606	637	638	645	651	672	675	677	704	705	711	712	714	723	729	729	729	729	729	729
Carcass ID Number	384	397	363	366	353	391	395	380	373	389	352	388	392	383	365	405	375	371	356	372	376	377	378	381	386
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin														X											
Histiocytic sarcoma										X															
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+	+	M	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars distalis, adenoma											X		X				X								
Pars distalis, histiocytic sarcoma										X															
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
C-cell, carcinoma									X																
Follicular cell, adenoma					X																				
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma															X										
Fibrous histiocytoma, metastatic, skin															X										
Histiocytic sarcoma										X															
Oviduct																					+	+			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, skeletal muscle													X												
Histiocytic sarcoma										X															
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland									X																
Histiocytic sarcoma																									
Lymph node			+	+		+		+	+	+		+	+		+	+			+						
Inguinal, histiocytic sarcoma																									
Lumbar, histiocytic sarcoma																									
Mediastinal, carcinoma, metastatic, thyroid gland									X																
Mediastinal, fibrous histiocytoma, metastatic, skin															X										
Mediastinal, hemangiosarcoma, metastatic, skeletal muscle													X												
Mediastinal, histiocytic sarcoma										X															
Pancreatic, fibrous histiocytoma, metastatic, skin															X										
Pancreatic, histiocytic sarcoma																									
Renal, fibrous histiocytoma, metastatic, skin															X										
Renal, histiocytic sarcoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	387	390	394	398	351	354	355	357	359	360	361	367	369	370	374	379	382	396	399	358	362	364	368	385	393	Total Tissues/ Tumors
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma, metastatic, skin																										1
Histiocytic sarcoma		X								X																3
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign														X												2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma											X															1
Parathyroid gland	M	M	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Pars distalis, adenoma																										3
Pars distalis, histiocytic sarcoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
C-cell, carcinoma																										1
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma														X												2
Fibrous histiocytoma, metastatic, skin																										1
Histiocytic sarcoma		X								X																3
Oviduct															+											3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, skeletal muscle																										1
Histiocytic sarcoma		X								X																3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1
Histiocytic sarcoma		X								X																2
Lymph node		+								+				+		+										15
Inguinal, histiocytic sarcoma										X																1
Lumbar, histiocytic sarcoma		X								X																2
Mediastinal, carcinoma, metastatic, thyroid gland																										1
Mediastinal, fibrous histiocytoma, metastatic, skin																										1
Mediastinal, hemangiosarcoma, metastatic, skeletal muscle																										1
Mediastinal, histiocytic sarcoma		X								X																3
Pancreatic, fibrous histiocytoma, metastatic, skin																										1
Pancreatic, histiocytic sarcoma		X																								1
Renal, fibrous histiocytoma, metastatic, skin																										1
Renal, histiocytic sarcoma		X								X																2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	344	400	435	466	536	606	637	638	645	651	672	675	677	704	705	711	712	714	723	729	729	729	729	729	729
Carcass ID Number	384	397	363	366	353	391	395	380	373	389	352	388	392	383	365	400	375	371	356	372	376	377	378	381	386
Hematopoietic System (continued)																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin															X										
Hemangiosarcoma, metastatic, skeletal muscle													X												
Histiocytic sarcoma										X															
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin															X										
Histiocytic sarcoma										X															
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma														X											
Subcutaneous tissue, fibrous histiocytoma															X										
Subcutaneous tissue, histiocytic sarcoma																									
Subcutaneous tissue, trichoepithelioma	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+					+					+												
Hemangiosarcoma													X												
Histiocytic sarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											
Alveolar/bronchiolar adenoma, multiple											X														
Carcinoma, metastatic, thyroid gland									X																
Fibrosarcoma, metastatic, skin														X											
Fibrous histiocytoma, metastatic, skin															X										
Granulosa cell tumor malignant, metastatic, ovary																						X			
Hemangiosarcoma, metastatic, liver						X																			
Hemangiosarcoma, metastatic, skeletal muscle													X												
Histiocytic sarcoma										X															
Mediastinum, fibrosarcoma, metastatic, skin														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	387	390	394	398	351	354	355	357	359	360	361	367	369	370	374	379	382	396	399	358	362	364	368	385	393	Total Tissues/ Tumors
Hematopoietic System (continued)																										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma		X								X																2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Hemangiosarcoma, metastatic, skeletal muscle																										1
Histiocytic sarcoma		X								X																3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Histiocytic sarcoma		X								X																3
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma										X																1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, fibrous histiocytoma																										1
Subcutaneous tissue, histiocytic sarcoma		X																								1
Subcutaneous tissue, trichoepithelioma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+																								4
Hemangiosarcoma																										1
Histiocytic sarcoma		X																								1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma														X												2
Alveolar/bronchiolar adenoma, multiple																										1
Carcinoma, metastatic, thyroid gland																										1
Fibrosarcoma, metastatic, skin																										1
Fibrous histiocytoma, metastatic, skin																										1
Granulosa cell tumor malignant, metastatic, ovary																										1
Hemangiosarcoma, metastatic, liver																										1
Hemangiosarcoma, metastatic, skeletal muscle																										1
Histiocytic sarcoma		X								X																3
Mediastinum, fibrosarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	344	405	435	466	536	606	637	638	645	651	672	675	677	704	705	711	712	714	723	729	729	729	729	729	729
Carcass ID Number	384	397	363	366	353	391	395	380	373	389	352	388	392	383	365	405	375	371	356	372	376	377	378	381	386
Special Senses System																									
Ear															+			+							
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X				X																
Carcinoma																				X					
Histiocytic sarcoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin															X										
Histiocytic sarcoma										X															
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X															
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X															
Lymphoma malignant			X					X	X			X				X							X		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Benzophenone: 1,250 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	387	390	394	398	351	354	355	357	359	360	361	367	369	370	374	379	382	396	399	358	362	364	368	385	393	Total Tissues/ Tumors
Special Senses System																										
Ear																										2
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																				X						3
Carcinoma																										1
Histiocytic sarcoma		X																								1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Histiocytic sarcoma		X								X																3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X								X																3
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X								X																3
Lymphoma malignant					X							X				X			X							10

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate[b]	2.2%	6.4%	4.3%	6.8%
Terminal rate[c]	1/40 (3%)	3/42 (7%)	2/41 (5%)	1/31 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	536
Poly-3 test[d]	P=0.281	P=0.307	P=0.501	P=0.288
Harderian Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	6.4%	4.3%	9.0%
Terminal rate	1/40 (3%)	3/42 (7%)	2/41 (5%)	2/31 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)	536
Poly-3 test	P=0.147	P=0.307	P=0.501	P=0.164
Small Intestine (Duodenum): Adenomatous Polyp				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.2%	0.0%	6.4%	0.0%
Terminal rate	1/40 (3%)	0/42 (0%)	3/41 (7%)	0/31 (0%)
First incidence (days)	729 (T)	—[e]	729 (T)	—
Poly-3 test	P=0.533N	P=0.499N	P=0.307	P=0.514N
Liver: Hepatocellular Adenoma				
Overall rate	5/50 (10%)	4/50 (8%)	10/50 (20%)	8/50 (16%)
Adjusted rate	10.8%	8.5%	21.4%	18.1%
Terminal rate	5/40 (13%)	3/42 (7%)	10/41 (24%)	7/31 (23%)
First incidence (days)	729 (T)	680	729 (T)	435
Poly-3 test	P=0.109	P=0.494N	P=0.131	P=0.243
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	10/50 (20%)	9/50 (18%)
Adjusted rate	10.8%	10.7%	21.4%	20.3%
Terminal rate	5/40 (13%)	4/42 (10%)	10/41 (24%)	7/31 (23%)
First incidence (days)	729 (T)	680	729 (T)	435
Poly-3 test	P=0.081	P=0.624N	P=0.131	P=0.165
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	5/50 (10%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	10.5%	4.3%	6.9%
Terminal rate	0/40 (0%)	4/42 (10%)	2/41 (5%)	1/31 (3%)
First incidence (days)	—	316	729 (T)	672
Poly-3 test	P=0.260	P=0.033	P=0.238	P=0.108
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	5/50 (10%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.1%	10.5%	4.3%	6.9%
Terminal rate	0/40 (0%)	4/42 (10%)	2/41 (5%)	1/31 (3%)
First incidence (days)	610	316	729 (T)	672
Poly-3 test	P=0.396	P=0.105	P=0.498	P=0.280
Ovary: Cystadenoma				
Overall rate	1/49 (2%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	6.4%	6.4%	4.6%
Terminal rate	0/40 (0%)	3/42 (7%)	3/41 (7%)	1/31 (3%)
First incidence (days)	708	729 (T)	729 (T)	705
Poly-3 test	P=0.433	P=0.308	P=0.307	P=0.477

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	2/49 (4%)	3/47 (6%)
Adjusted rate	4.3%	0.0%	4.4%	7.2%
Terminal rate	2/40 (5%)	0/42 (0%)	2/40 (5%)	0/30 (0%)
First incidence (days)	729 (T)	—	729 (T)	672
Poly-3 test	P=0.202	P=0.236N	P=0.687	P=0.451
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.2%	0.0%	8.4%	2.3%
Terminal rate	0/40 (0%)	0/42 (0%)	2/41 (5%)	0/31 (0%)
First incidence (days)	709	—	204	704
Poly-3 test	P=0.397	P=0.499N	P=0.187	P=0.745
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.2%	0.0%	8.4%	4.6%
Terminal rate	0/40 (0%)	0/42 (0%)	2/41 (5%)	0/31 (0%)
First incidence (days)	709	—	204	704
Poly-3 test	P=0.204	P=0.499N	P=0.187	P=0.477
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.2%	2.1%	8.4%	4.6%
Terminal rate	0/40 (0%)	1/42 (2%)	2/41 (5%)	0/31 (0%)
First incidence (days)	709	729 (T)	204	704
Poly-3 test	P=0.275	P=0.760N	P=0.187	P=0.477
Uterus: Stromal Polyp				
Overall rate	4/50 (8%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.6%	2.1%	0.0%	0.0%
Terminal rate	4/40 (10%)	1/42 (2%)	0/41 (0%)	0/31 (0%)
First incidence (days)	729 (T)	729 (T)	—	—
Poly-3 test	P=0.019N	P=0.177N	P=0.060N	P=0.070N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	5/50 (10%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	10.7%	2.1%	0.0%	0.0%
Terminal rate	4/40 (10%)	1/42 (2%)	0/41 (0%)	0/31 (0%)
First incidence (days)	709	729 (T)	—	—
Poly-3 test	P=0.008N	P=0.101N	P=0.030N	P=0.037N
All Organs: Histiocytic Sarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	5/50 (10%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	10.7%	6.9%
Terminal rate	0/40 (0%)	0/42 (0%)	4/41 (10%)	2/31 (7%)
First incidence (days)	—	—	718	651
Poly-3 test	P=0.032	—[f]	P=0.031	P=0.108
All Organs: Malignant Lymphoma				
Overall rate	8/50 (16%)	9/50 (18%)	5/50 (10%)	10/50 (20%)
Adjusted rate	16.8%	18.9%	10.6%	22.2%
Terminal rate	5/40 (13%)	6/42 (14%)	4/41 (10%)	5/31 (16%)
First incidence (days)	414	522	645	435
Poly-3 test	P=0.352	P=0.499	P=0.286N	P=0.347

Table D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
All Organs: Benign Neoplasms				
Overall rate	15/50 (30%)	17/50 (34%)	19/50 (38%)	19/50 (38%)
Adjusted rate	32.2%	35.5%	40.6%	40.7%
Terminal rate	14/40 (35%)	15/42 (36%)	18/41 (44%)	10/31 (32%)
First incidence (days)	708	316	704	344
Poly-3 test	P=0.214	P=0.451	P=0.264	P=0.261
All Organs: Malignant Neoplasms				
Overall rate	16/50 (32%)	13/50 (26%)	19/50 (38%)	21/50 (42%)
Adjusted rate	33.1%	26.8%	38.8%	45.4%
Terminal rate	9/40 (23%)	8/42 (19%)	13/41 (32%)	10/31 (32%)
First incidence (days)	414	522	204	435
Poly-3 test	P=0.064	P=0.326N	P=0.352	P=0.154
All Organs: Benign or Malignant Neoplasms				
Overall rate	25/50 (50%)	28/50 (56%)	32/50 (64%)	30/50 (60%)
Adjusted rate	51.7%	56.6%	65.4%	62.5%
Terminal rate	18/40 (45%)	22/42 (52%)	26/41 (63%)	16/31 (52%)
First incidence (days)	414	316	204	344
Poly-3 test	P=0.145	P=0.387	P=0.119	P=0.191

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE D4
Historical Incidence of Histiocytic Sarcoma in Untreated Female $B6C3F_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	0/50
Citral	0/99
p,p′-Dichlorodiphenyl sulfone	0/50
trans-Cinnamaldehyde	2/100
2-Methylimidazole	0/50
o-Nitrotoluene	0/60
p-Nitrotoluene	0/50
Overall Historical Incidence: Feed Studies	
Total (%)	2/459 (0.4%)
Mean ± standard deviation	0.3% ± 0.8%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	18/1,258 (1.4%)
Mean ± standard deviation	1.5% ± 2.2%
Range	0%-8%

[a] Data as of April 19, 2004

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Benzophenone[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	4	2	5	6
Natural deaths	6	6	4	12
Survivors				
Terminal sacrifice	40	42	41	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(49)	(50)	(50)	(50)
Cyst		1 (2%)	1 (2%)	
Infiltration cellular, lymphoid			1 (2%)	
Inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Mineralization			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic active				1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid		3 (6%)		1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		1 (2%)	4 (8%)
Basophilic focus	3 (6%)		1 (2%)	2 (4%)
Clear cell focus	3 (6%)	2 (4%)	4 (8%)	4 (8%)
Eosinophilic focus	2 (4%)	2 (4%)	7 (14%)	7 (14%)
Hematopoietic cell proliferation	18 (36%)	11 (22%)	20 (40%)	19 (38%)
Infarct				1 (2%)
Infiltration cellular, lymphoid	36 (72%)	38 (76%)	35 (70%)	35 (70%)
Inflammation, chronic active	44 (88%)	40 (80%)	41 (82%)	36 (72%)
Mineralization			1 (2%)	
Mixed cell focus	2 (4%)	5 (10%)	3 (6%)	2 (4%)
Tension lipidosis	4 (8%)	8 (16%)	3 (6%)	7 (14%)
Bile duct, hyperplasia	1 (2%)			
Hepatocyte, autolysis				1 (2%)
Hepatocyte, mitotic alteration			1 (2%)	
Hepatocyte, multinucleated				2 (4%)
Hepatocyte, necrosis	3 (6%)	5 (10%)	4 (8%)	
Hepatocyte, vacuolization cytoplasmic	41 (82%)	43 (86%)	39 (78%)	34 (68%)
Hepatocyte, centrilobular, degeneration				1 (2%)
Hepatocyte, centrilobular, hypertrophy		29 (58%)	44 (88%)	37 (74%)
Mesentery	(6)	(9)	(11)	(9)
Fat, fibrosis	1 (17%)	2 (22%)	8 (73%)	4 (44%)
Fat, hemorrhage				1 (11%)
Fat, infiltration cellular, lymphoid			4 (36%)	
Fat, inflammation, chronic active	1 (17%)	3 (33%)	7 (64%)	3 (33%)
Fat, mineralization		1 (11%)	2 (18%)	1 (11%)
Fat, necrosis	1 (17%)	4 (44%)	9 (82%)	4 (44%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)		
Infiltration cellular, lymphoid	20 (40%)	24 (48%)	33 (66%)	18 (36%)
Acinus, atrophy	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Acinus, cytoplasmic alteration		1 (2%)		
Duct, inflammation, chronic active			1 (2%)	
Duct, pigmentation			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	27 (54%)	34 (68%)	30 (60%)	28 (56%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)			1 (2%)
Epithelium, hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Epithelium, hyperplasia, squamous	1 (2%)	1 (2%)		1 (2%)
Epithelium, ulcer	1 (2%)			1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Epithelium, glands, hyperplasia	1 (2%)			
Glands, ectasia	18 (36%)	16 (32%)	22 (44%)	10 (20%)
Glands, mineralization		3 (6%)		2 (4%)
Tooth	(21)	(29)	(24)	(20)
Malformation			1 (4%)	
Gingiva, inflammation, chronic active	21 (100%)	29 (100%)	24 (100%)	20 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization				1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy			1 (2%)	
Hyperplasia, atypical			1 (2%)	1 (2%)
Inflammation, chronic active	2 (4%)	1 (2%)	1 (2%)	
Mineralization			1 (2%)	
Necrosis	1 (2%)			
Valve, inflammation, chronic active	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Accessory adrenal cortical nodule	1 (2%)			1 (2%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hemorrhage				1 (2%)
Hyperplasia	2 (4%)			
Hypertrophy	1 (2%)	1 (2%)		
Inflammation, chronic active	1 (2%)			
Mineralization				1 (2%)
Necrosis		1 (2%)		1 (2%)
Subcapsular, hyperplasia	49 (98%)	49 (98%)	49 (98%)	49 (100%)
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia			2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)		
Parathyroid gland	(42)	(41)	(40)	(40)
Cyst			3 (8%)	1 (3%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(49)	(47)
Pars distalis, angiectasis	2 (4%)	4 (8%)	5 (10%)	3 (6%)
Pars distalis, cyst	5 (10%)	3 (6%)	2 (4%)	
Pars distalis, hyperplasia	14 (28%)	14 (28%)	13 (27%)	3 (6%)
Pars distalis, hypertrophy			1 (2%)	
Pars distalis, pigmentation			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid		1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic active		1 (2%)	2 (4%)	
C-cell, hyperplasia				1 (2%)
Follicle, cyst		1 (2%)		
Follicle, degeneration	17 (34%)	19 (38%)	12 (24%)	9 (18%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Inflammation, chronic active	8 (16%)	1 (2%)		2 (4%)
Duct, cyst	1 (2%)			
Ovary	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		
Atrophy	48 (98%)	48 (96%)	46 (92%)	45 (90%)
Cyst	22 (45%)	17 (34%)	17 (34%)	14 (28%)
Hemorrhage	2 (4%)	5 (10%)	3 (6%)	4 (8%)
Infiltration cellular, lymphoid				1 (2%)
Inflammation, chronic active	2 (4%)	1 (2%)	1 (2%)	
Mineralization		4 (8%)	2 (4%)	
Pigmentation	6 (12%)	2 (4%)	3 (6%)	1 (2%)
Uterus	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	
Inflammation, chronic active	5 (10%)	4 (8%)	2 (4%)	2 (4%)
Endometrium, hyperplasia, cystic	47 (96%)	45 (90%)	44 (88%)	43 (86%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myelofibrosis	18 (36%)	30 (60%)	20 (40%)	26 (52%)
Myeloid cell, hyperplasia	1 (2%)			
Lymph node	(8)	(12)	(11)	(15)
Lumbar, hyperplasia, lymphoid	1 (13%)	1 (8%)		2 (13%)
Mediastinal, atrophy	1 (13%)			
Mediastinal, hyperplasia, lymphoid	2 (25%)	5 (42%)	4 (36%)	4 (27%)
Mediastinal, infiltration cellular, histiocyte	1 (13%)			
Mediastinal, inflammation, chronic active	1 (13%)			
Pancreatic, hyperplasia, lymphoid			2 (18%)	1 (7%)
Pancreatic, pigmentation			1 (9%)	
Renal, hemorrhage		1 (8%)		1 (7%)
Renal, hyperplasia, lymphoid		3 (25%)		1 (7%)
Lymph node, mandibular	(50)	(50)	(50)	(49)
Hyperplasia, lymphoid	4 (8%)	20 (40%)	12 (24%)	9 (18%)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Hyperplasia, lymphoid	6 (12%)	12 (24%)	6 (12%)	4 (8%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	16 (32%)	35 (70%)	32 (64%)	27 (54%)
Hemorrhage				2 (4%)
Inflammation, chronic active				1 (2%)
Necrosis				1 (2%)
Lymphoid follicle, hyperplasia, lymphoid	24 (48%)	36 (72%)	37 (74%)	22 (44%)
Thymus	(46)	(50)	(50)	(50)
Atrophy	8 (17%)	7 (14%)	3 (6%)	13 (26%)
Cyst	23 (50%)	35 (70%)	31 (62%)	24 (48%)
Ectopic parathyroid gland	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid		8 (16%)	5 (10%)	2 (4%)
Infiltration cellular, mast cell			1 (2%)	
Inflammation, chronic active	1 (2%)			
Thymocyte, necrosis		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosis		1 (2%)		
Subcutaneous tissue, infiltration cellular, lymphoid		4 (8%)	1 (2%)	
Subcutaneous tissue, infiltration cellular, polymorphonuclear	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Maxilla, fracture				1 (2%)
Skeletal muscle	(2)	(4)	(2)	(4)
Infiltration cellular, lymphoid		3 (75%)	2 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage				2 (4%)
Infiltration cellular, lymphoid		1 (2%)	2 (4%)	
Inflammation, chronic active				1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			
Infiltration cellular, lymphoid	33 (66%)	44 (88%)	44 (88%)	35 (70%)
Inflammation, chronic active		1 (2%)	1 (2%)	
Metaplasia, osseous				1 (2%)
Pigmentation				2 (4%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Alveolus, hemorrhage	2 (4%)			3 (6%)
Alveolus, infiltration cellular, histiocyte	1 (2%)			2 (4%)
Mediastinum, inflammation, chronic active	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)			
Olfactory epithelium, metaplasia				39 (78%)
Trachea	(50)	(50)	(50)	(50)
Glands, cyst		1 (2%)		
Glands, inflammation, chronic active		1 (2%)		

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm	312 ppm	625 ppm	1,250 ppm
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	2 (4%)			
Lens, cataract			1 (2%)	
Harderian gland	(50)	(50)	(50)	(49)
Hyperplasia		3 (6%)	3 (6%)	1 (2%)
Infiltration cellular, lymphoid	29 (58%)	27 (54%)	27 (54%)	23 (47%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet			1 (2%)	1 (2%)
Atrophy	1 (2%)			
Glomerulosclerosis				1 (2%)
Hydronephrosis	2 (4%)			1 (2%)
Infarct	4 (8%)	1 (2%)	3 (6%)	3 (6%)
Infiltration cellular, lymphoid	41 (82%)	45 (90%)	43 (86%)	39 (78%)
Inflammation, chronic active	1 (2%)			
Metaplasia, osseous	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Mineralization	15 (30%)	31 (62%)	36 (72%)	49 (98%)
Nephropathy	21 (42%)	33 (66%)	31 (62%)	30 (60%)
Artery, inflammation, chronic active	1 (2%)			1 (2%)
Cortex, cyst		1 (2%)		
Renal tubule, pigmentation	1 (2%)	1 (2%)	1 (2%)	
Urinary bladder	(50)	(49)	(50)	(50)
Infiltration cellular, lymphoid	37 (74%)	38 (78%)	40 (80%)	39 (78%)
Inflammation, chronic active				1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). Benzophenone was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of benzophenone. The high dose was limited by toxicity. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by benzophenone exposure. For benzophenone, the limiting factor was toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male mice were injected intraperitoneally three times at 24-hour intervals with benzophenone dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week toxicity study (NTP, 2000), peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per exposure group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as described for PCEs in the bone marrow micronucleus test. Results of the 14-week studies were accepted without repeat tests because additional test data could not be obtained.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

Results

Benzophenone showed no evidence of mutagenicity *in vitro* or *in vivo*. Benzophenone (1 to 1,000 µg/plate) did not induce mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver metabolic activation enzymes (Table E1; Mortelmans *et al*., 1986). Intraperitoneal injections of 200 to 500 mg benzophenone/kg body weight (three injections at 24-hour intervals) did not induce micronuclei in bone marrow PCEs of male $B6C3F_1$ mice (Table E2). A small increase in the frequency of micronucleated PCEs was noted in the 400 mg/kg group, but this increase was not significant. No increases in the frequencies of micronucleated NCEs were seen in peripheral blood of male or female $B6C3F_1$ mice administered benzophenone for 14 weeks in feed over a concentration range of 1,250 to 20,000 ppm (Table E3). No significant alterations in the percentage of PCEs among total erythrocytes were noted in either micronucleus test, indicating no toxicity to the bone marrow from benzophenone treatment.

Table E1
Mutagenicity of Benzophenone in *Salmonella typhimurium*[a]

		Revertants/Plate[b]					
		–S9		+10% hamster S9		+10% rat S9	
Strain	**Dose (µg/plate)**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
TA100	0	118 ± 12.3	118 ± 11.7	111 ± 1.8	133 ± 7.2	105 ± 8.5	146 ± 4.4
	1	113 ± 7.4					
	3	107 ± 8.1	125 ± 2.2	95 ± 4.6	130 ± 3.8		
	10	110 ± 10.3	132 ± 7.7	102 ± 7.3	136 ± 4.1	90 ± 6.6	131 ± 4.3
	33	100 ± 6.4	123 ± 2.8	84 ± 3.9	128 ± 10.7	96 ± 4.2	112 ± 11.7
	100	110 ± 4.7	114 ± 9.8	78 ± 6.3	154 ± 7.0	99 ± 7.5	124 ± 1.9
	166		52 ± 7.5[c]				
	333			80 ± 4.1	117 ± 8.7	86 ± 7.0	90 ± 6.6
	1,000					50 ± 6.1[c]	35 ± 10.9[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		383 ± 14.9	297 ± 16.9	1,784 ± 26.1	2,174 ± 37.4	922 ± 112.2	1,638 ± 60.4
TA1535	0	36 ± 1.9	32 ± 2.3	11 ± 2.1	16 ± 1.8	13 ± 3.5	6 ± 1.2
	1	33 ± 2.5					
	3	37 ± 0.7	30 ± 3.2	9 ± 1.7	9 ± 1.7		
	10	31 ± 0.7	30 ± 1.2	9 ± 1.8	10 ± 2.2	11 ± 2.7	12 ± 3.0
	33	26 ± 5.2	27 ± 2.0	10 ± 2.7	10 ± 1.5	8 ± 0.3	6 ± 3.7
	100	32 ± 3.8	22 ± 5.4	7 ± 0.6	11 ± 3.0	10 ± 2.7	8 ± 3.4
	166		0 ± 0.0[c]				
	333			6 ± 1.5	8 ± 0.9	8 ± 2.7	5 ± 0.3
	1,000					4 ± 1.0	1 ± 0.9[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		395 ± 21.7	404 ± 28.2	492 ± 17.2	691 ± 15.2	211 ± 18.1	535 ± 23.0
TA1537	0	4 ± 0.9	7 ± 0.3	9 ± 0.9	7 ± 0.6	7 ± 0.3	6 ± 1.2
	1	6 ± 2.1					
	3	5 ± 0.7	5 ± 1.8	8 ± 2.3	7 ± 2.4		
	10	4 ± 0.9	7 ± 0.6	5 ± 1.2	8 ± 2.6	6 ± 1.2	5 ± 0.7
	33	6 ± 1.7	6 ± 1.2	7 ± 1.5	8 ± 2.3	6 ± 1.2	13 ± 2.0
	100	4 ± 0.3	5 ± 1.8	7 ± 1.8	8 ± 2.7	8 ± 0.6	8 ± 0.6
	166		2 ± 0.3[c]				
	333			3 ± 1.5	5 ± 1.5	7 ± 0.9	5 ± 1.5
	1,000					4 ± 1.8	3 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		186 ± 19.4	443 ± 51.6	408 ± 11.7	125 ± 7.3	132 ± 20.3	509 ± 19.9

Table E1
Mutagenicity of Benzophenone in *Salmonella typhimurium*

		Revertants/Plate					
	Dose	–S9		+10% hamster S9		+10% rat S9	
Strain	(µg/plate)	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA98	0	98 ± 78.0	13 ± 2.6	36 ± 2.5	32 ± 0.0	23 ± 2.3	31 ± 3.3
	1	19 ± 0.3					
	3	19 ± 3.8	13 ± 4.8	34 ± 3.3	39 ± 1.5		
	10	19 ± 1.9	10 ± 2.4	30 ± 2.8	34 ± 4.5	33 ± 1.3	30 ± 0.7
	33	20 ± 2.3	17 ± 0.9	31 ± 2.7	36 ± 4.2	21 ± 2.4	27 ± 7.5
	100	14 ± 1.9	12 ± 2.2	30 ± 3.2	33 ± 4.4	28 ± 5.5	27 ± 1.2
	166		0 ± 0.0[c]				
	333			23 ± 1.0	15 ± 1.2	25 ± 4.5	14 ± 3.2
	1,000					15 ± 2.1	6 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		475 ± 5.4	431 ± 38.4	1,629 ± 25.7	1,901 ± 39.4	867 ± 11.9	1,221 ± 9.9

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al*. (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Administered Benzophenone by Intraperitoneal Injection[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Corn oil[d]		5	1.2 ± 0.41		38.2 ± 2.14
Benzophenone	200	5	1.5 ± 0.32	0.2817	47.9 ± 4.57
	300	5	1.5 ± 0.45	0.2817	39.8 ± 5.37
	400	5	2.2 ± 0.72	0.0430	48.7 ± 3.40
	500	5	1.7 ± 0.37	0.1764	42.0 ± 5.44
			P=0.085[e]		
Cyclophosphamide[f]	25	5	22.4 ± 1.85	0.0000	36.0 ± 2.80

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error.
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at P=0.006; positive control value is significant at P≤0.05 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs was tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
[f] Positive control

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Benzophenone in Feed for 14 Weeks[a]

	Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
NTP-2000 Feed[d]		5	0.90 ± 0.48		1.92 ± 0.19
Benzophenone	1,250	5	1.40 ± 0.19	0.1484	2.20 ± 0.23
	2,500	5	0.40 ± 0.19	0.9173	2.20 ± 0.29
	5,000	5	1.30 ± 0.34	0.1968	2.08 ± 0.23
	10,000	5	0.50 ± 0.16	0.8576	2.24 ± 0.21
			P=0.866[e]		
Female					
NTP-2000 Feed		5	0.60 ± 0.24		2.06 ± 0.21
Benzophenone	1,250	5	0.80 ± 0.44	0.2964	2.02 ± 0.23
	2,500	5	0.80 ± 0.34	0.2964	2.00 ± 0.27
	5,000	5	0.30 ± 0.20	0.8414	1.88 ± 0.10
	10,000	5	0.80 ± 0.12	0.2964	2.04 ± 0.18
	20,000	5	0.60 ± 0.29	0.5000	2.26 ± 0.36
			P=0.564		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte, PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control; significant at P=0.006 (males) or P=0.005 (females) (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs was tested by the one-tailed trend test; significant at $P \leq 0.025$ (ILS, 1990)

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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TABLE F2 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone 232

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF BENZOPHENONE

Benzophenone was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (10803KG) that was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the benzophenone studies are on file at the National Institute of Environmental Health Sciences.

Lot 10803KG, a white crystal with a geranium- or rose-like odor, was identified as benzophenone by melting point determination; infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; x-ray crystallography; and low and high resolution mass spectrometry by the analytical chemistry laboratory, and the study laboratory confirmed the identity by infrared spectroscopy. The melting point was within acceptable limits of the theoretical values for benzophenone. All spectra were consistent with the structure and matched reference spectra (NIST Standard Reference Database; *Sadtler*, 1979; *Aldrich*, 1981, 1983, 1985) of benzophenone. Infrared, nuclear magnetic resonance, and mass spectra are presented in Figures F1, F2, and F3.

The moisture content of lot 10803KG was determined at the analytical chemistry laboratory by Karl Fischer titration. The purity of lot 10803KG was determined by capillary gas chromatography using a Hewlett-Packard (Palo Alto, CA) gas chromatograph, a J&W (Folsom, CA) SE-30 (low polarity) or DB-17 (high polarity) (30 m × 0.25 mm ID, 0.25-µm film thickness) column, flame ionization detection, with helium (SE-30) or nitrogen (DB-17) as a carrier gas and a flow rate of 1 mL/minute. The study laboratory confirmed the purity of lot 10803KG by capillary gas chromatography using a similar system (low polarity column).

Karl Fischer titration indicated a moisture content of 0.426% (3.7% RSD). Gas chromatography by the analytical chemistry laboratory indicated one major peak that accounted for 100% of the total peak area with both columns. Major peak comparisons with a frozen reference sample of the same lot by the study laboratory using gas chromatography indicated a purity of 100.6% ± 0.5%. The overall purity of lot 10803KG was determined to be 99% or greater.

To ensure stability, the bulk chemical was stored at approximately 25° C in amber glass bottles sealed with Teflon®-lined lids. Periodic purity reanalyses of the bulk chemical were performed by the study laboratory using gas chromatography similar to the low polarity system described above. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared at least once a month by mixing benzophenone with feed (Table F1). The benzophenone was ground with a mortar and pestle then sieved through a 40-mesh screen. A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender for approximately 15 minutes. Formulations were stored in five-gallon white plastic buckets lined with plastic can liners at approximately 5° C for up to 35 days.

Homogeneity studies of the 312 and 1,250 ppm dose formulations were performed by the analytical chemistry laboratory with a Waters (Milford, MA) high performance liquid chromatography (HPLC) system, a reverse phase DuPont Zorbax C8 (25 cm × 4.6 mm ID) column, a Waters 490 UV detector (254 nm), a mobile phase of 35:65 acetonitrile:water, and a flow rate of 2.0 mL/minute. Stability studies of the 312 ppm dose formulation were

also performed by the analytical chemistry laboratory using the same HPLC system. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in sealed glass containers protected from light up to 5° C. Under simulated animal room dosing conditions, there were small losses after 3 days and significant losses after 7 days. To confirm these findings, the study laboratory performed simulated animal room stability studies of the 312 and 1,250 ppm dose formulations using an HPLC system similar to that described. Results indicated that twice weekly feeder changes should be acceptable, though there would be a slight decrease in concentration of benzophenone.

Periodic analyses of the dose formulations of benzophenone were conducted by the study laboratory using an HPLC system similar to that described above. During the 2-year studies, the dose formulations were analyzed at least every 11 weeks (Table F2). Of the dose formulations analyzed and used, all 63 for rats and all 60 for mice were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; 11 of 24 animal room samples analyzed for rats and 8 of 48 animal room samples analyzed for mice were within 10% of target concentrations. The decline in benzophenone concentration was not anticipated from animal room simulations with air and light performed during pre-study developmental work. After the decline was observed, additional experiments were performed in which benzophenone feed formulations were spiked with rodent urine and feces. Declines were approximately 5% with light and air and increased to approximately 15% in the presence of urine and feces. Contamination occurs when the animals crawl into or onto the feeders. The problem increases in cages where multiple animals are housed and are worst with female mice. Feeders were changed twice per week during the study to minimize the problem, but some contamination was unavoidable.

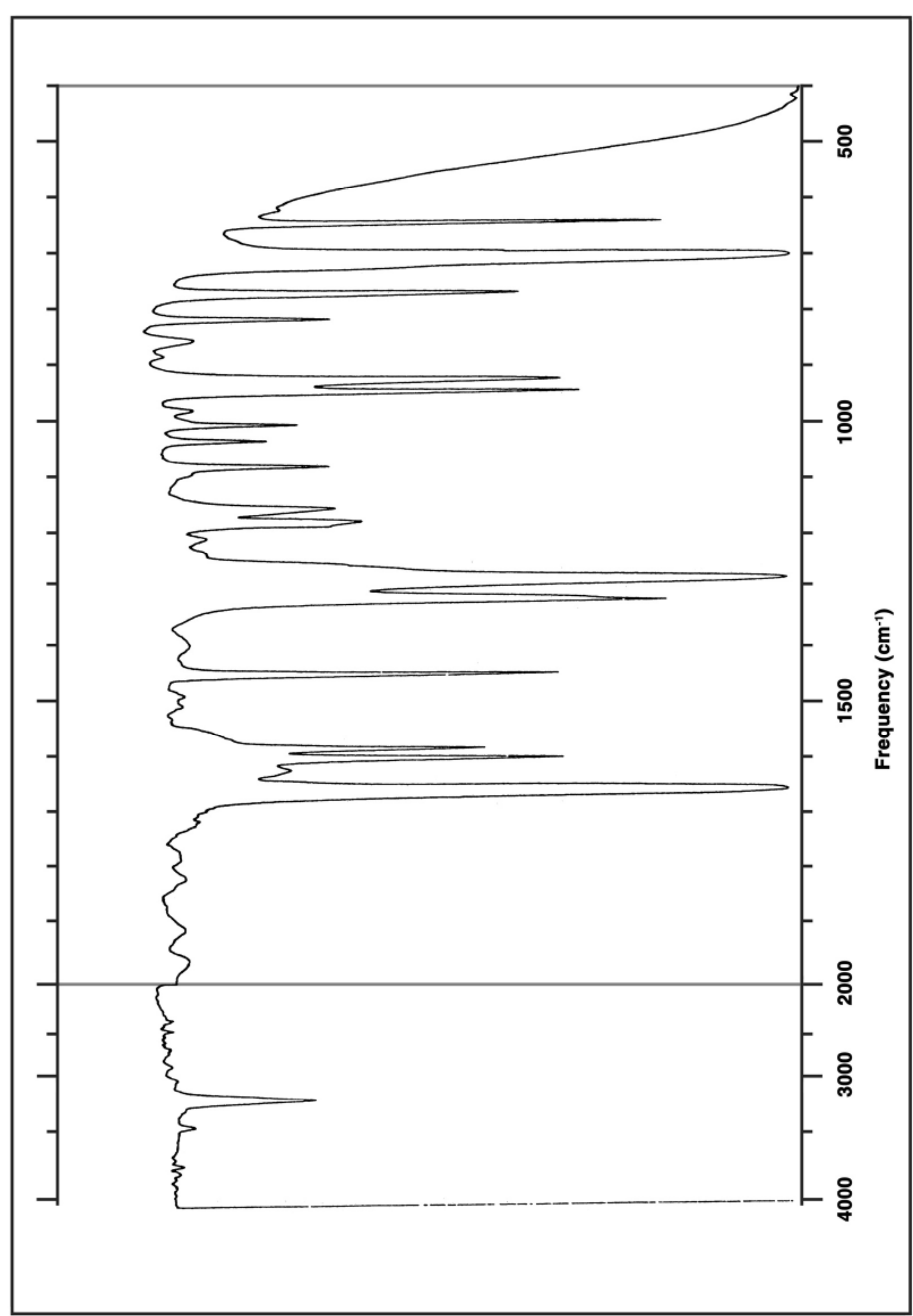

FIGURE F1
Infrared Absorption Spectrum of Benzophenone

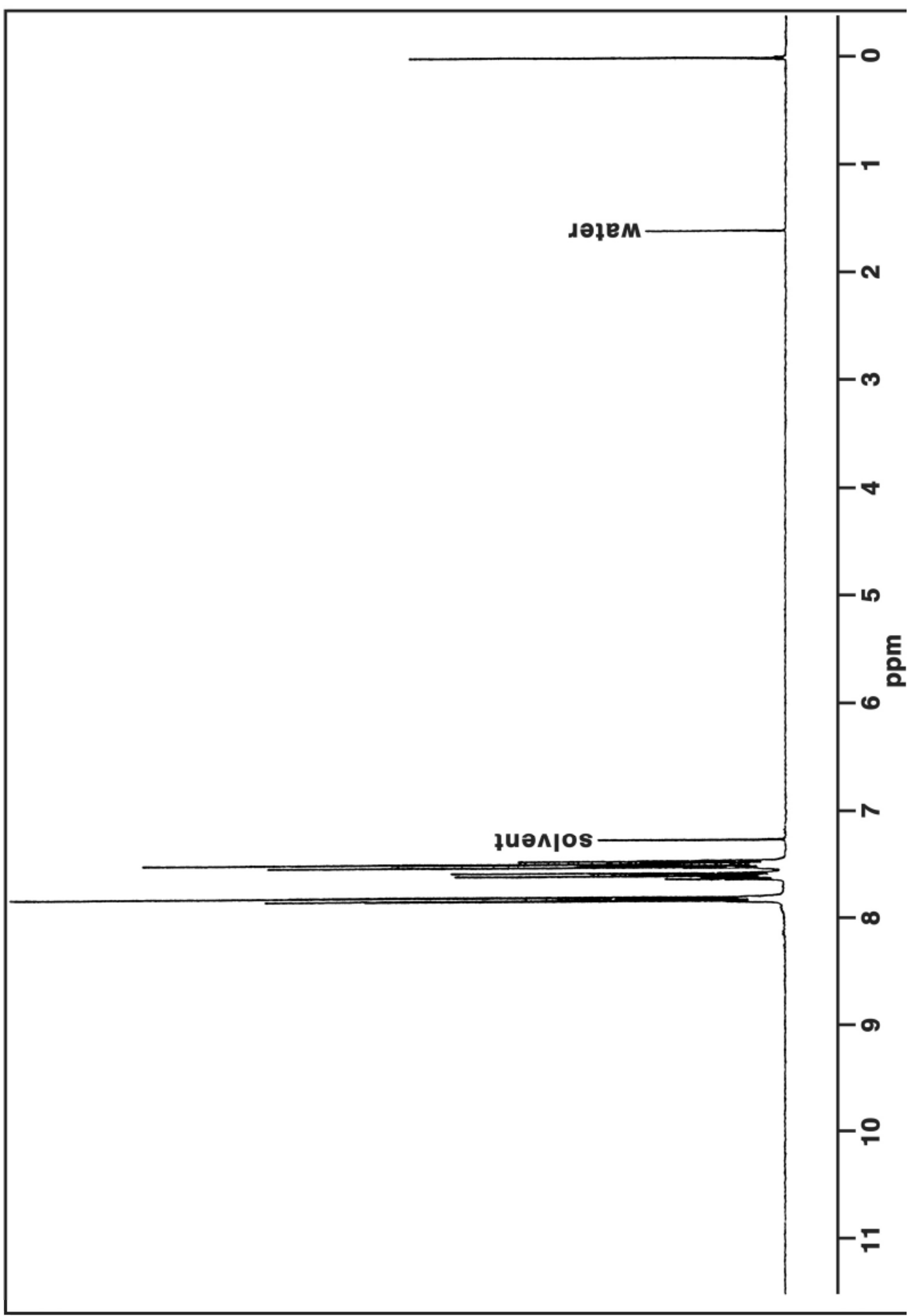

FIGURE F2
Nuclear Magnetic Resonance Spectrum of Benzophenone

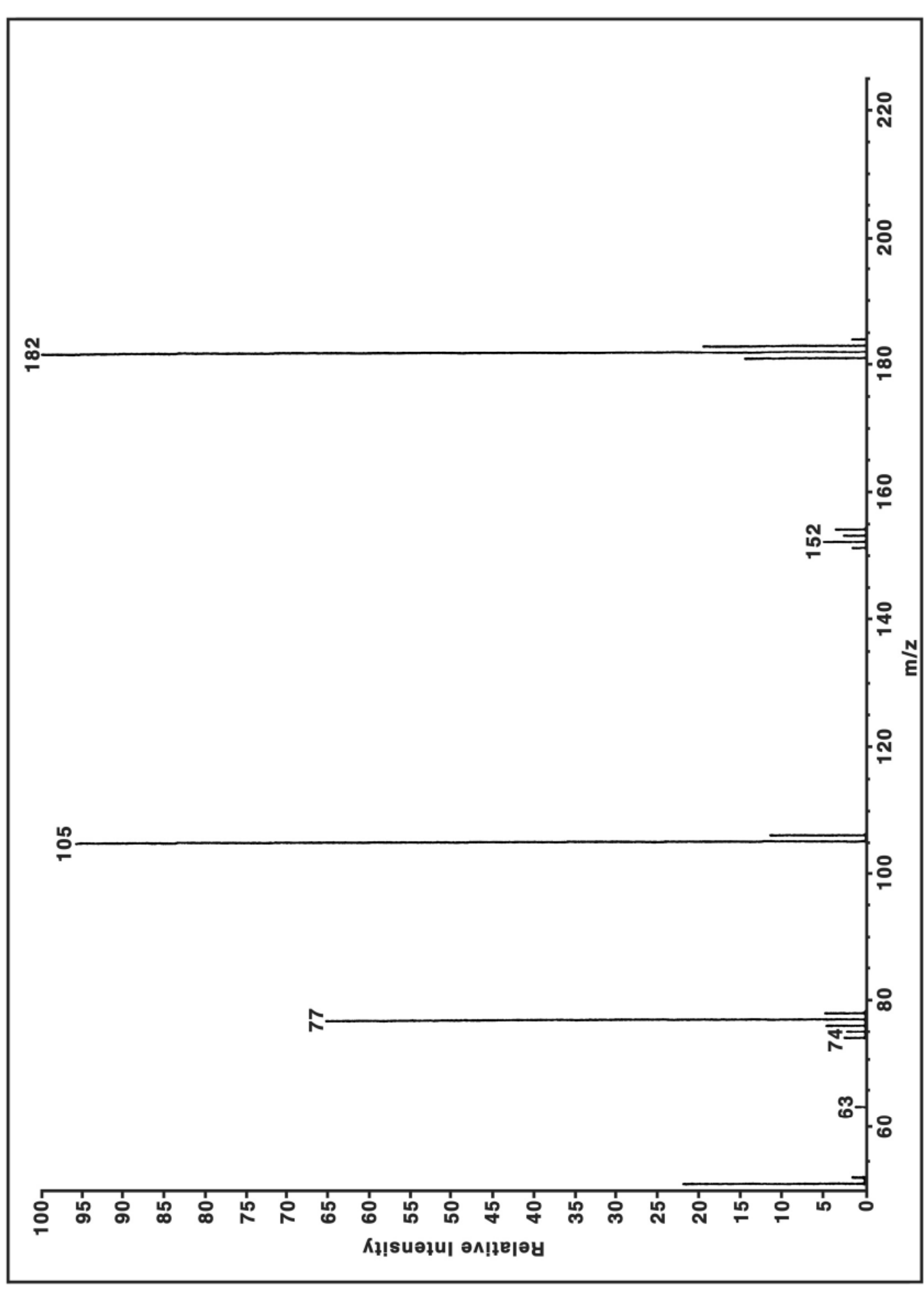

FIGURE F3
Low Resolution Mass Spectrum of Benzophenone

TABLE F1
Preparation and Storage of Dose Formulations in the Feed Studies of Benzophenone

Preparation
Benzophenone was ground with a mortar and pestle then sieved through a 40-mesh screen. A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender for approximately 15 minutes. Dose formulations were prepared at least once a month.

Chemical Lot Number
10803KG

Maximum Storage Time
35 days

Storage Conditions
Stored in 5-gallon white plastic buckets lined with plastic can liners at approximately 5° C

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Table F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
August 4, 1999	August 6-7, 1999	312	310.8	0
		625	566.4	–9
		1,250	1,197	–4
August 18, 1999	August 20-21, 1999	312	275.7[b]	–12
		312	302.1	–3
		625	611.5	–2
		625	618.3	–1
		1,250	1,234	–1
		1,250	1,197	–4
	September 23-24, 1999[c]	312	274.9	–12
		625	527.0	–16
		625	569.5	–9
		1,250	1,121	–10
		1,250	1,091	–13
August 27, 1999	August 27, 1999	312	309.0	–1
	September 23-24, 1999[c]	312	274.5	–12
November 4, 1999	November 5-6, 1999	312	314.6	+1
		312	319.5	+2
		625	642.0	+3
		625	607.2	–3
		1,250	1,258	+1
		1,250	1,233	–1
January 20, 2000	January 26, 2000	312	306.5	–2
		312	302.4	–3
		625	608.6	–3
		625	605.3	–3
		1,250	1,232	–1
		1,250	1,241	–1
April 6, 2000	April 10-11, 2000	312	282.4	–9
		312	320.8	+3
		625	601.6	–4
		625	600.9	–4
		1,250	1,193	–5
		1,250	1,181	–6
	May 18-20, 2000[d]	312	290.1	–7
		312	283.7	–9
		625	572.4	–8
		625	785.9	+26
		1,250	1,144	–8
		1,250	1,189	–5

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
June 22, 2000	June 26-27, 2000	312	298.4	–4
		312	305.1	–2
		625	620.7	–1
		625	607.7	–3
		1,250	1,283	+3
		1,250	1,242	–1
September 7, 2000	September 11, 2000	312	307.7	–1
		312	313.9	+1
		625	608.0	–3
		625	621.7	–1
		1,250	1,194	–4
		1,250	1,230	–2
November 21, 2000	November 28-29, 2000	312	315.4	+1
		312	295.6	–5
		625	600.9	–4
		625	620.8	–1
		1,250	1,200	–4
		1,250	1,246	0
	December 28-30, 2000[c]	312	283.4	–9
		312	274.0	–12
		625	572.8	–8
		625	568.6	–9
		1,250	1,108	–11
		1,250	1,103	–12
	December 28-30, 2000[e]	312	309.4	–1
		312	306.8	–2
		625	618.5	–1
		625	607.3	–3
		1,250	1,227	–2
		1,250	1,238	–1
February 8, 2001	February 13-14, 2001	312	304.8	–2
		312	302.2	–3
		625	615.2	–2
		625	620.0	–1
		1,250	1,253	0
		1,250	1,206	–4
April 26, 2001	April 30-May 1, 2001	312	310.1	–1
		312	303.8	–3
		625	622.9	0
		625	630.7	+1
		1,250	1,268	+1
		1,250	1,284	+3

Table F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
July 12, 2001	July 17, 2001	312	296.2	–5
		312	296.6	–5
		625	607.7	–3
		625	608.7	–3
		1,250	1,224	–2
		1,250	1,233	–1
	August 17-18, 2001[c]	312	263.1	–16
		312	261.0	–16
		625	534.6	–14
		625	543.1	–13
		1,250	1,146	–8
		1,250	1,261	+1
	August 17-18, 2001[e]	312	306.1	–2
		312	298.5	–4
		625	624.7	0
		625	619.2	–1
		1,250	1,164	–7
		1,250	1,168	–7
Mice				
August 18, 1999	August 20-21, 1999	312	275.7[b]	–12
		312	302.1	–3
		625	611.5	–2
		625	618.3	–1
		1,250	1,234	–1
		1,250	1,197	–4
	September 23-24, 1999[c]	312	274.3	–12
		625	512.4	–18
		625	559.1	–11
		1,250	1,053	–16
		1,250	1,033	–17
	September 23-24, 1999[d]	312	266.5	–15
		625	560.2	–10
		625	540.2	–14
		1,250	1,090	–13
		1,250	1,096	–12
August 27, 1999	August 27, 1999	312	309.0	–1
	September 23-24, 1999[c]	312	253.4	–19
	September 23-24, 1999[d]	312	264.8	–15

Table F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
November 4, 1999	November 5-6, 1999	312	314.6	+1
		312	319.5	+2
		625	642.0	+3
		625	607.2	–3
		1,250	1,258	+1
		1,250	1,233	–1
January 20, 2000	January 26, 2000	312	306.5	–2
		312	302.4	–3
		625	608.6	–3
		625	605.3	–3
		1,250	1,232	–1
		1,250	1,241	–1
April 6, 2000	April 10-11, 2000	312	282.4	–9
		312	320.8	+3
		625	601.6	–4
		625	600.9	–4
		1,250	1,193	–5
		1,250	1,181	–6
	May 18-20, 2000[c]	312	277.2	–11
		312	260.7	–16
		625	554.6	–11
		625	525.1	–16
		1,250	1,096	–12
		1,250	1,086	–13
	May 18-20, 2000[d]	312	285.3	–9
		312	287.4	–8
		625	570.1	–9
		625	572.2	–8
		1,250	1,135	–9
		1,250	1,143	–9
June 22, 2000	June 26-27, 2000	312	298.4	–4
		312	305.1	–2
		625	620.7	–1
		625	607.7	–3
		1,250	1,283	+3
		1,250	1,242	–1
September 7, 2000	September 11, 2000	312	307.7	–1
		312	313.9	+1
		625	608.0	–3
		625	621.7	–1
		1,250	1,194	–4
		1,250	1,230	–2

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
November 21, 2000	November 28-29, 2000	312	315.4	+1
		312	295.6	–5
		625	600.9	–4
		625	620.8	–1
		1,250	1,200	–4
		1,250	1,246	0
	December 28-30, 2000[c]	312	244.2	–22
		312	244.4	–22
		625	519.7	–17
		625	458.3	–27
		1,250	981.1	–22
		1,250	879.8	–30
	December 28-30, 2000[d]	312	276.2	–11
		312	280.9	–10
		625	522.0	–16
		625	534.4	–14
		1,250	1,117	–11
		1,250	1,102	–12
	December 28-30, 2000[e]	312	309.4	–1
		312	306.8	–2
		625	618.5	–1
		625	607.3	–3
		1,250	1,227	–2
		1,250	1,238	–1
February 8, 2001	February 13-14, 2001	312	304.8	–2
		312	302.2	–3
		625	615.2	–2
		625	620.0	–1
		1,250	1,253	0
		1,250	1,206	–4
April 26, 2001	April 30-May 1, 2001	312	310.1	–1
		312	303.8	–3
		625	622.9	0
		625	630.7	+1
		1,250	1,268	+1
		1,250	1,284	+3

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Benzophenone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
July 12, 2001	July 17, 2001	312	296.2	–5
		312	296.6	–5
		625	607.7	–3
		625	608.7	–3
		1,250	1,224	–2
		1,250	1,233	–1
	August 17-18, 2001[c]	312	213.0	–32
		312	216.8	–31
		625	425.5	–32
		625	422.2	–32
		1,250	842.1	–33
		1,250	856.6	–31
	August 17-18, 2001[d]	312	264.3	–15
		312	257.1	–18
		625	522.4	–16
		625	541.2	–13
		1,250	1,064	–15
		1,250	1,088	–13
	August 17-18, 2001[e]	312	306.1	–2
		312	298.5	–4
		625	624.7	0
		625	619.2	–1
		1,250	1,164	–7
		1,250	1,168	–7

[a] Results of duplicate analysis
[b] Discarded
[c] Animal room samples; females
[d] Animal room samples; males
[e] Animal room samples; dose formulation storage containers

APPENDIX G
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF BENZOPHENONE

TABLE G1 Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Benzophenone 240
TABLE G2 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Benzophenone 241
TABLE G3 Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Benzophenone 242
TABLE G4 Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Benzophenone 243

Table G1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Benzophenone

	0 ppm		312 ppm			625 ppm			1,250 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	17.6	108	17.9	109	51	17.0	109	98	14.6	110	166
2	18.2	144	19.0	148	40	19.0	146	81	21.0	142	185
6	19.5	266	19.4	268	23	20.0	266	47	19.9	264	94
10	18.8	319	18.3	318	18	18.7	319	37	18.8	316	74
14	16.9	352	17.1	352	15	16.5	350	29	18.8	353	67
18	17.2	379	17.4	380	14	17.6	372	30	17.3	372	58
22	18.9	394	18.4	392	15	19.0	388	31	18.5	388	60
26	19.1	416	19.1	413	14	17.8	407	27	17.1	407	53
30	18.3	426	17.8	427	13	18.1	424	27	19.8	423	59
34	17.5	442	17.2	443	12	16.7	438	24	18.3	436	53
38	17.6	453	18.0	455	12	18.7	449	26	18.3	447	51
41	18.5	457	18.6	460	13	18.7	457	26	18.4	450	51
46	18.0	472	17.9	463	12	17.5	468	23	18.9	462	51
50	17.6	478	17.7	476	12	18.2	473	24	17.7	465	48
54	20.3	482	20.0	479	13	19.9	479	26	19.3	466	52
58	17.5	489	17.7	488	11	16.9	486	22	16.2	471	43
62	17.8	494	17.2	493	11	17.6	491	22	17.7	472	47
66	17.1	499	17.3	495	11	17.3	497	22	17.4	472	46
70	16.3	492	17.1	494	11	17.5	485	23	16.9	458	46
74	17.3	498	16.7	491	11	17.2	490	22	16.1	449	45
78	18.5	499	18.6	493	12	17.2	484	22	14.7	436	42
82	18.6	500	18.5	496	12	17.4	479	23	15.8	424	47
86	16.7	502	16.0	494	10	16.7	481	22	14.8	411	45
90	17.1	497	16.8	486	11	16.9	471	22	15.9	384	52
94	15.7	487	16.6	482	11	16.3	463	22	14.4	379	48
98	16.6	478	16.2	478	11	16.0	444	23	14.9	343	54
102	16.5	477	16.3	460	11	15.2	422	23	15.1	306	62
Mean for weeks											
1-13	18.5	209	18.6	211	33	18.7	210	66	18.6	208	130
14-52	18.0	427	17.9	426	13	17.9	423	27	18.3	420	55
53-102	17.4	492	17.3	487	11	17.1	475	23	16.1	421	48

[a] Grams of feed consumed per animal per day
[b] Milligrams of benzophenone consumed per kilogram body weight per day

TABLE G2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Benzophenone

	0 ppm		312 ppm			625 ppm			1,250 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	12.9	101	13.0	101	40	12.9	100	81	11.5	100	144
2	13.2	120	13.1	120	34	13.0	119	68	13.8	118	146
6	12.4	169	12.2	169	23	12.4	165	47	11.3	161	87
10	12.5	189	11.8	186	20	12.0	184	41	11.5	179	80
14	10.9	202	10.0	195	16	10.5	191	34	10.1	187	67
18	11.1	214	10.2	205	15	10.4	201	33	10.0	193	65
22	12.2	219	11.3	210	17	10.8	206	33	10.4	198	65
26	10.8	224	10.6	214	16	10.2	210	30	9.6	202	59
30	10.9	234	10.9	222	15	10.0	216	29	10.0	210	60
34	10.9	241	10.3	227	14	10.1	221	29	9.6	213	57
38	12.2	249	11.4	235	15	11.3	228	31	10.5	219	60
41	11.2	254	11.9	240	15	11.4	233	30	10.9	222	61
46	12.2	264	11.2	249	14	11.2	237	30	10.9	222	61
50	12.6	275	12.1	258	15	11.6	245	30	10.6	231	57
54	12.6	283	12.8	268	15	12.3	253	30	11.3	236	60
58	11.8	290	11.8	273	13	11.4	258	28	10.1	239	53
62	13.2	302	13.5	290	15	13.1	272	30	11.7	250	58
66	13.2	313	12.6	298	13	11.7	279	26	11.3	256	55
70	12.7	318	12.5	306	13	12.4	287	27	11.7	265	55
74	13.4	328	12.6	313	12	12.1	294	26	11.8	272	54
78	12.8	331	12.7	317	13	12.4	300	26	11.8	276	54
82	13.6	338	13.3	324	13	13.2	309	27	12.1	284	53
86	12.8	344	12.3	328	12	12.4	313	25	11.8	287	51
90	13.3	347	12.9	332	12	12.1	312	24	11.2	287	49
94	13.9	346	13.3	333	13	13.0	315	26	11.7	289	51
98	13.1	349	12.8	337	12	12.0	314	24	11.2	291	48
102	12.6	345	13.3	337	12	12.0	313	24	13.0	295	55
Mean for weeks											
1-13	12.7	145	12.5	144	29	12.6	142	59	12.0	139	115
14-52	11.5	237	11.0	226	15	10.7	219	31	10.3	210	61
53-102	13.0	326	12.8	312	13	12.3	294	26	11.6	271	54

[a] Grams of feed consumed per animal per day
[b] Milligrams of benzophenone consumed per kilogram body weight per day

TABLE G3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Benzophenone

	0 ppm		312 ppm			625 ppm			1,250 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	5.0	23.3	5.6	23.1	76	5.2	22.9	143	4.9	23.3	261
2	5.2	23.9	6.5	24.2	84	6.0	23.9	156	5.3	24.0	277
3	4.7	25.2	5.2	24.6	66	5.3	25.0	134	4.9	25.1	245
6	4.9	28.5	5.1	28.7	55	4.9	28.5	107	4.9	28.3	217
10	4.9	32.0	5.2	31.8	52	5.0	31.7	99	5.1	31.3	204
14	5.2	34.9	5.0	34.4	46	5.1	35.3	91	5.0	33.9	183
18	4.8	37.4	5.1	37.5	42	5.0	36.9	85	5.2	35.9	180
22	4.8	38.9	4.8	38.0	39	4.8	38.5	77	5.0	38.3	162
26	5.0	40.9	5.4	40.2	42	5.0	41.0	76	5.1	40.6	158
30	4.5	41.6	4.6	41.3	35	4.4	41.6	66	4.4	41.4	133
34	5.1	43.1	5.2	42.9	38	4.8	41.6	73	5.1	41.9	151
37	5.1	43.6	5.2	43.3	37	5.0	42.9	73	5.1	42.2	151
42	4.8	44.6	4.9	43.9	35	4.9	44.1	69	4.7	43.2	137
46	4.9	45.0	4.7	44.4	33	4.9	44.0	69	4.8	43.6	137
50	4.8	46.0	5.2	45.8	35	5.0	45.0	70	4.9	45.0	137
54	4.8	45.7	5.1	44.9	36	5.0	44.1	71	4.8	44.6	134
58	5.3	45.9	5.4	44.7	37	5.2	44.4	74	5.2	45.6	142
62	5.0	45.9	5.2	44.7	36	4.9	45.4	67	5.1	45.8	138
66	4.9	44.1	5.0	44.8	35	5.0	45.4	69	4.8	45.3	133
70	4.8	43.8	4.9	44.3	34	4.9	44.8	69	4.5	44.7	126
74	4.9	44.0	5.2	45.0	36	5.1	44.9	71	5.1	45.2	141
78	5.1	43.1	5.3	44.1	38	5.1	44.5	71	4.9	43.8	140
84	5.2	40.7	5.3	40.8	41	5.3	40.9	81	5.4	40.4	168
86	5.1	41.7	5.2	41.5	39	5.2	42.2	76	5.2	41.3	156
90	5.0	41.9	5.2	41.7	39	4.9	41.7	74	4.9	41.3	149
94	5.2	41.4	5.1	40.8	39	5.0	41.0	76	5.1	39.8	161
98	4.5	41.2	4.9	39.8	38	4.7	40.3	73	4.9	39.5	155
102	4.6	40.3	4.7	39.5	37	4.6	40.3	71	4.4	38.4	142
Mean for weeks											
1-13	4.9	26.6	5.5	26.5	67	5.3	26.4	128	5.0	26.4	241
14-52	4.9	41.6	5.0	41.2	38	4.9	41.1	75	4.9	40.6	153
53-102	4.9	43.1	5.1	42.8	37	5.0	43.1	73	4.9	42.8	145

[a] Grams of feed consumed per animal per day
[b] Milligrams of benzophenone consumed per kilogram body weight per day

Table G4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Benzophenone

	0 ppm		312 ppm			625 ppm			1,250 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.7	18.8	3.8	18.6	63	3.6	18.6	122	3.5	18.5	233
2	3.8	19.1	3.7	19.5	60	3.8	18.6	128	3.6	19.5	228
6	3.6	22.5	3.5	22.4	48	3.7	22.7	101	3.7	22.8	204
10	3.8	25.0	3.6	25.7	44	3.7	24.0	96	4.0	25.7	197
14	3.8	27.2	4.0	28.4	44	3.9	26.9	92	4.0	28.0	181
18	3.6	29.5	3.9	30.8	39	3.8	30.3	79	4.0	30.3	164
22	4.3	32.8	4.9	33.7	45	4.6	33.3	86	4.5	32.2	173
26	4.1	33.2	4.0	35.1	36	4.3	33.6	80	4.0	33.0	150
30	3.8	35.3	4.2	36.6	36	3.9	35.5	69	3.7	34.4	136
34	3.8	36.5	4.1	38.0	34	3.8	36.9	64	3.8	35.1	134
37	3.8	38.3	4.2	39.7	33	3.9	38.0	64	3.9	36.2	133
42	3.8	39.8	4.1	40.6	31	3.7	39.3	59	3.7	37.2	125
46	3.6	41.2	3.7	42.0	27	3.5	40.0	54	3.4	37.2	114
50	4.0	41.8	3.9	42.9	28	3.8	41.1	58	3.9	37.6	128
54	3.6	43.3	3.8	42.8	28	3.6	41.4	55	4.1	37.2	136
58	4.1	43.7	4.2	43.5	30	3.8	40.2	58	4.1	37.8	135
62	3.9	44.6	3.9	43.5	28	3.9	41.4	59	3.8	37.9	125
66	4.1	44.4	3.8	43.1	27	3.9	41.4	58	3.8	38.5	125
70	4.0	44.4	4.0	43.3	29	4.0	41.7	59	4.0	38.9	130
74	4.2	45.6	4.5	44.2	32	4.4	42.2	65	4.4	39.4	140
78	4.9	46.2	5.0	43.8	36	5.1	41.5	77	4.8	39.5	151
84	3.8	43.5	4.1	41.4	31	4.1	39.5	65	4.5	37.4	149
86	4.3	43.9	4.2	42.0	32	4.7	41.0	71	4.4	37.8	146
90	4.2	44.4	4.0	41.1	31	4.0	40.6	61	4.1	38.4	133
94	3.9	44.4	4.1	41.1	31	4.1	41.1	63	4.2	38.6	136
98	3.8	45.1	4.0	41.3	30	4.2	41.4	63	4.0	38.4	131
102	3.5	43.5	3.8	40.4	29	3.9	40.2	60	3.8	37.2	126
Mean for weeks											
1-13	3.7	21.3	3.7	21.6	54	3.7	21.0	112	3.7	21.6	215
14-52	3.8	35.6	4.1	36.8	35	3.9	35.5	70	3.9	34.1	144
53-102	4.0	44.4	4.1	42.4	30	4.1	41.1	63	4.2	38.2	136

[a] Grams of feed consumed per animal per day
[b] Milligrams of benzophenone consumed per kilogram body weight per day

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

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Table H1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

Table H2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

Table H3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.3 ± 0.59	13.4 – 15.5	23
Crude fat (% by weight)	8.3 ± 0.34	7.4 – 9.1	23
Crude fiber (% by weight)	9.0 ± 0.55	7.9 – 10.2	23
Ash (% by weight)	5.2 ± 0.30	4.7 – 6.0	23
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.053	0.670 – 0.850	12
Cystine	0.223 ± 0.027	0.150 – 0.250	12
Glycine	0.702 ± 0.043	0.620 – 0.750	12
Histidine	0.343 ± 0.023	0.310 – 0.390	12
Isoleucine	0.534 ± 0.041	0.430 – 0.590	12
Leucine	1.078 ± 0.059	0.960 – 1.140	12
Lysine	0.729 ± 0.065	0.620 – 0.830	12
Methionine	0.396 ± 0.053	0.260 – 0.460	12
Phenylalanine	0.611 ± 0.038	0.540 – 0.660	12
Threonine	0.492 ± 0.045	0.430 – 0.590	12
Tryptophan	0.129 ± 0.016	0.110 – 0.160	12
Tyrosine	0.378 ± 0.054	0.280 – 0.460	12
Valine	0.658 ± 0.049	0.550 – 0.710	12
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.278	3.49 – 4.54	12
Linolenic	0.30 ± 0.038	0.21 – 0.35	12
Vitamins			
Vitamin A (IU/kg)	5,150 ± 1,183	2,960 – 7,560	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.3 ± 17.06	52.0 – 110.0	12
Thiamine (ppm)[b]	9.1 ± 3.41	6.3 – 23.0	23
Riboflavin (ppm)	6.4 ± 2.11	4.20 – 11.20	12
Niacin (ppm)	78.6 ± 10.86	66.4 – 98.2	12
Pantothenic acid (ppm)	23.1 ± 3.61	17.4 – 29.1	12
Pyridoxine (ppm)[b]	8.88 ± 2.05	6.4 – 12.4	12
Folic acid (ppm)	1.84 ± 0.56	1.26 – 3.27	12
Biotin (ppm)	0.337 ± 0.13	0.225 – 0.704	12
Vitamin B_{12} (ppb)	64.8 ± 50.9	18.3 – 174.0	12
Choline (ppm)[b]	3,094 ± 292	2,700 – 3,790	12
Minerals			
Calcium (%)	1.070 ± 0.070	0.953 – 1.220	23
Phosphorus (%)	0.609 ± 0.042	0.560 – 0.737	23
Potassium (%)	0.668 ± 0.023	0.627 – 0.694	12
Chloride (%)	0.368 ± 0.033	0.300 – 0.423	12
Sodium (%)	0.189 ± 0.016	0.160 – 0.212	12
Magnesium (%)	0.200 ± 0.009	0.185 – 0.217	12
Sulfur (%)	0.176 ± 0.026	0.116 – 0.209	12
Iron (ppm)	177 ± 46.2	135 – 311	12
Manganese (ppm)	53.4 ± 6.42	42.1 – 63.1	12
Zinc (ppm)	52.5 ± 6.95	43.3 – 66.0	12
Copper (ppm)	6.64 ± 1.283	5.08 – 9.92	12
Iodine (ppm)	0.535 ± 0.242	0.233 – 0.972	12
Chromium (ppm)	0.545 ± 0.125	0.330 – 0.751	12
Cobalt (ppm)	0.23 ± 0.041	0.20 – 0.30	12

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE H4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.21 ± 0.063	0.10 – 0.38	23
Cadmium (ppm)	0.05 ± 0.008	0.04 – 0.07	23
Lead (ppm)	0.08 ± 0.048	0.05 – 0.29	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.22 ± 0.052	0.15 – 0.36	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	10.9 ± 2.89	7.86 – 21.1	23
Nitrite nitrogen (ppm)[c]	<0.61		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	14.0 ± 15	10.0 – 80.0	23
Coliform (MPN/g)	2.1 ± 1.8	0.0 – 3.6	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	5.3 ± 1.29	2.3 – 7.8	23
N-Nitrosodimethylamine (ppb)[e]	2.3 ± 0.78	1.0 – 3.9	23
N-Nitrosopyrrolidine (ppb)[e]	3.0 ± 0.92	1.3 – 5.3	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.180 ± 0.103	0.039 – 0.536	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.214 ± 0.147	0.020 – 0.515	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX I
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from randomly selected rats and mice during the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
ELISA	
Mycoplasma arthriditis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	1, 6, 12, and 18 months, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
ELISA	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM (epizootic diarrhea of infant mice)	1, 6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis virus)	1, 6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	1, 6, 12, and 18 months, study termination
MHV (mouse hepatitis virus)	1, 6, 9, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	18 months
Mouse adenoma virus-FL	6 and 12 months, study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV (mouse hepatitis virus)	9 and 12 months
Parvovirus	1, 6, 12, and 18 months, study termination
PVM	6 and 12 months

RESULTS

All serology tests were negative.

APPENDIX J
TOXICOKINETIC STUDIES
IN F344/N RATS AND B6C3F$_1$ MICE

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TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION

A single dose of benzophenone was administered by intravenous injection or gavage to male and female F344/N rats and B6C3F$_1$ mice. Concentrations of benzophenone were determined in plasma at timepoints up to 24 hours after dosing. In addition, in the 2-year feed study of benzophenone, plasma samples were analyzed for benzophenone concentrations at 2 weeks and 3, 12, and 18 months in rats and at 12 months in mice. The results were analyzed to establish basic toxicokinetic parameters.

MATERIALS AND METHODS

Single-Dose Studies

Benzophenone was obtained from Aldrich Chemical Company, Inc (Milwaukee, WI) in one lot (10803KG) which was also used in the 2-year studies conducted at Battelle Columbus Laboratories (Columbus, OH). Lot 10803KG was characterized by infrared, ultraviolet/visible, nuclear magnetic resonance, and mass spectroscopy. It was found to be greater than 99% pure by capillary gas chromatography. Dose formulations for the gavage studies were prepared in 0.5% aqueous methylcellulose. Dose formulations for intravenous injection were prepared in Emulphor®:ethanol:deionized distilled water, 10:10:80.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic (Germantown, NY) and were 10 to 11 weeks old at the time of the studies. Animals were quarantined 7 days before use and were housed individually in polycarbonate cages on Ab-Sorb-Dri® cage litter. NTP-2000 feed and water were available *ad libitum*. Room environmental conditions included a temperature range of 69° to 75° F, relative humidity of 40% to 70%, and a 12:12 hour light/dark cycle.

Male and female rats were administered a single intravenous injection of a nominal dose of 2.5 mg benzophenone/kg body weight or a single gavage administration of a nominal dose of 2.5, 5, or 10 mg/kg. The dosing volume was 2 mL/kg by intravenous injection or 5 mL/kg by gavage. Male and female mice were administered a single intravenous injection of a nominal dose of 15 mg/kg or a single gavage administration of a nominal dose of 15, 30, or 60 mg/kg. The dosing volume was 4 mL/kg by intravenous injection or 10 mL/kg by gavage. The actual doses used in the rat and mouse studies (determined by dose analysis) are specified in the tables and figures that summarize the results.

At specified times after dosing, rats were anesthetized with a mixture of carbon dioxide and oxygen, and mice were euthanized with carbon dioxide. Blood samples were collected by retroorbital (rats) or cardiac (mice) puncture from five male and five female rats or one male and one female mouse per timepoint. Generally, two samples were taken from each rat (more than 2 hours apart), one from each eye. The times of blood sample collection after administration of benzophenone are given in Table J1. The samples were collected into heparinized microhematocrit tubes (rats) or heparinized syringes (mice) and the plasma was separated by centrifugation at 1000 × g for 10 minutes and then stored frozen until analysis using a validated method.

For analysis of benzophenone concentrations, 20 µL of a 0.508 mg/mL solution of butyrophenone in acetonitrile was added as an internal standard to a 0.3 mL aliquot of thawed plasma. The samples were mixed and allowed to stand at room temperature for approximately 15 minutes. A sodium chloride solution (0.1 mL of 330 mg sodium chloride/mL water) was added and mixed, followed by the addition of 0.4 mL acetonitrile with additional mixing. After centrifugation, the supernatant was analyzed using a validated high performance liquid chromatography (HPLC) method utilizing a Waters 845 HPLC (Waters Corp., Milford, MA). A Zorbax C8 column

(25 cm × 4.6 mm ID) was eluted with acetonitrile:water (1:1) at 1 mL per minute. Ultraviolet detection was used at 254 nm.

Plasma concentration data were analyzed by noncompartmental modeling techniques using Models 200 and 201 WinNonlin®, Version 1.0 (Scientific Consulting Inc., Cary, NC). These data were also analyzed by compartmental models (WinNonlin®) written to simultaneously solve gavage and intravenous data sets.

2-Year Feed Studies

For determination of plasma concentrations during the 2-year bioassay, groups of 10 male and 10 female rats and mice per dose were designated as the toxicokinetic groups. Blood samples were collected by orbital sinus bleeding from rats at 2 weeks and 3, 12, and 18 months, and from mice at 12 months. Exposure to benzophenone-dosed feed was continued during blood sampling. Samples were taken under CO_2/O_2 anesthesia using heparinized tubes at 1000, 1200, 1400, 1600, and 1800 hours, with n=0 to 2 at each time point. Rats were returned to exposure to benzophenone-dosed feed after each sampling until sacrifice after the 18-month time point. Mice were sacrificed after the 12-month time point. Blood samples were frozen and shipped to the analytical laboratory for analysis.

RESULTS

Rats

Single-Dose Studies

Mean plasma benzophenone concentration versus time data for male and female rats in the intravenous and gavage studies are plotted in Figures J1 and J2, respectively.

Noncompartmental parameter estimates are provided in Table J2. Area under the plasma concentration versus time curve (AUC) appears to be linear with dose for both sexes (AUC/Dose *ca.* 25). For males, bioavailability after a gavage dose ranged from 0.824 to 1.27, with an average value of 1.09. Estimates of elimination rate constants and half-lives (k_{elim} and $t_{½ elim}$, respectively) for males were similar for the intravenous and low gavage doses (k_{elim} *ca.* 0.00270 min^{-1}; $t_{½ elim}$ *ca.* 255 min), with slight decreases in k_{elim} and concomitant increases in $t_{½ elim}$ at the two higher gavage doses (k_{elim} *ca.* 0.00130 min^{-1}; $t_{½ elim}$ *ca.* 550 min). For female rats, estimates of k_{elim} and $t_{½ elim}$ were similar for the three gavage doses (k_{elim} *ca.* 0.00150 min^{-1}; $t_{½ elim}$ *ca.* 485 min). After intravenous administration to females, k_{elim} was slightly higher than after gavage administration, with a concomitant decrease in $t_{½ elim}$ (k_{elim} = 0.00280 min^{-1}; $t_{½ elim}$ = 247 min). Bioavailability after a gavage dose ranged from 1.05 to 1.39 in females, with an average value of 1.18. As shown in Figures J1 and J2, there was a great deal of fluctuation in mean plasma benzophenone concentrations at later time points, with secondary increases observed after initial decreases at most of the doses tested, regardless of the route of administration. This variation in concentration in the terminal portion of the curve resulted in extrapolation of up to 31% of the area from the last observed time point to infinity making the accuracy of the AUC and bioavailability estimates uncertain.

Mice

Mean plasma benzophenone concentration versus time data for male and female mice in the intravenous and gavage studies are plotted in Figures J3 and J4, respectively.

Noncompartmental parameter estimates are provided in Table J3; these estimates varied with dose and route of administration. Bioavailability of benzophenone in male and female mice is 50% or less. AUC/Dose, k_{elim}, and $t_{½ elim}$ are dose dependent in mice, with similar dependency for males and females. As shown in Figures J3 and J4, there was a great deal of fluctuation in plasma benzophenone concentrations at later time points, with clear secondary and even tertiary increases in concentration at all doses tested, regardless of the route of administration.

2-Year Feed Studies

For plasma samples from the 2-year feed studies, AUCs were estimated from the means (n=2) or the individual data points (n=1) using the trapezoidal rule from the time between 1000 and 1800 hours. The results are presented in Table J4. The AUCs are not uniformly dose proportional, although the 12- and 18-month data for rats appear to be dose proportional. The plasma concentrations of benzophenone were significantly higher ($P \leq 0.05$) in female rats and mice compared to males at the same exposure concentration for most of the durations of exposure. Statistical comparisons were by the z-test.

DISCUSSION

The present studies were designed to evaluate the toxicokinetics and estimate the internal dose of benzophenone when administered by intravenous injection or oral gavage to male and female rats and mice. The toxicokinetic studies were conducted to define the oral bioavailability of benzophenone when administered as a bolus gavage dose in aqueous methylcellulose and to establish a dose range over which plasma kinetics are linear.

After intravenous injection of a nominal dose of 2.5 mg benzophenone/kg to male and female rats, benzophenone initially rapidly cleared from the plasma, followed by a slight increase in plasma benzophenone concentration, and a secondary, slower elimination. Secondary maxima in mean plasma benzophenone concentration were observed during the terminal portion of the plasma concentration versus time curve.

After gavage administration of benzophenone to male rats, plasma concentration versus time curves for the three doses were roughly parallel between 200 and 600 minutes. Less well-defined secondary maxima in mean plasma benzophenone concentrations were observed between 5 and 120 minutes after gavage administration of 7.78 mg/kg (nominal dose = 10 mg/kg) to male rats and after gavage administration of 1.88 and 7.68 mg/kg (nominal doses of 2.5 and 10 mg/kg, respectively) to female rats. The similarity of all the intravenous plasma concentration versus time plots suggests that the secondary maxima may be due to enterohepatic recirculation and not artifacts. The characteristic pattern of plasma concentration increases due to enterohepatic recirculation may not be as obvious following gavage administration because the peaks are dampened somewhat by the absorption process, but there are discernable periodic increases in benzophenone concentration in the gavage plasma concentration versus time plots.

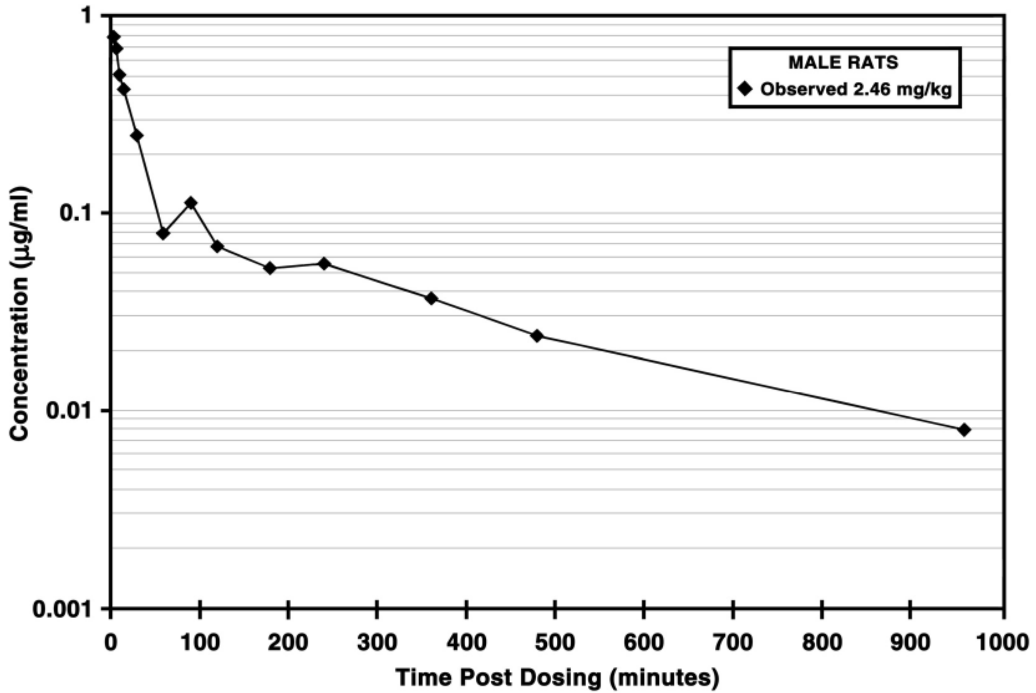
Based on the ratios of AUC for intravenous injection and gavage studies, average bioavailability was 1.09 and 1.18 for male and female rats, respectively. Overall, there were no apparent sex-related differences in noncompartmental pharmacokinetic parameter estimates for rats.

After intravenous injection of a nominal dose of 15 mg/kg to male and female mice, benzophenone was initially rapidly cleared from plasma, followed by a slower elimination phase. As with rats, fluctuations in mean plasma benzophenone concentration were observed in mice and were most likely due to enterohepatic recirculation. In mice, the AUCs were supralinear with respect to dose; as the dose was increased, the AUC/dose also increased. In rats, this parameter was more or less constant over the dose range. The nonlinearity in mice may be due to a first-pass effect of liver metabolism restricting the amount of benzophenone that gets into the general circulation. As the dose is increased, the first-pass metabolism becomes saturated. Mice appear to metabolize benzophenone more rapidly than rats; the doses are higher for mice, yet the half-lives and AUCs are smaller. As with rats, there were no obvious sex-related differences in noncompartmental pharmacokinetic parameter estimates for mice.

If benzophenone does undergo enterohepatic recirculation, none of the known metabolites are good candidates for the source of recirculated benzophenone. It may be explained (as shown in the following figure) by reduction to benzhydrol, conjugate formation, biliary excretion, deconjugation by gut flora, reabsorption, and oxidation to benzophenone.

Table J1
Blood Collection Times in the Single-Dose Toxicokinetic Studies of Benzophenone

	Route of Administration	Nominal Dose (mg/kg)	Sample Collection Times after Dosing (minutes)
Rats			
	Intravenous	2.5	4, 7, 10, 15, 30, 60, 90, 120, 180, 240, 360, 480, 960
	Gavage	2.5	2.5, 5, 7.5, 10, 15, 30, 60, 120, 180, 360, 480, 600, 960
	Gavage	5	2.5, 5, 7.5, 10, 15, 30, 60, 120, 180, 360, 600, 960, 1440
	Gavage	10	2.5, 5, 7.5, 10, 15, 30, 60, 120, 180, 360, 600, 960, 1440
Mice			
	Intravenous	15	4, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180
	Gavage	15	2.5, 5, 7.5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360
	Gavage	30	2.5, 5, 7.5, 10, 15, 30, 60, 90, 120, 180, 360, 480, 600
	Gavage	60	2.5, 5, 7.5, 10, 15, 30, 60, 120, 180, 360, 600, 960, 1440



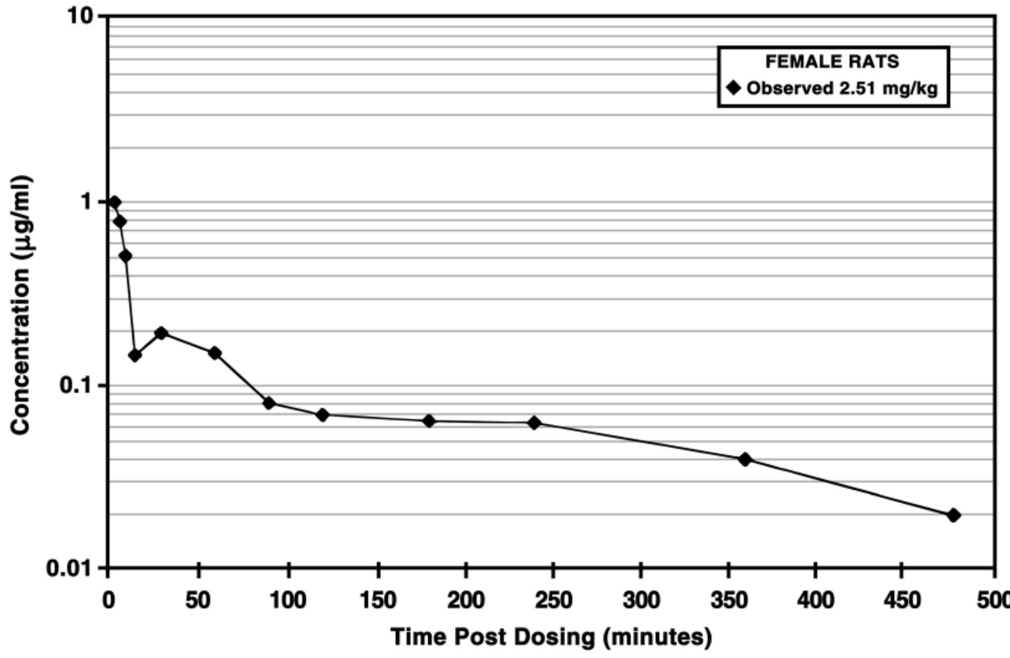
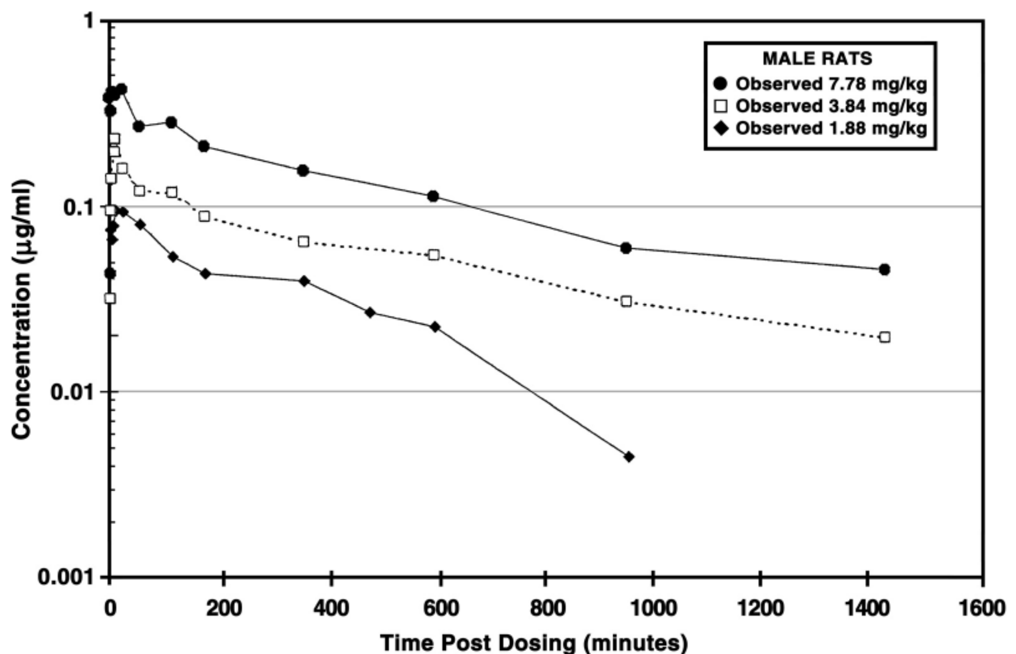


FIGURE J1
Plasma Concentrations of Benzophenone in F344/N Rats after a Single Intravenous Injection of Benzophenone



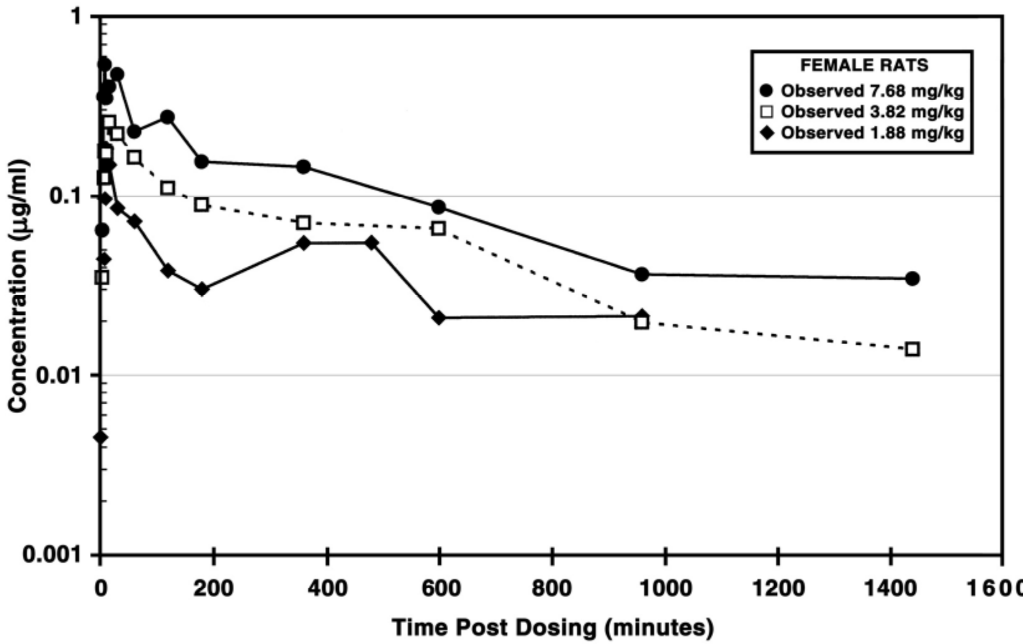
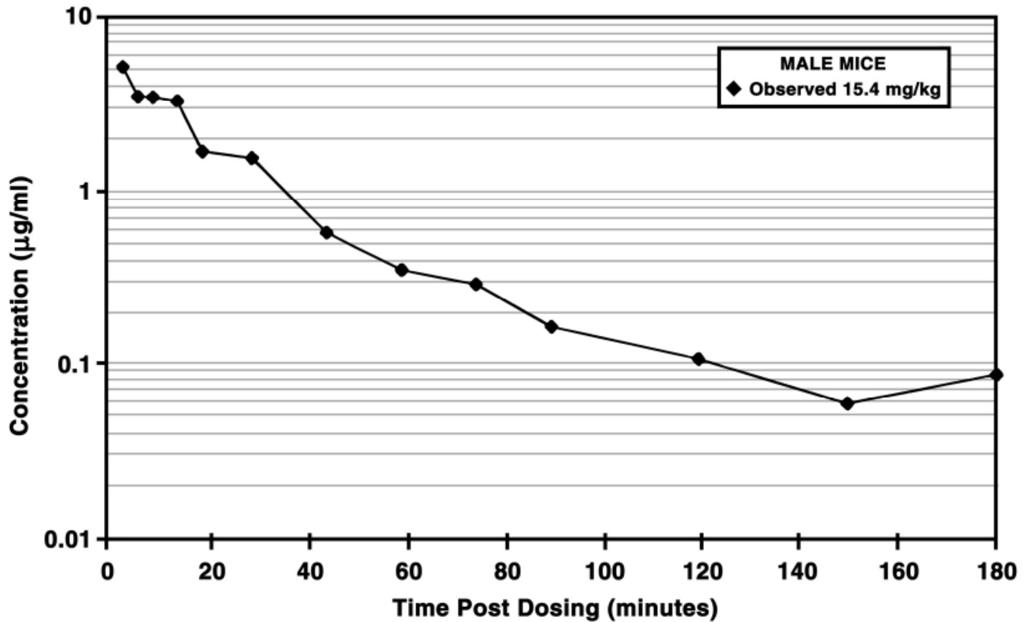


FIGURE J2
Plasma Concentrations of Benzophenone in F344/N Rats after a Single Gavage Dose of Benzophenone

Table J2
Noncompartmental Analysis of Plasma Concentration Versus Time Profiles for F344/N Rats Administered Single Intravenous or Gavage Doses of Benzophenone[a]

	Nominal Dose (mg/kg)	Actual Dose[b] (mg/kg)	k_{elim}[c] (min^{-1})	$t_{½ elim}$[d] (min)	AUC[e] (µg•min/mL)	AUC/ Dose	Bioavailability
Male							
Intravenous	2.5	2.46	0.00260	268	51.9	21.1	—
Gavage	2.5	1.88	0.00280	245	32.7	17.4	0.824
Gavage	5	3.84	0.00120	594	95.6	24.9	1.18
Gavage	10	7.78	0.00140	506	208	26.7	1.27
Female							
Intravenous	2.5	2.51	0.00280	247	51.6	20.6	—
Gavage	2.5	1.88	0.00120	567	53.8	28.6	1.39
Gavage	5	3.82	0.00180	395	86.8	22.7	1.10
Gavage	10	7.68	0.00140	499	166	21.6	1.05

[a] Toxicokinetic parameters were calculated from the plasma concentration-time curves, where each data point represented the mean of five samples
[b] Based on dose analysis
[c] k_{elim}=elimination rate constant
[d] $t_{½ elim}$=half-life
[e] AUC=area under the plasma concentration versus time curve



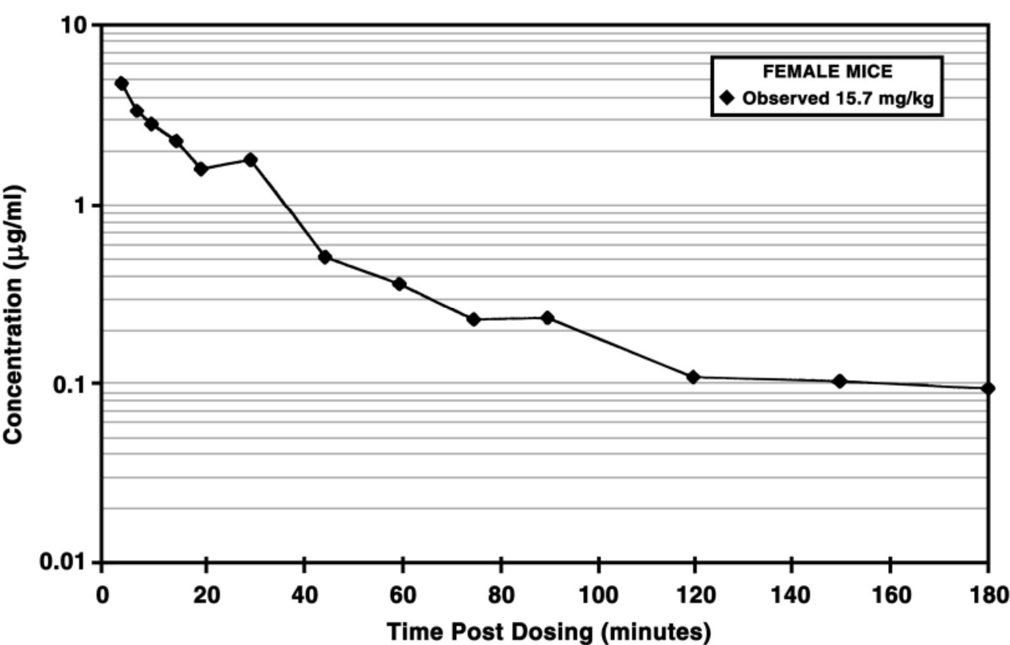
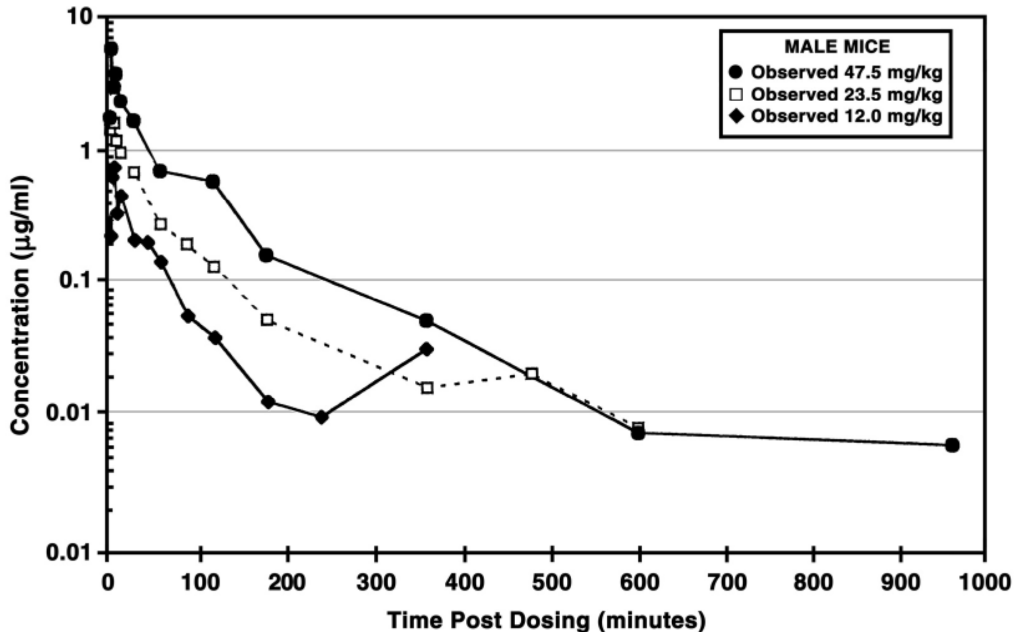


FIGURE J3
Plasma Concentrations of Benzophenone in B6C3F$_1$ Mice after a Single Intravenous Injection of Benzophenone



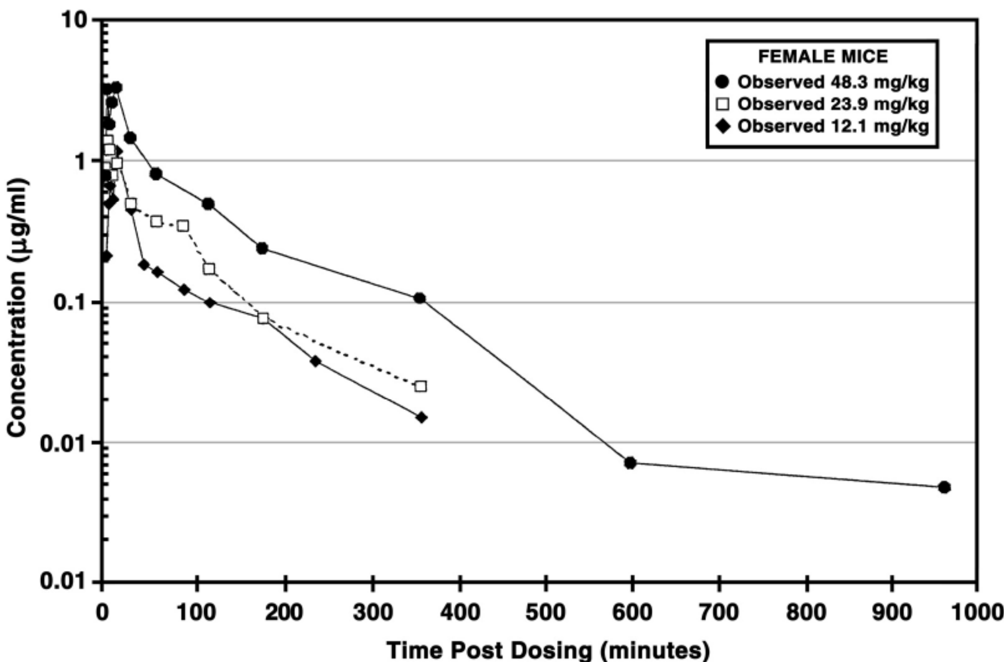


FIGURE J4
Plasma Concentrations of Benzophenone in B6C3F$_1$ Mice after a Single Gavage Dose of Benzophenone

Table J3
Noncompartmental Analysis of Plasma Concentration Versus Time Profiles for B6C3F$_1$ Mice Administered Single Intravenous or Gavage Doses of Benzophenone[a]

	Nominal Dose (mg/kg)	Actual Dose[b] (mg/kg)	k_{elim}[c] (min^{-1})	$t_{½ elim}$[d] (min)	AUC[e] (µg•min/mL)	AUC/ Dose	Bioavailability
Male							
Intravenous	15	15.4	0.0259	26.7	140	9.1	—
Gavage	15	12.0	0.0159	43.6	28.7	2.4	0.263
Gavage	30	23.5	0.00610	113	74.2	3.2	0.347
Gavage	60	47.5	0.00430	160	205	4.3	0.475
Female							
Intravenous	15	15.7	0.0128	54.0	137	8.7	—
Gavage	15	12.1	0.00790	87.5	49.2	4.1	0.468
Gavage	30	23.9	0.00940	73.9	75.9	3.2	0.365
Gavage	60	48.3	0.00640	108	211	4.4	0.501

[a] Toxicokinetic parameters were calculated from the plasma concentration-time curves, where each data point represented one sample
[b] Based on dose analysis
[c] k_{elim}=elimination rate constant
[d] $t_{½ elim}$=half-life
[e] AUC=area under the plasma concentration versus time curve

Table J4
Area Under the Plasma Concentration Versus Time Curves at 2 Weeks, and 3, 12, and 18 Months in the 2-Year Feed Studies of Benzophenone[a]

	312 ppm	625 ppm	1,250 ppm
Rats			
2 Weeks			
Male	0.981 ± 0.062	1.07 ± 0.07	1.34 ± 0.09
Female	1.181 ± 0.109	1.43 ± 0.08	1.91 ± 0.08
P Value[b]	0.054	0.0004	<0.0001
3 Months			
Male	1.300 ± 0.069	2.16 ± 0.09	2.80 ± 0.06
Female	2.062 ± 0.104	2.47 ± 0.25	5.35 ± 0.40
P Value	<0.0001	0.124	<0.0001
12 Months			
Male	0.597 ± 0.063	1.12 ± 0.07	2.30 ± 0.19
Female	1.939 ± 0.123	3.85 ± 0.46	5.97 ± 0.64
P Value	<0.0001	<0.0001	<0.0001
18 Months			
Male	0.626 ± 0.131	1.45 ± 0.24	3.86 ± 0.14
Female	1.702 ± 0.234	3.46 ± 0.23	6.75 ± 0.63
P Value	<0.0001	<0.0001	<0.0001
Mice			
12 Months			
Male	0.177 ± 0.007	0.085 ± 0.019	0.230 ± 0.082
Female	0.508 ± 0.275	0.287 ± 0.050	0.533 ± 0.129
P Value	0.114	<0.0001	0.024

[a] Data reflect the interval from 1000 to 1800 hours at each analysis and are given in µg • hour/mL as the mean ± standard deviation
[b] P Value from z-test, comparing males and females

Oxybenzone HEL Monograph - 5 of 7

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Joe DiNardo <jmjdinardo@aol.com>

Sun 11/26/2017 7:21 AM

To:IEM Committee <IEM.Committee@mauicounty.us>; County Clerk <County.Clerk@mauicounty.us>;

Cc:cadowns@haereticus-lab.org <cadowns@haereticus-lab.org>;

17 attachments (2 MB)

30 Nakajima Activity related to the carcinogenicity of plastic additives in the benzophenone group.docx; 31 Cuquerella Benzophenone photosensitized DNA damage.docx; 32 Zhao Substituent contribution to the genotoxicity of benzophenone-type UV filters.docx; 33 Hanson Sunscreen enhancement of UV-induced reactive oxygen species in the skin.docx; 34 Kerdivel Estrogenic potency of benzophenone.docx; 35 In Benzophenone-1 and nonylphenol stimulated MCF-7 breast cancer.docx; 36 Kim Growth and migration of LNCaP prostate cancer cells are promoted by triclosan and benzophenone-1.docx; 37 Phiboonchaiyanan Benzophenone metastasis.docx; 38 Alamer and Darbre Human Breast Cancer Cells J Appl Toxicol.docx; 39 Szczurko Photocontact allergy to oxybenzone- ten years of experience.docx; 40 Langan Photocontact allergy to oxybenzone.docx; 41 Chuah Photopatch testing in Asians- a 5-year experience in Singapore.docx; 42 French NTP Report.docx; 43 Schlumpf Developmental Toxicity of UV Filters.pdf; 44 Gulati NTP Reproductive assessment.pdf; 45 Louis Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples fecundity.docx; 46 Louis Urinary concentrations of benzophenone-type ultraviolet light filters and semen quality.docx;

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,
Craig Downs – Executive Director – Haereticus Environmental Laboratory
Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:
- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.
- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.
- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:
1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone
2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

30) J UOEH. 2006 Jun 1;28(2):143-56.

Activity related to the carcinogenicity of plastic additives in the benzophenone group.

Nakajima D1, Asada S, Kageyama S, Yamamoto T, Kuramochi H, Tanaka N, Takeda K, Goto S.
Author information
1 Research Centerfor Environmental Risk, National Institutefor Environmental Studies, Onogawa, Tsukuba, Ibaraki 305-8506, Japan.

Abstract
This study examines the activities relating to the carcinogenicity of six types of benzophenone derivatives (benzophenone, 2-hydroxy-4-octyloxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 2,4-dihydroxybenzophenone, 2,2'-dihydroxy-4-methoxybenzophenone and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone) currently used in plastic products as additives to serve as ultraviolet absorbing agents. The evaluation of the initiation activity used a light absorption umu-test, a luminescent umu-test and the Ames test. The promotion activity was examined by a Bhas assay, a method that uses Bhas 42 cells for the formation of transformation foci. The luminescent umu-test indicated positive initiation activity of 2-hydroxy-4-methoxybenzophenone, and pseudo-positive activity of 2,4-dihydroxybenzophenone and 2,2'-dihydroxy-4-methoxybenzophenone. In the Ames test, 2-hydroxy-4-octyloxybenzophenone showed pseudo-positive initiation activity. Conversely, 2,4-dihydroxybenzophenone indicated weak promotion activity at 10 microg/ml concentration.

31) Acc Chem Res. 2012 Sep 18;45(9):1558-70. Epub 2012 Jun 14.

Benzophenone photosensitized DNA damage.

Cuquerella MC1, Lhiaubet-Vallet V, Cadet J, Miranda MA.
Author information

Abstract
Although the carcinogenic potential of ultraviolet radiation is well-known, UV light may interact with DNA by direct absorption or through photosensitization by endogenous or exogenous chromophores. These chromophores can extend the "active" fraction of the solar spectrum to the UVA region and beyond, which means that photosensitizers increase the probability of developing skin cancer upon exposure to sunlight. Therefore researchers would like to understand the mechanisms involved in photosensitized DNA damage both to anticipate possible photobiological risks and to design tailor-made photoprotection strategies. In this context, photosensitized DNA damage can occur through a variety of processes including electron transfer, hydrogen abstraction, triplet-triplet energy transfer, or generation of reactive oxygen species. In this Account, we have chosen benzophenone (BP) as a classical and paradigmatic chromophore to illustrate the different lesions that photosensitization may prompt in nucleosides, in oligonucleotides, or in DNA. Thus, we discuss in detail the accumulated mechanistic evidence of the BP-photosensitized reactions of DNA or its building blocks obtained by our group and others. We also include ketoprofen (KP), a BP-derivative that possesses a chiral center, to highlight the stereodifferentiation in the key photochemical events, revealed through the dynamics of the reactive triplet excited state ((3)KP*). Our results show that irradiation of the BP chromophore in the presence of DNA or its components leads to nucleobase oxidations, cyclobutane pyrimidine dimer formation, single strand breaks, DNA-protein cross-links, or abasic sites. We attribute the manifold photoreactivity of BP to its well established photophysical properties: (i) it absorbs UV light, up to 360 nm; (ii) its intersystem crossing quantum yield (ϕ(ISC)) is almost 1; (iii) the energy of its nπ* lowest triplet excited state (E(T)) is ca. 290 kJ mol(-1); (iv) it produces singlet oxygen ((1)O(2)) with a quantum yield (ϕ(Δ)) of ca. 0.3. For electron transfer and singlet oxygen reactions, we focused on guanine, the nucleobase with the lowest oxidation potential. Among the possible oxidative processes, electron transfer predominates. Conversely, triplet-triplet energy transfer occurs mainly from (3)BP* to thymine, the base with the lowest lying triplet state in DNA. This process results in the formation of cyclobutane pyrimidine dimers, but it also competes with the Paternò-Büchi reaction in nucleobases or nucleosides, giving rise to oxetanes as a result of crossed cycloadditions. Interestingly, we have found significant stereodifferentiation in the quenching of the KP triplet excited state by both 2'-deoxyguanosine and thymidine. Based on these results, this chromophore shows potential as a (chiral) probe for the investigation of electron and triplet energy transport in DNA.

32) Ecotoxicol Environ Saf. 2013 Sep;95:241-6. doi: 10.1016/j.ecoenv.2013.05.036.
Epub 2013 Jun 20.

Substituent contribution to the genotoxicity of benzophenone-type UV filters.

Zhao H1, Wei D, Li M, Du Y.
Author information
1 State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China. wyuchemys10zhm@126.com
Erratum in
Ecotoxicol Environ Saf. 2013 Dec;98:391.

Abstract
Benzophenones (BPs) are widely used in UV filters, fragrance enhancers, and plastic additives. In this study, the genotoxicity of 14 BPs was tested using the SOS/umu assay, and the related substituent contribution was disclosed. The results of this study revealed that the major contributor to the genotoxicity of the BPs was the ortho,para-di-substitution, and the increasing hydroxy substitution on the benzene ring. In addition, the higher the dispersion of the substituent species on the two benzene rings, the lower the genotoxicity exhibited by the compound. Furthermore, 2 dimensional and 3 dimenional quantitative structure-activity relationships (2D- and 3D-QSAR) studies indicated that hydrogen-bond interactions and electrostatic effects were determinants for the genotoxicity of the BPs. The current results provide useful information for the assessment of the potential ecological risk and health effects of BP-type UV filters.

33) Free Radic Biol Med. 2006 Oct 15;41(8):1205-12. Epub 2006 Jul 6.

Sunscreen enhancement of UV-induced reactive oxygen species in the skin.

Hanson KM1, Gratton E, Bardeen CJ.
Author information
1 Department of Chemistry, University of California at Riverside, Riverside, CA 92506, USA. Kerry.hanson@ucr.edu

Abstract
The number of UV-induced (20 mJ cm(-2)) reactive oxygen species (ROS) generated in nucleated epidermis is dependent upon the length of time the UV filter octocrylene, octylmethoxycinnamate, or benzophenone-3 remains on the skin surface. Two-photon fluorescence images acquired immediately after application of each formulation (2 mg cm(-2)) to the skin surface show that the number of ROS produced is dramatically reduced relative to the skin-UV filter control. After each UV filter remains on the skin surface for t=20 min, the number of ROS generated increases, although it remains below the number generated in the control. By t=60 min, the filters generate ROS above the control. The data show that when all three of the UV filters penetrate into the nucleated layers, the level of ROS increases above that produced naturally by epidermal chromophores under UV illumination.

34) PLoS One. 2013 Apr 4;8(4):e60567. doi: 10.1371/journal.pone.0060567. Print 2013.

Estrogenic potency of benzophenone UV filters in breast cancer cells: proliferative and transcriptional activity substantiated by docking analysis.

Kerdivel G1, Le Guevel R, Habauzit D, Brion F, Ait-Aissa S, Pakdel F.
Author information
1 Transcription, Environment and Cancer Group, Institut de Recherche sur la Santé, Environnement et Travail (IRSET), INSERM U1085, Université de Rennes 1, Rennes, France.

Abstract
The results from recent studies show that some benzophenones (BPs) and their hydroxylated metabolites can function as weak estrogens (E2) in the environment. However, little is known about the structure-activity relationship of these molecules. We have examined the effects of exposure to ten different BPs on the proliferation of estrogen receptor (ER)-positive breast cancer cells and on the transcriptional activity of E2-target genes. We analyzed two genes that are tightly linked with estrogen-mediated proliferation, the CXCL12 and amphiregulin genes and two classical estrogen-responsive genes, the pS2 and progesterone receptor. Significant differences in the BPs efficiency to induce cell proliferation and endogenous E2-target gene expressions were observed. Using ERE-, Sp1-, AP1- and C3-reporter genes that contain different ER-binding sites in their promoter, we also showed significant differences in the BPs efficiency in activation of the ER transactivation. Together, our analyzes showed that the most active molecule is 4-hydroxy-BP. Docking analysis of the interaction of BPs in the ligand-binding pocket of ERα suggests that the minimum structural requirement for the estrogenic activity of BPs is a hydroxyl (OH) group in the phenyl A-ring that allows interaction with Glu-353, Arg-394 or Phe-404, which enhances the stability between BPs and ERα. Our modeling also indicates a loss of interaction between the OH groups of the phenyl B-ring and His-524. In addition, the presence of some OH groups in the phenyl B-ring can create repulsion forces, which may constrain helix 12 in an unfavorable position, explaining the differential estrogenic effects of BPs. These results, together with our analysis of BPs for their potency in activation of cell proliferation and ER-mediated transcription, report an improved understanding of the mechanism and structure-activity relationship of BPs.

35) J Toxicol Environ Health A. 2015;78(8):492-505. doi:
10.1080/15287394.2015.1010464.

Benzophenone-1 and nonylphenol stimulated MCF-7 breast cancer growth by regulating cell cycle and metastasis-related genes via an estrogen receptor α-dependent pathway.

In SJ1, Kim SH, Go RE, Hwang KA, Choi KC.
Author information
1 a Laboratory of Biochemistry and Immunology, College of Veterinary Medicine , Chungbuk National University , Cheongju , Chungbuk , Republic of Korea.

Abstract
Endocrine-disrupting chemicals (EDC) are defined as environmental compounds that produce adverse health manifestations in mammals by disrupting the endocrine system. Benzophenone-1 (2,4-dihydroxybenzophenone, BP1) and nonylphenol (NP), which are discharged from numerous industrial products, are known EDC. The aim of this study was to examine the effects of BP1 and NP on proliferation and metastasis of MCF-7 human breast cancer cells expressing estrogen receptors (ER). Treatment with BP1 (10^{-5}-10^{-7} M) and NP (10^{-6}-10^{-7} M) promoted proliferation of MCF-7 cells similar to the positive control 17 -beta-estradiol (E2). When ICI 182,780, an ER antagonist, was co-incubated with E2, BP1, or NP, proliferation of MCF-7 cells returned to the level of a control. Addition of BP1 or NP markedly induced migration of MCF-7 cells similar to E2. To elucidate the underlying molecular mechanisms produced by these EDC, alterations in transcriptional and translational levels of proliferation and metastasis-related markers, including cyclin D1, p21, and cathepsin D, were determined. Data showed increase in expression of cyclin D1 and cathepsin D and decrease in p21 at both transcriptional and translational levels. However, BP1- or NP-induced alterations of these genes were blocked by ICI 182,780, suggesting that changes in expression of these genes may be regulated by an ERα-dependent pathway. In conclusion, BP1 and NP may accelerate growth of MCF-7 breast cancer cells by regulating cell cycle-related genes and promote cancer metastasis through amplification of cathepsin D.

36) Environ Toxicol Pharmacol. 2015 Mar;39(2):568-76. doi:
10.1016/j.etap.2015.01.003. Epub 2015 Jan 20.

Growth and migration of LNCaP prostate cancer cells are promoted by triclosan and benzophenone-1 via an androgen receptor signaling pathway.

Kim SH1, Hwang KA1, Shim SM2, Choi KC3.
Author information
1 Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 361-763 Republic of Korea.
2 Department of Food Science & Technology and Carbohydrate Bioproduct Research Center, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul, 143-747, Republic of Korea.
3 Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 361-763 Republic of Korea.
Electronic address: kchoi@cbu.ac.kr.

Abstract
Prostate cancer (PCa) is a global health concern in human males. Recently, it has been known that endocrine-disrupting chemicals (EDCs) may act as an exogenous factor to enhance cancer progression. Triclosan (TCS) and 2,4-dihydroxybenzophenone (BP-1) were reported to bioaccumulate in human bodies through the skin absorption. However, there has been insufficient evidence on the findings that the intervention of EDCs may promote the cancer progression in PCa. In the present study, to verify the risk of TCS and BP-1 to a PCa progression, cancer cell proliferation and migration were investigated in LNCaP PCa cells. TCS and BP-1 increased LNCaP cell proliferative activity and migration as did dihydrotestosterone (DHT). This phenomenon was reversed by the treatment with bicalutamide, a well known AR antagonist, suggesting that TCS and BP-1 acted as a xenoandrogen in LNCaP cells via AR signaling pathway by mimicking the action of DHT. A Western blot assay was performed to identify the alterations in the translational levels of cell growth- and metastasis-related markers, i.e., c-fos, cyclin E, p21, and cathepsin D genes. The expressions of genes related with G1/S transition of cell cycle and metastasis were increased by DHT, TCS, and BP-1, while the expression of p21 protein responsible for cell cycle arrest was reduced by DHT, TCS, and BP-1. Taken together, these results indicated that TCS and BP-1 may enhance the progression of PCa by regulating cell cycle and metastasis-related genes via AR signaling pathway.

37) Cell Biol Toxicol. 2017 Jun;33(3):251-261. doi: 10.1007/s10565-016-9368-3.
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Benzophenone-3 increases metastasis potential in lung cancer cells via epithelial to mesenchymal transition.

Phiboonchaiyanan PP1, Busaranon K2, Ninsontia C1,2, Chanvorachote P3,4.
Author information
1 Cell-based Drug and Health Products Development Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand.
2 Departments of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand.
3 Cell-based Drug and Health Products Development Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand. pithi_chan@yahoo.com.
4 Departments of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand.
pithi_chan@yahoo.com.

Abstract
Exposure to compounds with cancer-potentiating effects can contribute to the progression of cancer. Herein we have discovered for the first time that benzophenone-3 (BP-3), a chemical used as sunscreen in various cosmetic products, enhances the ability of lung cancer cells to undergo metastasis. The exposure of the lung cancer cells to BP-3 at non-toxic concentrations significantly increased the number of anoikis resistant cells in a dose-dependent manner. Also, BP-3 increased the growth rate as well as the number of colonies accessed by anchorage-independent growth assay. We found that the underlying mechanisms of such behaviors were the epithelial to mesenchymal transition (EMT) process of cancer cells, and the increase in caveolin-1 (Cav-1) expression. As both mechanistic events mediated anoikis resistance via augmentation of cellular survival signals, our results further revealed that the BP-3 treatment significantly up-regulated extracellular-signal-regulated kinase (ERK). Also, such compounds increased the cellular levels of anti-apoptotic Bcl-2 and Mcl-1 proteins. As the presence of a substantial level of BP-3 in plasma of the consumers has been reported, this finding may facilitate further investigations that lead to better understanding and evidence concerning the safety of use in cancer patients.

38 J Appl Toxicol. 2017 Oct 9. doi: 10.1002/jat.3525. [Epub ahead of print]

Effects of exposure to six chemical ultraviolet filters commonly used in personal care products on motility of MCF-7 and MDA-MB-231 human breast cancer cells in vitro.

Alamer M1, Darbre PD1.
Author information
1 School of Biological Sciences, University of Reading, Reading, RG6 6UB, UK.

Abstract
Benzophenone (BP)-1, BP-2, BP-3, octylmethoxycinnamate (OMC), 4-methylbenzilidenecamphor and homosalate are added to personal care products to absorb ultraviolet light. Their presence in human milk and their oestrogenic activity suggests a potential to influence breast cancer development. As metastatic tumour spread is the main cause of breast cancer mortality, we have investigated the effects of these compounds on migration and invasion of human breast cancer cell lines. Increased motility of oestrogen-responsive MCF-7 human breast cancer cells was observed after long-term exposure (>20 weeks) to each of the six compounds at ≥10-7 m concentrations using three independent assay systems (scratch assay, live cell imaging, xCELLigence technology) and increased invasive activity was observed through matrigel using the xCELLigence system. Increased motility of oestrogen-unresponsive MDA-MB-231 human breast cancer cells was observed after 15 weeks of exposure to each of the six compounds by live cell imaging and xCELLigence technology, implying the increased migratory activity was not confined to oestrogen-responsive cells. Molecular mechanisms varied between compounds and cell lines. Using MCF-7 cells, reduction in E-cadherin was observed following 24 weeks' exposure to 10-5 m BP-1 and 10-5 m homosalate, and reduction in β-catenin was noted following 24 weeks' exposure to 10-5 m OMC. Using MDA-MB-231 cells, increased levels of matrix metalloproteinase 2 were observed after 15 weeks exposure to 10-7 m OMC and 10-7 m 4-methylbenzilidenecamphor. Although molecular mechanisms differ, these results demonstrate that exposure to any of these six compounds can increase migration and invasion of human breast cancer cells.

39) Photodermatol Photoimmunol Photomed. 1994 Aug;10(4):144-7.

Photocontact allergy to oxybenzone: ten years of experience.

Szczurko C1, Dompmartin A, Michel M, Moreau A, Leroy D.
Author information
1 Service de Dermatologie, Centre Hospitalier Universitaire, Caen, France.

Abstract
Intolerance of sunscreen agents has often been reported in the literature. This mainly comprises photosensitization to sunscreens such as oxybenzone. The aims of this study were to establish the incidence of photocontact allergy to oxybenzone and its relationship with the use of other cosmetics. From 1982 to 1992 we performed photopatch tests on 283 patients with suspected photodermatosis. Forty-six patients (16%) had positive reactions: 61 positive photopatch tests and 9 positive patch tests. Photocontact allergy to sunscreens was divided into 2 main groups: 35 cases to oxybenzone and 17 cases to para-aminobenzoic acid and its derivatives. Among our 35 cases of photoallergy to oxybenzone, more than one third had photoallergy to a daily moisturizer that contained oxybenzone.

40) Contact Dermatitis. 2006 Mar;54(3):173-4.
Photocontact allergy to oxybenzone and contact allergy to lignocaine and prilocaine.

Langan SM1, Collins P.
Author information
1 City of Dublin Skin and Cancer Hospital, Hume Street, Dublin 2, UK.
sineadlangan@hotmail.com

Case reported on photocontact allergy to oxybenzone.

41) Photodermatol Photoimmunol Photomed. 2013 Jun;29(3):116-20. doi: 10.1111/phpp.12034.

Photopatch testing in Asians: a 5-year experience in Singapore.

Chuah SY1, Leow YH, Goon AT, Theng CT, Chong WS.
Author information
1 Department of Dermatology, National Skin Centre, Singapore. sychuah@nsc.gov.sg

Abstract
BACKGROUND:
Photopatch testing is important for diagnosing photoallergic contact dermatitis. We aimed to evaluate the use of photopatch test at the National Skin Centre, Singapore.
METHODS:
All patients who had photopatch tests done between 2007 and 2011 at the National Skin Centre were included.
RESULTS:
Twenty-two patients were included. The mean age was 40.2. Female : male ratio was 3.4. The ethnic groups were Chinese (68%), Malay (4%), Indian (14%) and others (14%). Ten out of 22 patients (45.5%) had a positive photopatch test. There were 20 positive photopatch test reactions found in these 10 patients, and all 20 positive reactions were of current relevance. The frequencies of the positive photopatch test reactions were 2-hydroxy-4-methoxybenzophenone (oxybenzone) (n = 6), 2-hydroxymethoxymethylbenzophenone (mexenone) (n = 3), 2-ethylhexyl-4-dimethylaminobenzoate (n = 1), ketoprofen gel (n = 1) and the patient's own product (n = 9).
CONCLUSIONS:
Our study suggests that sunscreen is the most common photoallergen to date as opposed to musk ambrette, which was the most common photoallergen in our earlier study in 1991-1993. This finding is similar to the recent European Multicentre Photopatch Test Study.

42) Toxic Rep Ser. 1992 Oct;21:1-E14.

NTP technical report on the toxicity studies of 2-Hydroxy-4-methoxybenzophenone (CAS No. 131-57-7) Adminstered Topically and in Dosed Feed to F344/N Rats and B6C3F1 Mice.

French JE.

Abstract
2-Hydroxy-4-methoxybenzophenone (HMB) occurs naturally in flower pigments and is synthesized for use in sunscreens, as a UV stabilizer in various cosmetic products, and in plastic surface coatings and polymers. Toxicity studies of HMB were performed in F344/N rats and B6C3F1 mice, by administering HMB in feed and by topical application, in studies of 2 weeks' (5 animals/sex, dose and species) and 13 weeks' (10 animals/sex, dose and species) duration. Assessments included hematology, clinical chemistry, urinalysis, reproductive toxicity, and histopathologic evaluations. In both 2- and 13-week dosed feed studies, rats received diets containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. One high-dose female rat died during the 2-week study. Body weight gains of high-dose male and female rats were reduced in the 13-week study. Liver and kidney weights were increased in dosed rats in both studies. In the 2-week studies, enlarged livers were associated with a marked hepatocyte cytoplasmic vacuolization in rats receiving diets containing concentrations of 6250 ppm HMB or higher; renal lesions, consisting of dilated tubules and regeneration of tubular epithelial cells, were found primarily in high-dose rats. In the 13-week studies, kidney lesions progressed to include papillary degeneration, or necrosis, and inflammation, while the liver lesion appeared to regress; liver enzymes in serum remained elevated. Rats receiving a diet with 50000 ppm HMB showed markedly lower epididymal sperm density and an increase in the length of the estrous cycle at the end of the 13-week studies. In 2-week dermal studies, rats received topical applications of 1.25 to 20 mg of HMB in an acetone or lotion vehicle. The only effects noted were small and variable increases in liver and kidney weights, reaching statistical significance primarily in the higher dose groups. In 13-week studies, rats received topical doses from 12.5 to 200 mg/kg HMB in acetone. Kidney weights were elevated in dosed groups of female rats. No other findings were attributed to HMB treatment. In 2- and 13-week dosed feed studies, mice received feed containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. A dose- related increase in liver weight associated with hepatocyte cytoplasmic vacuolization was the only finding in mice in the 2- week studies. Decreased body weight gains were dose-related in mice in the 13-week studies; mild increases in liver weights were seen in dosed mice of both sexes. Kidney weights were increased variably in dosed females. Microscopic lesions were noted only in the kidneys of males receiving 50000 ppm HMB; these included eosinophilic protein casts in dilated renal tubules and a mild inflammation associated with the dilated tubules. Mice in the highest dose group exhibited a decrease in epididymal sperm density and an increase in length of the estrous cycle. In 2-week

dermal studies, mice received topical applications from 0.5 to 8 mg HMB in an acetone or lotion vehicle. The only effects noted were minimal, variable increases in liver and kidney weights, primarily in the higher dose groups. In 13-week studies, mice received topical doses of 22.75 to 364 mg/kg in acetone. Kidney weights were increased variably in dosed male mice. Epididymal sperm density was decreased at all 3 dose levels evaluated (22.75, 91, and 200 mg/kg). The genetic toxicity of HMB also was evaluated in mutagenicity studies with Salmonella typhimurium, in cytogenetic studies with Chinese hamster ovary (CHO) cells, and by evaluation of micronucleated erythrocytes in peripheral blood smears from mice in the 13-week studies. HMB was weakly mutagenic in Salmonella with metabolic activation, and induced sister-chromatid exchanges and chromosomal aberrations in CHO cells in the presence of a metabolic activation system. There was no increase in the frequency of micronucleated erythrocytes in the blood of mice receiving HMB. In summary, HMB produced generally similar effects following topical and oral administration to rats and mice. Consistent findings included decreases in epididymal sperm density, lengthened estrous cycle, and increased liver and kidney weights. Mice in the dosed feed studies exhibited microscopic changes in the kidneys, comprising tubular dilatation with eosinophilic protein casts. Dilatation, tubular regeneration, papillary degeneration, and inflammation were noted in the kidneys of rats; and liver lesions consisting of an apparently reversible hepatocyte cytoplasmic vacuolization occurred in both rats and mice. A no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6250 ppm HMB in the diet for rats and mice. A NOAEL was not reached for decreased epididymal sperm density in the 13- week dermal study in mice (<23 mg/kg/day). Synonyms: Oxybenzone; 4-Methoxy-2-hydroxy-benzophenone; Cyasorb UV; Uvinul M 40; (2-hydroxy-4-methoxyphenyl)phenyl-methanone; NSC-7778; Spectra-sorb UV; Syntase 62; UF 3; USAF CY-9; NCI-C60957.

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REVIEW ARTICLE

Developmental toxicity of UV filters and environmental exposure: a review

Margret Schlumpf,* Stefan Durrer,* Oliver Faass,* Colin Ehnes,* Michaela Fuetsch,* Catherine Gaille,* Manuel Henseler,* Luke Hofkamp,† Kirsten Maerkel,* Sasha Reolon,* Barry Timms,† Jesus A. F. Tresguerres‡ and Walter Lichtensteiger*

*GREEN Tox and Institute of Anatomy, University of Zurich, Zurich, Switzerland, †University of South Dakota, Vermillion, SD, USA, and ‡Complutense University, Madrid, Spain

Keywords:
3-benzylidene camphor, 4-methylbenzylidene camphor, brain, cosmetics, developmental toxicity, estrogenic activity, prostate, sexual behaviour, uterus, UV filters

Correspondence:
Margret Schlumpf, GREEN Tox, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
E-mail: margret.schlumpf@access.uzh.ch

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Summary

Several ultraviolet (UV) filters exhibit estrogenic, some also anti-androgenic activity. They are present in waste water treatment plants, surface waters and biosphere including human milk, suggesting potential exposure during development. Developmental toxicity was studied in rats for the UV filters 4-methylbenzylidene camphor (4-MBC, 0.7, 7, 24, 47 mg/kg/day) and 3-benzylidene camphor (3-BC, 0.07, 0.24, 0.7, 2.4, 7 mg/kg/day) administered in chow to the parent generation before mating, during pregnancy and lactation, and to the offspring until adulthood. Neonates exhibited enhanced prostate growth after 4-MBC and altered uterine gene expression after both chemicals. 4-MBC and 3-BC delayed male puberty and affected reproductive organ weights of adult offspring. Effects on the thyroid axis were also noted. Expression and oestrogen sensitivity of oestrogen-regulated genes and nuclear receptor coregulator levels were altered at mRNA and protein levels in adult uterus, prostate and brain regions involved in gonadal control and sexual behaviour. Female sexual behaviour was impaired by both filters; 3-benzylidene camphor caused irregular cycles. Classical endpoints exhibited lowest observed adverse effect levels (LOAELs) and no observed adverse effect levels (NOAELs) of 7/0.7 mg/kg for 4-MBC and 0.24/0.07 mg/kg for 3-BC. Molecular endpoints were affected by the lowest doses studied. Our data indicate that the potential risk posed by endocrine active UV filters warrants further investigations.

Ultraviolet filters in environment and biosphere

Ultraviolet (UV) filters are either organic molecules (chemical UV filters) absorbing light in the UV range (UVA 400–320 nm, UVB 320–280 nm) or physical UV filters like titanium dioxide and zinc oxide, which mainly scatter and reflect UV rays. Only substances listed in cosmetics directives are allowed for use. Technical UV filters used in plastics and other products need not be declared, in spite of considerable structural similarities with authorized cosmetic UV filters.

With increasing use of UV filters, the possible environmental impact of sunscreen ingredients deserves consideration. There is good evidence that pharmaceuticals and ingredients of personal care products, including chemical UV filters, can spread to the biosphere and reach the food chain (Balmer *et al.*, 2005; Kupper *et al.*, 2006; Nakata *et al.*, 2007; Schmid *et al.*, 2007). Recent studies indicate that chemical UV filters are present at high concentrations in sewage sludge (Kupper *et al.*, 2006). Highest levels (January, July) were observed for octocrylene [320–18740 μg/kg dry material (d.m.)] and octyltriazone (700–27700 μg/kg d.m.); levels of 4-methylbenzylidene camphor (4-MBC) and ethylhexyl cinnamate (EHMC, OMC) were 150–4980 μg/kg d.m. and 10–390 μg/kg d.m., respectively. UV filters are released into surface waters by waste water treatment plants (WWTPs) and by swimming (Balmer *et al.*, 2005), and accumulate in fish (Nagtegaal *et al.*, 1997; Buser *et al.*, 2006). UV filter levels in fish from rivers receiving inputs from WWTPs were considerably higher (4-MBC: 1800 ng/g) than in fish from lakes with inputs from WWTPs (Buser *et al.*, 2006),

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suggesting increased availability of these contaminants for fish in rivers.

Humans may be exposed to UV filters directly by use of cosmetics, or indirectly through the food chain. Exposure of populations is most reliably assessed by measuring internal exposure levels. In a monitoring study on human milk conducted in collaboration with a Swiss university hospital, we detected chemical UV filters in 75% of the samples, besides synthetic perfumes, brominated flame retardants and other pollutants (Schlumpf *et al.*, unpublished data).

Endocrine activity of UV filters in vitro and in vivo

Following our report on estrogenic activity of UV filters used in sunscreens in vitro and in the uterotrophic assay (Schlumpf *et al.*, 2001a), this finding was confirmed in a number of in vitro and in vivo test systems on mammals and fish (Holbech *et al.*, 2002; Schreurs *et al.*, 2002; Tinwell *et al.*, 2002; Inui *et al.*, 2003; Mueller *et al.*, 2003; Jarry *et al.*, 2004; Klammer *et al.*, 2005; Kunz *et al.*, 2006). Certain UV filters also display anti-androgenic activity in vitro (Ma *et al.*, 2003; Schreurs *et al.*, 2005). Interactions with the thyroid axis were also observed (Schlumpf *et al.*, 2004b; Schmutzler *et al.*, 2004; Maerkel *et al.*, 2007). Chronic studies in ovariectomized rats (Seidlova-Wuttke *et al.*, 2005, 2006) and developmental toxicity studies (below) demonstrated that different hormone targets are affected by UV filters.

Role of oestrogens in early sexual differentiation

Ontogenesis is a highly sensitive life stage for estrogenic or anti-androgenic chemicals because sex hormones exert organizing actions on the developing organism. Development of the male phenotype depends on testosterone (T), but is not exclusively mediated by androgen receptors (AR). In some targets such as brain, T is converted to estradiol (E2) (MacLusky & Naftolin, 1981; Lauber & Lichtensteiger, 1994), and the developmental action (male sexual brain differentiation) is achieved by E2 acting on oestrogen receptors (ER) (MacLusky & Naftolin, 1981). Recent observations in aromatase knockout mice indicate that development of the female brain also depends on E2, in contrast to the classical theory (Bakker *et al.*, 2002). Certain peripheral male tissues such as prostate can convert T to dihydrotestosterone acting on AR as well as to E2 acting on ER (George, 1993; Pezzi *et al.*, 2003), providing sites of action for estrogenic as well as anti-androgenic chemicals. With the exception of tissues with local conversion of T to E2, endogenous oestrogen levels are very low in foetuses of both sexes in rodents (Habert & Picon, 1984) and also comparatively low in humans (Siler-Khodr, 1998), thus facilitating competition of weak xenoestrogens for ER.

Developmental toxicity of 4-MBC and 3-benzylidene camphor

The potential relevance of exposure to estrogenic UV filters was investigated in a mammalian model, the rat, using 4-MBC and 3-benzylidene camphor (3-BC) (Schlumpf *et al.*, 2004b; Durrer *et al.*, 2005, 2007; Maerkel *et al.*, 2005, 2007). Both compounds exhibit ER beta preference (Schlumpf *et al.*, 2004a), but are also active in ER alpha-typical tests, 4-MBC possibly because of a hydroxylated metabolite (Völkel *et al.*, 2006). Male and female rats of the parent generation and their offspring were exposed to 4-MBC (47, 24, 7.0, 0.7 mg/kg/day) or 3-BC (7.0, 2.4, 0.7, 0.24, 0.07 mg/kg/day) administered in chow at least 10 weeks before mating, during pregnancy and lactation, and until adulthood of the F1 generation. 47 mg/kg 4-MBC corresponds to 40% of uterotrophic lowest observed adverse effect level (LOAEL), 3-BC was dosed according to uterotrophic potency relative to 4-MBC. The present report focuses on data from neonatal and young adult (12-week-old) offspring; 13-month-old offspring were also studied.

Early postnatal period, low-dose effects, and puberty

Both UV filters reduced survival rate at higher doses (Schlumpf *et al.*, 2001b, 2004b). A decrease in thymus weight at postnatal day (PN) 14 (day of birth = PN 1) signals an impairment of the immune system. Recent studies on low-dose effects in the neonate revealed that oestrogen target gene mRNA levels in early postnatal uterus (PN 6), determined by real-time reverse transcription polymerase chain reaction (RT PCR) (Durrer *et al.*, 2005), are affected at doses as low as 0.07 mg/kg 3-BC and 0.7 mg/kg 4-MBC (Table 2). The same doses also affected gene expression in PN 6 brain (M. Fuetsch, C. Gaille, unpublished data). The changes in mRNAs encoding for vascular epithelial growth factor, inducible nitric oxide synthase and, in part, endothelial cell nitric oxide synthase suggest that angiogenesis and blood flow may be influenced. Effects on the developing prostate were demonstrated in the PN 1 male. 4-MBC increased number of ducts in dorsal prostate and duct volume in ventral prostate (Table 1, Hofkamp *et al.*, 2007, and unpublished data).

Puberty was delayed in males (preputial separation), but unaffected in females (Tables 1 and 2, Durrer *et al.*,

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Table 1 Effect of 4-methylbenzylidene camphor and 3-benzylidene camphor on selected endpoints in male rat offspring

	4-methylbenzylidene camphor (mg/kg p.o.)				3-benzylidene camphor (mg/kg p.o.)				
	0.7	7	24	47	0.07	0.24	0.7	2.4	7
Puberty (preputial separation)		Delayed	Delayed	Delayed		Ø	Ø	Delayed	Delayed
Adult body weight	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	↓
Testis									
Postnatal day 14, testis relative weight		↓	↓	↓					
Adult F1, testis relative weight	Ø	Ø	Ø	↑		Ø	Ø	Ø	Ø
Prostate									
Postnatal day 1, duct number (dorsal) and duct volume (ventral)	Ø	↑			Ø	Ø			
Adult F1, ventral lobe relative weight-	Ø	↓	↓	↓	Ø	↓	Ø	Ø	Ø
Gene expression, adult F1 prostate, mRNA/protein									
Androgen receptor (AR) dorsolateral prostate (DP)	Ø/Ø	↓/Ø	↓/↓	↓/(↓)		↑/↓	Ø/↓	Ø/–	↑/–
Androgen receptor (AR) ventral prostate (VP)	Ø/Ø	Ø/(↓)	↓/↓	Ø/–		↑/↑	↑/Ø	↑/–	Ø/–
AR mRNA down-regulation by estradiol in VP		↓	↓						
N-CoR protein, DP	↓	↓	(↓)			↓	↓		
N-CoR protein, VP	Ø	(↓)	↓			Ø	Ø		
Central nervous system, adult F1, mRNA									
Gene expression in ventromedial hypothalamic nucleus									
Oestrogen receptor-alpha		Ø	↓	↓		↑	↑	Ø	Ø
Progesterone receptor		Ø	Ø	Ø		Ø	Ø	↑	↑

Ø, no statistically significant change; blank (or) –, not analysed; PN 1, day of birth; Adult F1 offspring, 12 weeks of age, studied under baseline conditions, females in diestrus. Data from Schlumpf *et al.*, 2001b, 2004b, Durrer *et al.*, 2007; Maerkel *et al.*, 2005, 2007, Hofkamp *et al.*, 2007; Lichtensteiger *et al.*, 2007; O. Faass, M. Fuetsch, C. Ehnes, C. Gaille, unpublished data.

2007; Schlumpf *et al.*, 2004b). 4-MBC and 3-BC thus mimic the typical effect of E2 only in males, but differ from E2 in females, where E2 advances puberty (Biegel *et al.*, 1998). Body weight at puberty was slightly reduced in females but normal in males, indicating that the delay of male puberty did not result from nutritional effects. Adult body weights remained unaffected by 4-MBC but slightly reduced by the highest dose of 3-BC, possibly as a result of estrogenic activity (Biegel *et al.*, 1998).

Reproductive organs of adult offspring: regulation of target gene expression

Exposure to 4-MBC and 3-BC affected reproductive organ weights (Tables 1 and 2, Durrer *et al.*, 2005, 2007; Schlumpf *et al.*, 2004b). Testes of 4-MBC-exposed offspring showed decreased weight at PN 14 (Schlumpf *et al.*, 2001b) and increased relative weight at the highest dose in adulthood. The adult finding is reminiscent of neonatal administration of weak oestrogens (Atanassova *et al.*, 2000; Putz *et al.*, 2001). In contrast, the decrease in prostate weight of 4-MBC-exposed offspring resembles the effect of perinatal administration of the potent ER agonist diethylstilbestrol (vom Saal *et al.*, 1997; Atanassova *et al.*, 2000). This suggests a differential sensitivity of male target organs. Interactions of higher 4-MBC doses with the thyroid axis were also noted (Maerkel *et al.*, 2007).

Gene expression was affected in ventral and dorsolateral prostate and uterus at mRNA and protein levels in a tissue-specific manner (Durrer *et al.*, 2005, 2007). The 4-MBC-induced decrease in prostate weight was accompanied by a decrease in AR, ER alpha, and insulin-like growth factor-I (IGF-I) (Table 1). In 4-MBC-exposed uterus, affected target genes include ER alpha and progesterone receptor (PR) (Table 2). Effect patterns differed between the two camphor derivatives also at the molecular level, in spite of close structural relationship and similar actions in acute assays.

To assess changes in sensitivity to oestrogens, adult offspring were gonadectomized and injected with a single dose of E2 (10 or 50 μg/kg s.c.). 4-MBC reduced up-regulation of PR and IGF-I and down-regulation of ER alpha and AR mRNA in uterus 6 h after E2, and down-regulation of AR and IGF-I mRNA in ventral prostate (Table 1, Durrer *et al.*, 2005, 2007). Decreased steroid receptor coactivator-1 (SRC-1) protein levels accompanied the reduced up-regulation of oestrogen target genes in uterus, while reduced repression of genes in prostate was paralleled by reduced nuclear receptor corepressor (N-CoR) protein (Durrer *et al.*, 2005, 2007). This identifies nuclear

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Table 2 Effect of 4-methylbenzylidene camphor and 3-benzylidene camphor on selected endpoints in female rat offspring

	4-methylbenzylidene camphor (mg/kg p.o.)				3-benzylidene camphor (mg/kg p.o.)				
	0.7	7	24	47	0.07	0.24	0.7	2.4	7
Puberty (vaginal opening)		Ø	Ø	Ø		Ø	Ø	Ø	Ø
Adult body weight	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	↓
Ovary									
Adult F1, ovary relative weight	Ø	Ø	↑	↑					
Uterus									
Postnatal day 6, uterus relative weight	Ø	Ø			Ø	Ø			
Adult F1, uterus relative weight	Ø	Ø	↑	Ø		Ø	Ø	Ø	↓
Postnatal day 6 uterus, gene expression, mRNA									
Oestrogen receptor-alpha	↓	Ø			↓	↓			
ecNOS	Ø	Ø			↓	(↓)			
iNOS	↓	↓			↓	↓			
VEGF	↓	Ø			↓	↓			
Adult F1 uterus, gene expression, mRNA/protein									
Progesterone receptor (PR-A protein)	Ø/↓	Ø/Ø	↓/Ø	↓/Ø		Ø/↑	Ø/ Ø	Ø/ Ø	↑/Ø
PR mRNA up-regulation by estradiol	Ø	↓	↓						
SRC-1 protein	↓	Ø	(↓)	↓		Ø	(↓)	↓	Ø
Central nervous system, adult F1, mRNA									
Gene expression in ventromedial hypothalamic nucleus									
Oestrogen receptor-alpha		↓	↓	↓		Ø	Ø	↑	↑
Progesterone receptor		↓	↓	↓		↑	Ø	↓	↓
PR mRNA up-regulation by estradiol		↑	Ø						
Female sexual behaviour (proceptive and lordosis behaviour)		↓	↓					↓	↓
Oestrous cycle		Ø	Ø	Ø		irregular	irregular	irregular	irregular

VEGF, vascular epithelial growth factor; iNOS, inducible nitric oxide synthase; ecNOS, endothelial cell nitric oxide synthase; SRC-1, steroid receptor coactivator-1; Ø, no statistically significant change; blank, not analysed; PN 1, day of birth; Adult F1 offspring, 12 weeks of age, studied under baseline conditions, females in diestrus. Data from Schlumpf *et al.*, 2001b, 2004b, Durrer *et al.*, 2005; Maerkel *et al.*, 2005, 2007, Lichtensteiger *et al.*, 2007; O. Faass, M. Fuetsch, C. Ehnes, C. Gaille, unpublished data.

receptor coregulators as targets of endocrine receptors and suggests that they are involved in changes in oestrogen sensitivity.

Sexually dimorphic gene expression in brain and female sexual behaviour

On the basis of data on brain differentiation outlined above, we hypothesized that the female brain should be particularly sensitive to estrogenic chemicals. Sexual behaviour of female offspring exposed to 4-MBC (7 and 24 mg/kg) or 3-BC (2.4 and 7 mg/kg) was strongly impaired (Table 2, Lichtensteiger *et al.*, 2007; Faass *et al.*, unpublished data). The treatment affected proceptive behaviour (jumping and ear wiggling) displayed to attract the male, as well as receptive (lordosis) behaviour (decreased lordosis quotient LQ = number of lordosis responses/number of mounts × 100). In 4-MBC-exposed offspring, female sexual behaviour was disturbed in the presence of normal oestrous cycles, whereas 3-BC exposure caused irregular cycles. The two functions thus are differentially affected.

Gene expression was analysed by real-time RT PCR in adult male and female offspring in two brain regions involved in the control of gonadal function and sexual behaviour, medial pre-optic region (MPO) and ventromedial hypothalamic nucleus (VMH) (Tables 1 and 2, Maerkel *et al.*, 2005, 2007; Lichtensteiger *et al.*, 2007; Faass *et al.*, unpublished data). Both compounds caused sex- and region-specific changes in ER, nuclear receptor coregulator and target gene mRNA levels in MPO and VMH. A drop of PR mRNA in female VMH down to male levels emerged as a common feature observed after all doses of 4-MBC and after the higher two doses of 3-BC. Reduced PR mRNA in female VMH was correlated with impaired female sexual behaviour. A similar relationship was observed with a brominated flame retardant, PBDE 99, and with a polychlorinated biphenyl (PCB) mixture (Lichtensteiger *et al.*, 2004). Lordosis behaviour is directly correlated with expression of PR mRNA in female rat VMH (Pollio *et al.*, 1993; Ogawa *et al.*, 1994). Loss of

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sexual dimorphism of PR in female VMH thus appears to represent a signal of altered regulation of PR that is linked with behavioural impairment across different endocrine disrupters.

Considerations regarding human risk

These data indicate that pre- and postnatal exposure to 4-MBC and 3-BC can interfere with sexual development at brain and reproductive organ levels. One approach of risk analysis is the comparison of external exposures: Classical endpoints exhibited lowest observed adverse effect levels/no observed adverse effect levels (LOAELs/NOAELs) of 7/0.7 mg/kg for 4-MBC and 0.24/0.07 mg/kg for 3-BC. Molecular endpoints were affected by the lowest doses studied (Tables 1 and 2). The classical LOAEL and NOAEL of 4-MBC were 30 and three times, respectively, above an estimated human exposure level of 0.23 mg/kg (SCCNFP 1998). As estimates of external human exposure are based upon indirect calculations, a comparison of internal exposure levels from animal experiments and epidemiological studies would seem to be more reliable. Analysis of human milk provides information on internal exposure of mother and foetus and on quality and magnitude of pollution of the food provided to the nursing infant. At the LOAEL dose of 7 mg/kg 4-MBC, levels in rat milk (Schlumpf *et al.*, 2004b) were only 13.4 times the highest value so far found in our ongoing human milk monitoring study (19 ng/g milk fat, Schlumpf *et al.*, unpublished data). With both approaches, the margin of safety (MOS = NOAEL/exposure × 100), normally set at 100, would not be met. This indicates that the potential risk posed by UV filters warrants further investigations.

Acknowledgements

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Panel discussion

S. Swan

What is the half-life of these UV filters in the human body?

M. Schlumpf

The manufacturer's data suggest a relatively short half life of several weeks. The substances can enter the food chain. The half-life in animals is poorly documented but is different for different animals.

T. Søeborg

When you compare levels of these substances in human and rat milk should you not expose them in the same way? I suspect that humans are exposed topically and rats orally.

M. Schlumpf

It is impossible to apply these substances to rats topically because of their fur. Not all human exposure is through the skin: to some degree cosmetics are absorbed from the food chain. The content of perfumes is not declared because of manufacturer's confidentiality but all contain two to four UV filters to protect the colour. Lipstick contains many UV filters. Some of these compounds enter the food chain but the magnitude of this route is not known.

A. Soto

These substances can be screened by E-screen and androgen bioassay. You measure the total xenobiotics burden in milk, but have you tried to assess the total burden by activity, such as oestrogenic activity, antiandrogen activity, either by using bioassays or estimation per level and relative potency?

M. Schlumpf

This is a good suggestion for the future. At present we are fully occupied in the analysis of xenobiotics in milk.

A. Soto

Has Dr Margaret Schlumpf in her studies on UV filters assessed human fat for the burden of these chemicals? UV filters are found in fish, and samples of human fat are readily available.

M. Schlumpf

We looked at the concentration of these substances in milk but not fat.

P. Foster

The concentration of a wide variety of chemicals in addition to sunscreens, including chlorinated pesticides, are present in human milk often at a high TDI which is greater than the recommended exposure level. Should we therefore state that breast feeding is not recommended?

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M. Schlumpf

This is a difficult question to answer. It has been policy for nurses to encourage strongly absolute breast feeding and now we tell the mothers that there are possible dangers associated with breast milk. Nurses and mothers are confused and it is now difficult to give clear guidelines and recommendations. The whole aspect of breast feeding must be considered in depth.

A.A. Jensen

After 3–4 months of breast feeding, the baby has the same serum concentration of pollutants as the mother, and after 4 months the levels are higher in the baby than the mother by a factor of up to 2–3 times. Perhaps the old advice from WHO is best which recommends 4 months breast feeding. The situation is different in developing countries where bottle feeding is relatively very expensive, and the impurities in the water supply may be more dangerous than the contaminants in breast milk. In developed countries, it is perhaps not wise to allow the baby to have higher concentrations of endocrine disrupters than the mother.

A. Soto

It is possible, therefore, that the recommendation in USA for 6 months breast feeding is unnecessary. This recommendation was brought in around 1950 when breast feeding was unusual, and no terrible consequences have been identified. Perhaps a compromise approach is advisable.

H. Leffers

Perhaps obesity is linked to ‘‘non breast feeding’’ the children.

E. Gregorasczczuk

There are data to indicate that a woman clears her body stores of Dioxins, PCBs and brominated compounds through lactation. The amount of these substances passed to the infant is dependent on the age of the mother, the rank of the baby (first or subsequent born), and the time between pregnancies. The first child receives the entire body load accumulated since the mother was born, and the second baby receives the amount accumulated between pregnancies.

P. Bjerregaard

The Baltic sea in the 1970s and 1980s was heavily contaminated with PCBs, DDT and other chemicals but these levels have markedly reduced in the last 20 years. In the early 1980s, a Danish committee discussed the recommendations for breast feeding and only a minority advised against it because of the levels of PCBs and DDT in breast milk. Infants breast fed at that time, especially if fish was a large part of the diet, are now suitable for assessing possible brain damage caused by PCBs in infancy. The situation is much better today now that these substances are much less in the environment and breast milk.

S. Swan

I am alarmed by the direction of this discussion. The adverse effects of low level exposure to endocrine disrupting chemicals in breast milk are under investigation at present. However, the nutritional, immunological and psychological benefits of breast feeding are well established and not disputed. The alternatives to breast milk may not be advantageous. What is the hormonal content of casein-based formula milk, cow’s milk or soy formula substitutes? This is a very complicated situation and even water must be considered as a source of pollutants. We are not yet in a position to recommend changes to breast feeding which has well established health advantages.

M. Schlumpf

We must be very careful about assessing the data before acting. Milk can be contaminated with cosmetics and UV filters, but these can be controlled during lactation to reduce the exposure of the infants.

H. Patisaul

Soy infant formula is used by about one third of mothers in USA and this proportion is increasing, but this product contains phytoestrogens which can give levels in the infant’s plasma 7,000 time greater than the endogenous oestradiol level in the mother. This is much higher than the levels encountered in the endocrine disrupters under discussion at this Workshop, and in vitro studies indicate that these phytoestrogens have much higher binding affinity for ER. This is a complex issue. The infants must drink something and I do not know of anything better than breast milk.

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2-Hydroxy-4-methoxybenzophenone

CAS #131-57-7
Swiss CD-1 mice, at 0.0, 1.25, 2.5, and 5.0% in feed
Robert Chapin, NTP/NIEHS Project Officer
Dushyant Gulati and Robin Mounce,
Environmental Health Research and Testing
Started 9/28/88; Completed 1/8/91
NTIS #PB91158477

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2-Hydroxy-4-methoxybenzophenone (HMBP), a common ingredient in sunscreens and tanning agents, was found in a preliminary study in B6C3F1 mice to reduce epididymal sperm density by approximately 25%, and to slightly increase estrous cycle length. This stimulated a further evaluation of its potential to cause reproductive toxicity in Swiss CD-1 mice using the RACB protocol. Data on food and water consumption, body weights, and clinical signs during a 2-week dose-range-finding study (Task 1) were used to set exposure concentrations for the Task 2 continuous cohabitation phase at 0.0, 1.25, 2.5, and 5.0% in feed. Based on body weight and feed consumption data, the estimated average daily dose values were approximately 1.8, 4, and 9 g/kg body weight.

For the first generation, body weights dropped in females in both the middle and high doses (13 and 18%) but in males only at the high concentration (by 8%). Interestingly, food consumption increased in all groups for weeks 1, 2, 6, and 10, but only in the high dose group at week 14.

The number of live pups per litter dropped by 22 and 23%, respectively, in the medium and high dose groups, although there was no change in average litters per pair or in the adjusted live pup weights. Cumulative days to litter were increased for only the high dose animals, by 11, 9, and 7% for litters 1, 2, and 3, respectively. As the number of most severely affected pairs stopped having litters, the days to deliver for litters 4 and 5 became the same as controls.

The Task 3 crossover was not conducted in this study.

There were adverse effects noted during the rearing of the last litter for second-generation evaluation. There were unexpected deaths of lactating dams (n=5, 4, and 9 in low to high dose groups). These deaths were not preceded by clinical signs of illness; most happened after the pups were greater than or equal to 14 days of age. Because of this, preweaning mortality was increased by approximately 30% and approximately 33%, respectively. At weaning, pups from the remaining dams in the middle dose and high dose groups had lower body weights, by approximately 20 and 40%, respectively. The loss of these animals did not jeopardize the ability to obtain sufficient numbers of nonsibling animals for the Task 4 mating trial.

The F_1 mice from control, middle, and high dose levels were evaluated for reproductive success. While there was no change in the mating, pregnancy, or fertility indices, number of pups per litter or in the viability of those pups, F_2 pup weight adjusted for litter size was reduced by 7 and 10%, respectively, while F_1 dam weights in those dose groups were reduced by 7 and 15%, respectively.

After the delivery of the F_2 litters, and the collection of estrous cycle data from the females, the control and high dose F_2 mice were killed and necropsied. Male body weight and testis weight were reduced by 14 and 6%, respectively. Adjusted liver weight was increased by 50%; adjusted prostate weight was increased by approximately 30%. Epididymal sperm number, motility, and morphology were similar in the two groups. Female body weight was reduced by 11%, while adjusted liver weight and kidney weight were increased by approximately 30 and 10%, respectively. Estrous cycle length was unchanged by exposure to 5% HMBP in the diet. There were no remarkable microscopic findings.

These data show that these high levels of 2-hydroxy-4-methoxybenzophenone in the diet of Swiss CD-1 mice were correlated with reduced pup weight and number and increased mortality of lactating dams. These reproductive effects occurred at levels of 2-hydroxy-4-methoxybenzophenone that reduced body weight, and, at the high dose, increased liver and kidney weights.

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **PB91158477**
Chemical: **2-Hydroxy-4-methoxybenzophenone**
CAS#: **131-57-7**
Mode of exposure: **Feed**
Species/strain: **Swiss CD-1 mice**

F_0 generation — Dose concentration →	1.25%	2.5%	5.0%
General toxicity	**Male, female**	**Male, female**	**Male, female**
Body weight	— , —	— , ↓	↓ , ↓
Kidney weight[a]	•	•	•
Liver weight[a]	•	•	•
Mortality	— , —	— , —	— , —
Feed consumption	↑/— , ↑/—	↑/— , ↑/—	↑ , ↑
Water consumption	•	•	•
Clinical signs	— , —	— , —	— , —

Reproductive toxicity			
x̄ litters/pair	—	—	—
# live pups/litter; pup wt./litter	— , —	↓ , —	↓ , —
Cumulative days to litter	—	—	↑
Absolute testis, epididymis weight[a]	•	•	•
Sex accessory gland weight[a] (prostate, seminal vesicle)	•	•	•
Epidid. sperm parameters (#, motility, morphology)	•	•	•
Estrous cycle length	•	•	•

Determination of affected sex (crossover)	Male	Female	Both
Dose level	•	•	•

F_1 generation — Dose concentration →	1.25%	2.5%	5.0%
General toxicity	**Male, female**	**Male, female**	**Male, female**
Pup growth to weaning	— , —	↓ , ↓	↓ , ↓
Mortality	— , —	↑ , ↑	↑ , ↑
Adult body weight	•	↓ , ↓	↓ , ↓
Kidney weight[a]	•	•	— , ↑
Liver weight[a]	•	•	↑ , ↑
Feed consumption	•	↑ , ↑	↑ , ↑
Water consumption	•	•	•
Clinical signs	— , ↑	— , ↑	— , ↑

Reproductive toxicity			
Fertility index	•	—	—
# live pups/litter; pup wt./litter	•	— , ↓	— , ↓
Absolute testis, epididymis weight[a]	•	•	↓ , ↑
Sex accessory gland weight[a] (prostate, seminal vesicle)	•	•	↑ , —
Epidid. sperm parameters (#, motility, morphology)	•	•	— , — , —
Estrous cycle length	•	•	—

Summary information	
Affected sex?	**Unclear**
Study confounders:	**Lactating dam death**
NOAEL reproductive toxicity:	**1.25%**
NOAEL general toxicity:	**≤1.25%**
F_1 more sensitive than F_0?	**Unknown**
Postnatal toxicity:	**Yes**

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); — , —, no change in males or females. [a]Adjusted for body weight.

45) Am J Epidemiol. 2014 Dec 15;180(12):1168-75. doi: 10.1093/aje/kwu285. Epub 2014 Nov 13.

Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples' fecundity.

Buck Louis GM, Kannan K, Sapra KJ, Maisog J, Sundaram R.

Abstract
Concern has arisen about benzophenone (BP) ultraviolet (UV) radiation filters, given their use in sunscreen and personal-care products and their reported estrogenic and antiandrogenic activity. We recruited 501 couples who were discontinuing use of contraceptives in order to become pregnant for the Longitudinal Investigation of Fertility and the Environment (LIFE) Study (Michigan and Texas, 2005-2009). Couples provided urine specimens and completed daily journals until they either achieved pregnancy or had tried for 12 months. Women used fertility monitors to time sexual intercourse and digital pregnancy tests. Urinary concentrations of 5 UV filters (ng/mL) were determined using triple-quadrupole mass spectrometry: 2,4-dihydroxybenzophenone (also called BP-1); 2,2',4,4'-tetrahydroxybenzophenone (BP-2); 2-hydroxy-4-methoxybenzophenone (BP-3); 2,2'-dihydroxy-4-methoxybenzophenone (BP-8); and 4-hydroxybenzophenone. Fecundability odds ratios were estimated for each UV filter (dichotomized at the 75th percentile) and adjusted for age, creatinine concentration, body mass index (weight (kg)/height (m)(2)), cotinine concentration, season, and site, while accounting for time off contraception. Separate models were fitted for each UV filter and partner; final models included partners' concentrations. Male partners' concentrations of BP-2 and 4-hydroxybenzophenone were associated with reduced fecundity in adjusted models (fecundability odds ratio (FOR) = 0.69 (95% confidence interval (CI): 0.50, 0.95) and FOR = 0.74 (95% CI: 0.54, 1.00), respectively). In models adjusting for both partners' concentrations, male BP-2 concentration remained associated with reduced fecundity (FOR = 0.69, 95% CI: 0.49, 0.97). These data suggest that male exposure to select UV filters may diminish couples' fecundity, resulting in a longer time to pregnancy.
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Urinary concentrations of benzophenone-type ultraviolet light filters and semen quality.

Buck Louis GM1, Chen Z2, Kim S2, Sapra KJ3, Bae J4, Kannan K5.
Author information
1 Office of the Director, Division of Intramural Population Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, Maryland. Electronic address: louisg@mail.nsih.gov.
2 Biostatistics and Bioinformatics Branch, Division of Intramural Population Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, Maryland.
3 Office of the Director, Division of Intramural Population Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, Maryland.
4 Office of the Director, Division of Intramural Population Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, Maryland; Department of Preventive Medicine, Catholic University of Daegu School of Medicine, Daegu, Republic of Korea.
5 Wadsworth Center, New York State Department of Health, Albany, New York; Department of Environmental Health Sciences, School of Public Health, The University at Albany-SUNY, Albany, New York.

Abstract
OBJECTIVE:
To assess benzophenone-type ultraviolet (UV) filter concentrations, chemicals used in sunscreen and personal care products, and semen endpoints.
DESIGN:
Cohort.
SETTING:
Not applicable.
PATIENT(S):
A total of 413 men provided semen and urine samples, 2005-2009. Five UV filters were quantified (ng/mL) in urine using liquid chromatography-triple quadrupole mass spectrometry: BP-1 (2,4-dihydroxybenzophenone), BP-2 (2,2',4,4'-tetrahydroxybenzophenone), BP-3 (2-hydroxy-4-methoxybenzophenone), BP-8 (2,2'-dihydroxy-4-methoxybenzophenone), and 4-OH-BP (4-hydroxybenzophenone). Using linear regression, β-coefficients (β) and 95% confidence intervals (CIs) for each chemical dichotomized at the 75th percentile and Box-Cox transformed semen endpoint were estimated, after adjusting for age, body mass index, cotinine, season, and site.
INTERVENTION(S):
None.
MAIN OUTCOME MEASURE(S):
Thirty-five semen endpoints.
RESULT(S):
BP-2 was associated with diminished sperm concentration (β = -0.74; 95% CI -1.41, -0.08), straight (β = -4.57; 95% CI -8.95, -0.18) and linear movement (β = -3.15; 95% CI -6.01, -0.30), more immature sperm (β = 0.38; 95% CI 0.15, 0.62), and a decreased percentage of other tail abnormalities (β = -0.16; 95% CI -0.31, -0.01). BP-8 was associated with decreased hypo-osmotic swelling (β = -2.57; 95% CI -4.86, -0.29) and higher acrosome area (β = 1.14; 95% CI 0.01, 2.26). No associations were observed for BP-1, BP-3, or 4OH-BP.
CONCLUSION(S):
The findings suggest that specific UV filters may be associated with some aspects of semen endpoints, but await future corroboration.