County Clerk

From: Joe DiNardo <jmjdinardo@aol.com>
Sent: Sunday, November 26, 2017 6:47 AM
To: IEM Committee; County Clerk
Cc: cadowns@haereticus-lab.org
Subject: Dermatology Paper - Oxybenzone Review 4 of 4
Attachments: 22 CDC Fourth Report Updated Benzophenone-3 Tables Volume1 Jan2017.pdf; 23 Meeker et al EnvSciTech 2013 urine_conc_UV_filt_in_Puerto_Rican_women.pdf; 24 Tina Harmer Lassen Danish Men.docx; 25 BP3 Metabolites Env Int 2014_Kim and Choi.pdf; 26 Huo Oxybenzone mechanism for causing Hirschsprung Disease in human infants.pdf; 27 IARC monograph 101 Benzophenone.pdf

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:

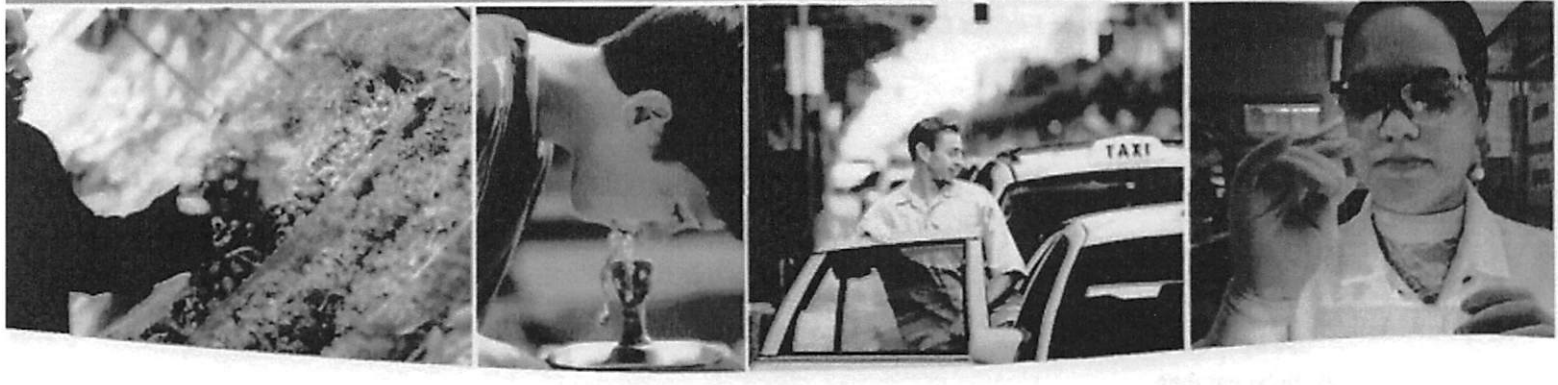
1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone

2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

2017

Fourth National Report on Human Exposure to Environmental Chemicals

Updated Tables, January 2017, Volume One



U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Complete Table of Contents - Volumes One and Two

Volume One

What's New and Different?

The *Updated Tables, January 2017,* include chemicals that have results available from the NHANES survey periods 2005-2006, 2007-2008, 2009-2010, and 2013-2014. No new 2011-2012 data were released for this *Updated Tables*. New chemicals measured for the first time include the herbicide atrazine and five of its metabolites, the insect repellent DEET and two of its metabolites, triclocarban, branched and linear isomers of both PFOS and PFOA, and six blood VOCs: 1,1,1,2-tetrachloroethane; 1,2,3-trichloropropane; 1,2-dibromoethane; furan; isopropylbenzene (cumene); and nitromethane. A new chemical group created in 2013-2014 is the Personal Care and Consumer Product Chemicals and Metabolites, which reflects the chemical uses and combines three previous groups: Environmental Phenols, Parabens, and Other Pesticide Metabolites. The chemical group previously called Perfluorinated Compounds: Surfactants has been renamed as Perfluoroalkyl and Polyfluoroalkyl Substances: Surfactants.

Chemicals with updated data in this release are

- adducts of hemoglobin (acrylamide and glycidamide);
- blood VOCs, including disinfection by-products;
- urinary PBA, benzophenone-3, triclosan, four parabens, 2,4-dichlorophenol, and 2,5-dichlorophenol;
- whole blood metals and mercury species;
- serum metals;
- urinary metals and arsenic species;
- urinary perchlorate, nitrate, and thiocyanate;
- serum perfluoroalkyl and polyfluoroalkyl substances (PFAS);
- urinary polycyclic aromatic hydrocarbon metabolites;
- serum polychlorinated dibenzo-*p*-dioxins (pooled);
- serum polychlorinated dibenzofurans (pooled);
- serum dioxin-like polychlorinated biphenyls: coplanar PCBs (pooled).

CAS Registry Numbers (CAS Nos.)

With this *Updated Tables*, we are including CAS registry numbers in the Table of Contents placed along with the chemical name. Not all chemicals have a CAS No. assigned, and there is no number for chemicals that have values calculated from two or more species (e.g., arsenic) or isomers (e.g., PFOS, PFOA).

Updated Tables in Two Volumes

In this release, we have expanded the *Updated Tables* to **two volumes**, each as a separate PDF. This change was made because the file size of the single PDF had become so large that it could not easily be shared or sent as an attachment. **Volume One** contains data tables for most of the chemicals measured in the U.S. general population. **Volume Two** contains data tables for the persistent organic pollutants and pesticides previously measured in individual samples and are currently measured in pooled samples. **Volume Two** also contains data tables for the special sample of adult cigarette smokers and nonsmokers, including recently released data

Urinary Benzophenone-3 (2003 – 2010)

Geometric mean and selected percentiles of urine concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

	Survey years	Geometric mean (95% conf. interval)	50th Percentile (95% conf. interval)	75th Percentile (95% conf. interval)	90th Percentile (95% conf. interval)	95th Percentile (95% conf. interval)	Sample size
Total	03-04	**22.9** (18.1-28.9)	**18.1** (15.5-23.2)	**94.0** (67.5-123)	**370** (225-570)	**1040** (698-1390)	2517
	05-06	**19.4** (16.5-22.8)	**14.1** (12.5-17.5)	**68.8** (54.5-87.2)	**433** (294-597)	**1150** (936-1510)	2548
	07-08	**18.3** (13.7-24.3)	**14.3** (10.7-19.8)	**64.1** (42.8-116)	**365** (223-524)	**801** (471-2080)	2604
	09-10	**22.3** (18.0-27.7)	**16.4** (12.7-20.5)	**81.2** (55.0-126)	**493** (320-742)	**1750** (931-3120)	2749
Age group							
6-11 years	03-04	**21.2** (16.4-27.3)	**17.2** (14.9-25.9)	**66.7** (38.7-102)	**158** (106-246)	**246** (154-618)	314
	05-06	**21.2** (14.3-31.3)	**19.4** (11.7-26.1)	**58.8** (40.5-102)	**259** (115-433)	**852** (355-2200)	356
	07-08	**24.1** (15.0-38.7)	**17.2** (11.8-25.0)	**79.9** (32.9-168)	**392** (129-1610)	**1410** (165-43600)	389
	09-10	**21.7** (15.6-30.2)	**14.6** (12.0-19.7)	**52.7** (27.2-119)	**470** (80.7-1900)	**1570** (153-6440)	415
12-19 years	03-04	**22.9** (18.0-29.3)	**20.1** (16.1-25.1)	**67.1** (45.2-93.8)	**170** (137-240)	**407** (183-717)	715
	05-06	**21.2** (15.8-28.4)	**14.9** (12.1-21.4)	**64.3** (39.7-95.3)	**227** (130-382)	**633** (252-1260)	702
	07-08	**21.5** (16.0-28.8)	**15.0** (11.9-21.3)	**59.3** (36.7-111)	**214** (113-613)	**613** (206-1350)	401
	09-10	**23.3** (16.6-32.5)	**16.6** (13.1-19.7)	**62.3** (35.1-129)	**423** (184-843)	**988** (550-2670)	420
20 years and older	03-04	**23.1** (18.0-29.6)	**18.1** (14.7-23.3)	**109** (72.1-140)	**450** (315-733)	**1220** (769-1750)	1488
	05-06	**19.0** (16.5-21.8)	**13.5** (12.0-16.3)	**70.9** (55.2-87.2)	**543** (390-638)	**1200** (954-1690)	1490
	07-08	**17.3** (13.0-23.0)	**13.7** (10.0-19.5)	**64.1** (41.5-117)	**371** (240-516)	**801** (516-1790)	1814
	09-10	**22.3** (17.9-27.7)	**16.5** (12.3-22.5)	**86.7** (63.1-129)	**505** (339-760)	**1890** (1240-3170)	1914
Gender							
Males	03-04	**16.8** (13.2-21.3)	**13.7** (11.4-16.8)	**55.3** (33.2-86.6)	**178** (134-324)	**567** (238-1350)	1229
	05-06	**14.6** (10.8-19.7)	**11.8** (8.80-15.9)	**43.4** (31.0-68.8)	**227** (103-552)	**909** (479-1190)	1270
	07-08	**11.9** (8.84-16.0)	**9.40** (7.70-12.3)	**37.6** (22.6-54.7)	**172** (79.9-376)	**471** (208-1410)	1294
	09-10	**15.3** (12.7-18.6)	**12.2** (10.0-15.2)	**52.8** (33.9-72.6)	**223** (129-332)	**610** (318-1270)	1399
Females	03-04	**30.7** (23.7-39.8)	**26.0** (20.2-34.1)	**137** (106-172)	**596** (403-769)	**1340** (776-1790)	1288
	05-06	**25.5** (21.7-29.9)	**17.9** (14.3-23.1)	**129** (82.9-166)	**638** (478-900)	**1410** (945-2620)	1278
	07-08	**27.6** (20.4-37.3)	**25.6** (17.5-33.9)	**117** (72.6-211)	**535** (361-757)	**1260** (643-2280)	1310
	09-10	**32.0** (23.7-43.3)	**23.0** (16.4-29.8)	**148** (73.3-260)	**1020** (540-1800)	**3200** (1760-4400)	1350
Race/ethnicity							
Mexican Americans	03-04	**16.5** (10.9-25.1)	**11.9** (8.50-18.3)	**45.5** (25.9-78.2)	**178** (76.4-412)	**412** (178-2180)	613
	05-06	**14.3** (10.7-19.1)	**10.2** (7.40-14.1)	**45.6** (24.1-75.7)	**214** (166-313)	**635** (337-1090)	637
	07-08	**12.1** (9.49-15.3)	**9.90** (7.70-12.4)	**32.6** (25.0-48.3)	**142** (85.0-268)	**409** (214-778)	531
	09-10	**17.2** (13.7-21.6)	**12.5** (10.5-14.5)	**41.9** (30.2-57.4)	**351** (248-546)	**1130** (493-2140)	566
Non-Hispanic blacks	03-04	**12.8** (9.38-17.4)	**10.2** (7.40-14.4)	**34.3** (22.8-50.6)	**127** (90.8-176)	**247** (143-499)	652
	05-06	**12.4** (9.77-15.6)	**9.40** (7.30-11.8)	**33.0** (22.3-46.5)	**208** (115-291)	**556** (269-1060)	678
	07-08	**8.55** (6.75-10.8)	**7.40** (5.00-9.70)	**25.5** (17.5-34.5)	**117** (66.8-217)	**422** (195-786)	597
	09-10	**14.0** (10.9-18.1)	**8.90** (7.30-10.5)	**30.9** (21.9-50.5)	**276** (143-437)	**705** (320-3000)	516
Non-Hispanic whites	03-04	**27.7** (20.3-37.8)	**24.4** (16.8-32.0)	**121** (83.6-162)	**507** (316-769)	**1340** (733-2070)	1092
	05-06	**21.6** (18.5-25.2)	**16.0** (13.5-19.0)	**82.4** (64.2-114)	**552** (392-754)	**1370** (945-2050)	1038
	07-08	**23.1** (15.0-35.5)	**18.7** (11.0-33.7)	**101** (47.4-206)	**459** (241-749)	**1160** (487-2620)	1077
	09-10	**26.1** (19.0-35.9)	**21.1** (14.6-29.7)	**94.9** (59.8-186)	**610** (325-1240)	**2130** (934-3820)	1206

Limit of detection (LOD, see Data Analysis section) for Survey years 03-04, 05-06, 07-08 and 09-10 are 0.3, 0.4, 0.4, and 0.4 respectively.

Biomonitoring Summary

http://www.cdc.gov/biomonitoring/Benzophenone-3_BiomonitoringSummary.html

Factsheet

http://www.cdc.gov/biomonitoring/Benzophenone-3_FactSheet.html

Urinary Benzophenone-3 (2011 - 2014)

Geometric mean and selected percentiles of urine concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

	Survey years	Geometric mean (95% conf. interval)	50th Percentile (95% conf. interval)	75th Percentile (95% conf. interval)	90th Percentile (95% conf. interval)	95th Percentile (95% conf. interval)	Sample size
Total	11-12	**23.2** (17.2-31.3)	**18.7** (13.6-27.0)	**83.8** (50.8-142)	**461** (290-861)	**1200** (861-1610)	2489
	13-14	**25.2** (20.4-31.2)	**21.0** (16.8-25.3)	**87.9** (72.7-102)	**417** (269-642)	**1210** (853-1760)	2686
Age group							
6-11 years	11-12	**18.7** (12.3-28.2)	**13.2** (8.20-20.8)	**55.4** (28.0-108)	**336** (134-653)	**657** (236-1270)	396
	13-14	**26.0** (16.5-40.9)	**19.2** (14.4-30.0)	**77.6** (39.0-206)	**363** (171-720)	**824** (271-1840)	409
12-19 years	11-12	**27.8** (15.4-50.2)	**21.6** (10.8-42.1)	**109** (34.0-255)	**433** (159-1160)	**1120** (261-1990)	388
	13-14	**29.2** (23.3-36.4)	**26.2** (19.7-36.4)	**87.2** (57.8-137)	**261** (167-504)	**537** (269-1340)	462
20 years and older	11-12	**23.1** (17.8-30.0)	**19.2** (14.4-27.0)	**83.8** (52.8-137)	**482** (305-805)	**1250** (975-1640)	1705
	13-14	**24.6** (19.7-30.6)	**20.1** (16.0-24.7)	**90.6** (73.4-102)	**482** (294-801)	**1360** (875-2740)	1815
Gender							
Males	11-12	**17.2** (12.1-24.5)	**16.0** (9.40-23.6)	**50.2** (40.1-78.7)	**295** (141-448)	**594** (301-1360)	1259
	13-14	**19.0** (15.8-22.9)	**16.8** (14.4-19.8)	**55.0** (46.4-63.1)	**231** (176-259)	**576** (340-864)	1285
Females	11-12	**31.0** (22.7-42.2)	**22.2** (16.0-34.1)	**137** (79.9-243)	**781** (467-1220)	**1720** (1260-2290)	1230
	13-14	**32.9** (24.9-43.5)	**26.2** (19.4-37.2)	**120** (94.9-195)	**733** (447-1230)	**1950** (1200-3460)	1401
Race/ethnicity							
Mexican Americans	11-12	**16.8** (10.5-26.8)	**13.4** (7.20-25.2)	**46.1** (25.2-115)	**305** (111-644)	**885** (305-1740)	316
	13-14	**25.9** (20.5-32.7)	**19.0** (15.0-24.2)	**69.3** (44.1-86.6)	**256** (204-538)	**893** (518-1230)	438
Non-Hispanic blacks	11-12	**14.5** (11.4-18.5)	**10.9** (8.00-14.1)	**41.4** (28.8-59.5)	**316** (192-433)	**895** (475-1270)	665
	13-14	**10.9** (7.48-15.8)	**9.60** (6.60-13.1)	**26.9** (19.6-39.9)	**104** (60.3-205)	**448** (118-1070)	609
Non-Hispanic whites	11-12	**26.8** (17.8-40.5)	**22.2** (14.8-36.5)	**101** (52.8-216)	**512** (255-1130)	**1230** (805-1830)	813
	13-14	**29.7** (22.7-38.8)	**24.9** (19.5-32.9)	**108** (94.0-131)	**523** (340-853)	**1470** (1150-2860)	988
All Hispanics	11-12	**21.2** (15.2-29.4)	**16.3** (10.6-22.7)	**57.9** (38.2-126)	**408** (261-684)	**1020** (527-1610)	571
	13-14	**28.2** (23.8-33.4)	**20.1** (16.5-23.9)	**81.5** (64.7-98.3)	**415** (219-824)	**1120** (738-1780)	690
Asians	11-12	**16.7** (10.5-26.5)	**11.9** (7.30-21.1)	**66.4** (37.6-122)	**486** (157-1350)	**1640** (770-2780)	352
	13-14	**18.6** (12.4-28.0)	**17.0** (8.30-26.8)	**64.2** (36.1-107)	**254** (114-532)	**532** (283-1770)	289

Limit of detection (LOD, see Data Analysis section) for Survey years 11-12 and 13-14 are 0.4 and 0.4, respectively.

Biomonitoring Summary

http://www.cdc.gov/biomonitoring/Benzophenone-3_BiomonitoringSummary.html

Factsheet

http://www.cdc.gov/biomonitoring/Benzophenone-3_FactSheet.html

Urinary Benzophenone-3 (creatinine corrected) (2003 – 2010)

Geometric mean and selected percentiles of urine concentrations (in µg/g of creatinine) for the U.S. population from the National Health and Nutrition Examination Survey.

	Survey years	Geometric mean (95% conf. interval)	50th Percentile (95% conf. interval)	75th Percentile (95% conf. interval)	90th Percentile (95% conf. interval)	95th Percentile (95% conf. interval)	Sample size
Total	03-04	**22.2** (17.6-28.0)	**16.2** (12.7-21.6)	**82.0** (58.7-108)	**415** (283-577)	**1080** (686-1600)	2514
	05-06	**18.9** (15.8-22.6)	**13.5** (11.2-16.8)	**60.4** (47.6-81.4)	**445** (346-553)	**1210** (794-1970)	2548
	07-08	**18.4** (13.9-24.4)	**13.5** (10.2-17.8)	**67.9** (45.2-117)	**338** (215-584)	**935** (525-1800)	2604
	09-10	**23.3** (18.7-29.1)	**16.1** (12.8-19.8)	**79.1** (52.2-124)	**579** (355-1040)	**2340** (1380-3010)	2749
Age group							
6-11 years	03-04	**25.8** (19.5-34.1)	**22.4** (14.4-33.7)	**84.6** (41.0-131)	**171** (132-365)	**427** (171-710)	314
	05-06	**23.3** (15.9-34.1)	**17.5** (11.6-28.3)	**67.7** (39.7-105)	**249** (106-631)	**868** (264-2080)	356
	07-08	**29.7** (19.0-46.6)	**19.3** (12.4-32.3)	**103** (51.1-159)	**442** (133-1550)	**1430** (187-29100)	389
	09-10	**28.3** (20.4-39.4)	**18.8** (14.7-21.1)	**53.9** (33.2-132)	**565** (104-3410)	**1780** (253-8230)	415
12-19 years	03-04	**17.2** (13.7-21.5)	**12.9** (10.4-16.5)	**43.6** (29.5-57.7)	**136** (91.7-239)	**350** (173-646)	713
	05-06	**15.8** (11.6-21.6)	**12.1** (9.16-17.0)	**42.5** (28.6-58.7)	**164** (101-438)	**625** (225-956)	702
	07-08	**16.7** (12.4-22.4)	**12.1** (8.57-17.6)	**55.7** (30.4-74.8)	**211** (88.2-505)	**505** (163-902)	401
	09-10	**18.7** (13.4-26.1)	**13.6** (10.1-19.7)	**39.9** (25.5-91.6)	**412** (89.8-750)	**1240** (629-2540)	420
20 years and older	03-04	**22.8** (17.8-29.1)	**16.2** (12.7-21.9)	**93.2** (66.0-130)	**491** (361-700)	**1330** (880-1880)	1487
	05-06	**19.0** (16.3-22.2)	**13.4** (11.2-16.2)	**64.2** (49.3-87.0)	**509** (406-628)	**1480** (816-2200)	1490
	07-08	**17.7** (13.3-23.6)	**12.6** (9.93-17.5)	**67.7** (44.1-127)	**362** (249-584)	**960** (581-1970)	1814
	09-10	**23.6** (18.8-29.5)	**16.3** (12.7-20.2)	**83.6** (58.3-146)	**602** (364-1230)	**2470** (1350-3080)	1914
Gender							
Males	03-04	**13.6** (10.8-17.1)	**10.3** (8.36-12.9)	**40.0** (24.9-62.5)	**169** (93.3-316)	**381** (229-685)	1228
	05-06	**11.7** (8.74-15.8)	**9.43** (6.72-12.5)	**31.9** (19.7-49.4)	**176** (77.1-406)	**553** (317-948)	1270
	07-08	**10.0** (7.37-13.7)	**7.50** (5.74-10.0)	**28.7** (18.5-47.3)	**142** (65.8-225)	**332** (181-960)	1294
	09-10	**13.7** (11.4-16.6)	**10.7** (8.53-12.7)	**44.6** (29.7-57.4)	**186** (123-287)	**420** (287-1230)	1399
Females	03-04	**35.5** (27.1-46.4)	**28.2** (20.2-37.0)	**144** (101-224)	**686** (491-1130)	**1850** (1220-2580)	1286
	05-06	**30.0** (25.3-35.6)	**20.9** (17.0-29.0)	**129** (88.0-175)	**707** (527-912)	**1880** (1150-2770)	1278
	07-08	**33.0** (25.0-43.7)	**26.2** (19.1-41.1)	**156** (90.2-235)	**617** (385-935)	**1500** (746-2710)	1310
	09-10	**38.6** (28.1-53.0)	**25.7** (17.5-35.2)	**161** (76.3-378)	**1580** (955-2410)	**3250** (2540-4640)	1350
Race/ethnicity							
Mexican Americans	03-04	**15.1** (9.44-24.0)	**11.1** (6.95-16.0)	**40.7** (18.3-85.8)	**158** (87.4-362)	**595** (118-1860)	612
	05-06	**12.9** (10.2-16.2)	**8.63** (6.16-12.5)	**32.1** (23.1-51.8)	**216** (135-408)	**948** (441-1220)	637
	07-08	**11.8** (8.87-15.6)	**8.97** (7.00-12.4)	**33.6** (26.0-43.2)	**154** (93.4-249)	**413** (217-932)	531
	09-10	**17.1** (13.4-21.7)	**12.2** (9.64-14.5)	**40.4** (26.4-60.3)	**288** (155-603)	**1310** (441-2180)	566
Non-Hispanic blacks	03-04	**8.78** (6.49-11.9)	**6.80** (5.27-9.00)	**19.7** (13.5-33.4)	**79.8** (46.8-139)	**185** (79.8-536)	651
	05-06	**8.69** (7.02-10.8)	**6.29** (5.52-7.78)	**21.2** (14.5-34.5)	**118** (74.9-245)	**448** (208-876)	678
	07-08	**6.65** (5.24-8.42)	**5.09** (4.15-6.10)	**17.8** (13.2-24.2)	**86.6** (57.5-122)	**297** (113-903)	597
	09-10	**10.2** (8.12-12.7)	**6.46** (5.40-7.67)	**23.1** (15.5-38.2)	**160** (79.9-396)	**653** (305-1580)	516
Non-Hispanic whites	03-04	**28.3** (20.6-38.8)	**22.0** (14.6-32.7)	**116** (73.5-175)	**510** (380-760)	**1330** (852-2410)	1091
	05-06	**22.7** (19.4-26.7)	**16.2** (13.0-19.7)	**77.3** (56.5-106)	**538** (406-679)	**1500** (828-2430)	1038
	07-08	**24.1** (15.6-37.2)	**18.1** (11.0-30.0)	**103** (53.6-198)	**494** (227-960)	**1170** (522-2710)	1077
	09-10	**29.3** (20.7-41.5)	**22.1** (14.6-33.1)	**99.2** (54.3-241)	**767** (412-1550)	**2590** (1510-3250)	1206

Biomonitoring Summary

http://www.cdc.gov/biomonitoring/Benzophenone-3_BiomonitoringSummary.html

Factsheet

http://www.cdc.gov/biomonitoring/Benzophenone-3_FactSheet.html

Urinary Benzophenone-3 (creatinine corrected) (2011 - 2014)

Geometric mean and selected percentiles of urine concentrations (in µg/g of creatinine) for the U.S. population from the National Health and Nutrition Examination Survey.

	Survey years	Geometric mean (95% conf. interval)	50th Percentile (95% conf. interval)	75th Percentile (95% conf. interval)	90th Percentile (95% conf. interval)	95th Percentile (95% conf. interval)	Sample size
Total	11-12	**26.4** (19.3-36.2)	**18.8** (13.3-27.3)	**102** (57.8-162)	**613** (330-1020)	**1680** (1010-2500)	2487
	13-14	**25.2** (20.5-31.0)	**19.8** (15.7-24.7)	**79.8** (66.2-103)	**467** (299-603)	**1190** (847-1810)	2684
Age group							
6-11 years	11-12	**26.9** (17.9-40.2)	**18.8** (13.0-28.6)	**67.8** (37.8-135)	**552** (138-1010)	**1010** (297-1670)	395
	13-14	**32.8** (21.5-50.1)	**28.8** (16.7-45.7)	**90.0** (46.9-189)	**401** (152-868)	**868** (432-1700)	409
12-19 years	11-12	**27.1** (14.2-51.8)	**16.7** (8.69-44.3)	**102** (29.9-331)	**631** (117-3050)	**1720** (331-3900)	388
	13-14	**23.7** (17.8-31.4)	**21.6** (15.8-28.7)	**63.0** (49.1-86.2)	**221** (116-440)	**506** (221-647)	462
20 years and older	11-12	**26.3** (19.8-34.8)	**19.8** (13.9-26.6)	**107** (68.1-156)	**640** (371-957)	**1890** (1080-2500)	1704
	13-14	**24.7** (20.3-30.0)	**18.8** (15.2-23.1)	**82.3** (69.5-102)	**529** (317-790)	**1420** (915-2660)	1813
Gender							
Males	11-12	**16.1** (11.3-22.8)	**11.9** (8.77-18.1)	**48.8** (31.6-84.5)	**237** (120-442)	**716** (267-1030)	1258
	13-14	**15.9** (13.2-19.3)	**13.8** (10.9-17.5)	**44.0** (33.1-58.5)	**181** (146-229)	**432** (299-532)	1284
Females	11-12	**42.7** (30.6-59.5)	**30.1** (19.7-49.4)	**186** (107-374)	**1150** (771-1910)	**2720** (1660-4230)	1229
	13-14	**38.9** (29.2-51.8)	**29.7** (21.3-39.9)	**153** (83.6-242)	**905** (603-1250)	**2550** (1440-3500)	1400
Race/ethnicity							
Mexican Americans	11-12	**18.9** (12.4-29.0)	**13.8** (8.75-22.0)	**45.3** (23.5-132)	**369** (132-820)	**1030** (371-2330)	316
	13-14	**26.4** (20.2-34.4)	**18.2** (15.6-23.2)	**59.1** (40.6-82.7)	**296** (122-1090)	**1180** (473-2130)	438
Non-Hispanic blacks	11-12	**11.3** (8.82-14.5)	**7.48** (6.04-9.49)	**27.6** (18.0-42.1)	**269** (159-376)	**747** (376-1010)	665
	13-14	**8.02** (5.57-11.5)	**6.43** (4.68-8.60)	**16.9** (11.6-24.7)	**93.5** (51.1-204)	**332** (144-818)	609
Non-Hispanic whites	11-12	**32.6** (21.1-50.4)	**25.5** (15.3-41.1)	**128** (70.8-248)	**793** (334-1320)	**1970** (1030-3500)	811
	13-14	**31.2** (24.5-39.6)	**25.3** (20.1-31.5)	**115** (77.3-159)	**569** (432-765)	**1270** (871-2550)	987
All Hispanics	11-12	**23.7** (17.6-32.0)	**15.9** (11.4-21.7)	**65.0** (38.6-132)	**425** (265-916)	**1850** (784-2330)	571
	13-14	**28.0** (23.2-33.7)	**18.8** (16.3-22.5)	**66.8** (50.9-84.5)	**338** (190-1020)	**1380** (974-2740)	690
Asians	11-12	**22.3** (14.3-34.9)	**14.2** (8.24-27.8)	**73.1** (41.6-153)	**624** (282-1640)	**1860** (641-4410)	352
	13-14	**23.1** (16.3-32.8)	**20.2** (12.9-34.4)	**67.5** (48.4-102)	**253** (131-537)	**657** (287-1900)	288

Biomonitoring Summary

http://www.cdc.gov/biomonitoring/Benzophenone-3_BiomonitoringSummary.html

Factsheet

http://www.cdc.gov/biomonitoring/Benzophenone-3_FactSheet.html

Appendix C. Limit of Detection Table

The analytical limit of detection (LOD) for each of the different chemical measurements is presented in the table below. The LOD is the concentration at which the measurement has a 95% probability of being greater than zero (Taylor, 1987). As analytical methods improve, LODs will often change. For this reason, LOD results are reported by survey periods (e.g., 1999-2000, 2001-2002, etc.).

Reference: Taylor JK. Quality Assurance of Chemical Measurements. Chelsea (MI): Lewis Publishing. 1987

Chemical, *matrix, units*	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014
Adducts of Hemoglobin *in packed rbcs, pmol/g hemoglobin*								
Acrylamide			3.0	0.11				
Glycidamide			4.0	0.66				
Tobacco Smoke								
Cotinine *in serum, ng/mL*	0.05	0.05	0.015	0.015	0.015	0.015	0.015	
NNAL *in urine, pg/mL*					1	0.6	0.6	
Disinfection By-Products *in blood, pg/mL*								
Bromodichloromethane		0.233	0.62	0.62	0.62			
Dibromochloromethane		0.271	0.62	0.62	0.62			
Tribromomethane (Bromoform)		0.596	1.5	1.0	1.0			
Trichloromethane (Chloroform)		2.37	2.11	2.1	2.1			
Personal Care and Consumer Product Chemicals and Metabolites *in urine, µg/L*								
Benzophenone-3			0.3	0.4	0.4	0.4	0.4	0.4
Bisphenol A			0.4	0.4	0.4	0.4	0.4	0.2
4-*tert*-Octylphenol				0.2	0.2	0.2		
Triclocarban								0.1
Triclosan			2.3	2.3	2.3	2.3	2.3	1.7
Butyl paraben				0.2	0.2	0.2	0.2	0.1
Ethyl paraben				1	1	1	1	1
Methyl paraben				1	1	1	1	1
n-Propyl paraben				0.2	0.2	0.2	0.2	0.1
2,4-Dichlorophenol			0.17	0.2	0.2	0.2	0.2	0.1
2,5-Dichlorophenol			0.12	0.2	0.2	0.2	0.2	0.1
Fungicides and Metabolites *in urine, µg/L*								
ortho-Phenylphenol			0.1	0.1	0.1	0.2		
Ethylene thiourea			0.1	0.24	0.21			
Pentachlorophenol			0.5					
Propylene thiourea			0.1	0.37	0.36			
Herbicides and Metabolites *in urine, µg/L*								
Atrazine					0.5			
Atrazine mercapturate					0.5			
Desethyl atrazine					0.25			
Desisopropyl atrazine					0.25			
Desisopropyl atrazine mercapturate					0.1			
Diaminochlorotriazine					0.5			
2,4-Dichlorophenoxyacetic acid	0.952	0.2	0.1		0.4	0.15		
2,4,5-Trichlorophenoxyacetic acid	1.2	0.1	0.1		0.10	0.1		
Sulfonyl Urea Herbicides *in urine, µg/L*								
Urinary Bensulfuron-methyl			0.05	0.05	0.05			
Urinary Chlorsulfuron			0.06	0.06	0.06			
Urinary Ethametsulfuron-methyl			0.1	0.1	0.1			
Urinary Foramsulfuron			0.05	0.05	0.05			
Urinary Halosulfuron			0.1	0.1	0.1			
Urinary Mesosulfuron-methyl			0.06	0.06	0.06			
Urinary Metsulfuron-methyl			0.05	0.05	0.05			
Urinary Nicosulfuron			0.1	0.1	0.1			
Urinary Oxasulfuron			0.06	0.06	0.06			
Urinary Primisulfuron-methyl			0.07	0.07	0.07			
Urinary Prosulfuron			0.05	0.05	0.05			
Urinary Rimsulfuron			0.05	0.05	0.05			
Urinary Sulfometuron-methyl			0.05	0.05	0.05			
Urinary Sulfosulfuron			0.1	0.1	0.1			
Urinary Thifensulfuron-methyl			0.08	0.08	0.08			
Urinary Triasulfuron			0.07	0.07	0.07			
Urinary Triflusulfuron-methyl			0.05	0.05	0.05			
Insect Repellent and Metabolites *in urine, µg/L*								
N,N-Diethyl-*meta*-toluamide (DEET)					0.089	0.089		
3-(Diethylcarbamoyl) benzoic acid (DCBA)					0.83	0.475		
N,N-Diethyl-3-(hydroxymethyl) benzamide (DHMB)					0.083	0.083		

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

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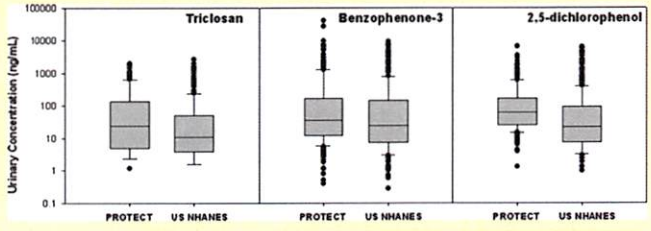
Supporting Information

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

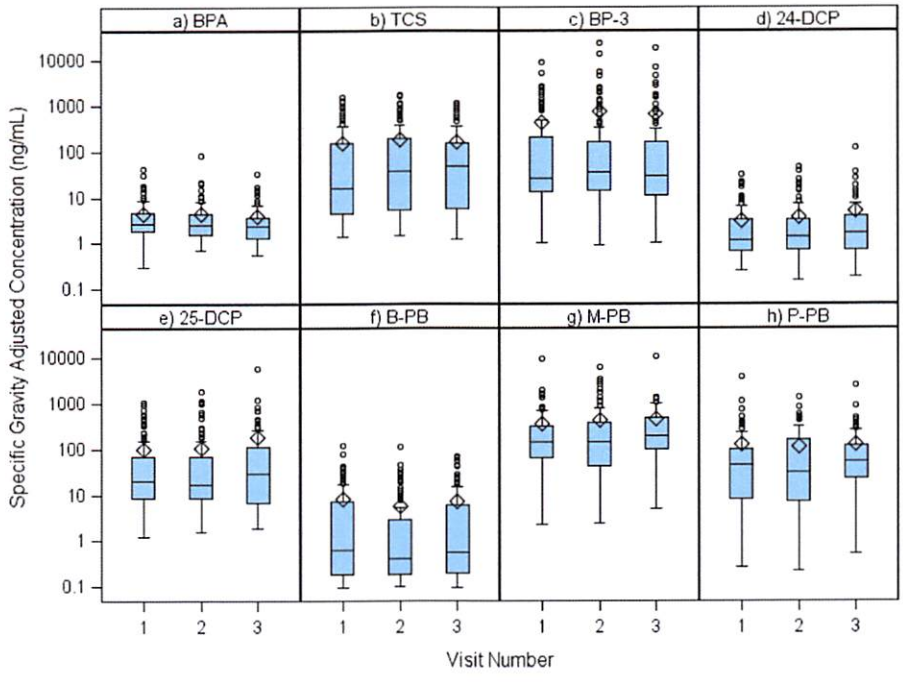

Figure 1. Boxplots comparing SG-adjusted concentrations of urinary biomarkers across study visits. Visit 1 (18 ± 2 weeks gestation), Visit 2 (22 ± 2 weeks), Visit 3 (26 ± 2 weeks).

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.

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Notes

The authors declare no competing financial interest.

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Tina Harmer Lassen, Hanne Frederiksen, Tina Kold Jensen, Jørgen Holm Petersen, Katharina M. Main, Niels E. Skakkebæk, Niels Jørgensen and Selma Kløve Kranich. Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples. Environmental Research
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Abstract
Human exposure to modern non-persistent chemicals is difficult to ascertain in epidemiological studies as exposure patterns and excretion rates may show temporal and diurnal variations. The aim of this study was to assess the temporal variability in repeated measurements of urinary excretion of bisphenol A (BPA) and seven other phenols. All analytes were determined using TurboFlow-LC–MS/MS. Two spot, three first morning and three 24-h urine samples were collected from 33 young Danish men over a three months period. Temporal variability was estimated by means of intraclass correlation coefficients (ICCs). More than 70% of the urine samples had detectable levels of BPA, triclosan (TCS), benzophenone-3 (BP-3) and sum of 2,4-dichlorophenol and 2,5-dichlorophenol (ΣDCP). We found low to moderate ICCs for BPA (0.10–0.42) and ΣDCP (0.39–0.72), whereas the ICCs for BP-3 (0.69–0.80) and TCS (0.55–0.90) were higher. The ICCs were highest for the two spot urine samples, which were collected approximately 4 days apart, compared with the 24-h urine samples and the first morning urine samples, which were collected approximately 40 days apart. A consequence of the considerable variability in urinary excretion of BPA may be misclassification of individual BPA exposure level in epidemiological studies, which may lead to attenuation of the association between BPA and outcomes. Our data do not support that collection of 24-h samples will improve individual exposure assessment for any of the analysed phenols.

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Review

Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review

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ABSTRACT

Benzophenone-3 (BP-3) has been widely used in sunscreens and many other consumer products, including cosmetics. The widespread use of BP-3 has resulted in its release into the water environment, and hence its potential impact on aquatic ecosystem is of concern. To better understand the risk associated with BP-3 in aquatic ecosystems, we conducted a thorough review of available articles regarding the physicochemical properties, toxicokinetics, environmental occurrence, and toxic effects of BP-3 and its suspected metabolites. BP-3 is lipophilic, photostable, and bioaccumulative, and can be rapidly absorbed via oral and dermal routes. BP-3 is reported to be transformed into three major metabolites in vivo, i.e., benzophenone-1 (BP-1), benzophenone-8 (BP-8), and 2,3,4-trihydroxybenzophenone (THB). BP-1 has a longer biological half-life than its parent compound and exhibits greater estrogenic potency in vitro. BP-3 has been detected in water, soil, sediments, sludge, and biota. The maximum detected level in ambient freshwater and seawater is 125 ng/L and 577.5 ng/L, respectively, and in wastewater influent is 10,400 ng/L. The major sources of BP-3 are reported to be human recreational activities and wastewater treatment plant (WWTP) effluents. BP-3 and its derivatives have been also detected in fish lipid. In humans, BP-3 has been detected in urine, serum, and breast milk samples worldwide. BP-1 has also been detected in placental tissues of delivering women. While sunscreens and cosmetics are known to be major sources of exposure, the fact that BP-3 has been detected frequently among young children and men suggests other sources. An increasing number of in vitro studies have indicated the endocrine disrupting capacity of BP-3. Based on a receptor binding assay, BP-3 has shown strong anti-androgenic and weak estrogenic activities but at the same time BP-3 displays anti-estrogenic activity as well. Predicted no effect concentration (PNEC) for BP-3 was derived at 1.32 μg/L. The levels observed in ambient water are generally an order of magnitude lower than the PNEC, but in wastewater influents, hazard quotients (HQs) greater than 1 were noted. Considering limited ecotoxicological information and significant seasonal and spatial variations of BP-3 in water, further studies on environmental monitoring and potential consequences of long-term exposure in aquatic ecosystem are warranted.

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Contents

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1. Introduction

Exposure to ultraviolet (UV) radiation may pose a public health threat, including the risk of various skin diseases such as sunburn, photo-aging and skin cancers (Pathak, 1987). While solar UVC (wavelength range 200–280 nm) is absorbed by ozone in the stratosphere, UVA (320–400 nm) and UVB (290–320 nm) can reach the earth surface and therefore may influence humans and ecosystems (Clydesdale et al., 2001; de Gruijl, 2002). Long-wave UVA comprises more than 90% of solar light, and can penetrate deep into both the epidermis and dermis of the skin eventually causing premature photo-aging. UVB is a minor component in proportion, but is more active and more capable of causing sunburn than UVA. UVB is considered to be responsible for inducing DNA damage and ultimately skin cancer (Svobodová et al., 2003). The concern over the deleterious effects of solar UV light has increased the demand for sunscreen products. Consequently, sunscreen products, commonly referred to as UV filters, have been widely used to reduce sunlight exposure and to protect human skin.

Based on composition, UV filters can be classified roughly into two groups, i.e., organic (or chemical) and inorganic (or physical). Inorganic filters include mineral particles such as TiO_2 and ZnO, and function by reflecting and scattering UV light from the skin. On the other hand, organic filters usually possess aromatic structures that can absorb and stabilize the solar UV radiation (Gasparro et al., 1998). Benzophenones (BPs), camphors, and cinnamates are among well-known organic UV filters. These chemical filters are generally used in combination because no single active agent, used at levels currently permitted by legislation, would provide sufficient protection against UV (Díaz-Cruz et al., 2008; U.S. FDA Department of Health and Human Services, 2013a). UV filters are also used in other cosmetic products such as lipsticks, skin lotions, facial creams, and fragrances (Environmental Working Group, 2013; Liao and Kannan, 2014), and in various personal care products, including shampoos, body washes, toilet soaps, hair sprays, and insect repellents (Liao and Kannan, 2014; National Library of Medicine, 2011), because they can prevent polymer degradation or pigmentation.

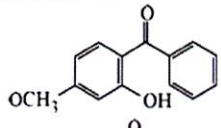
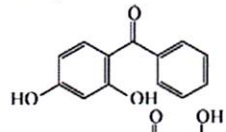
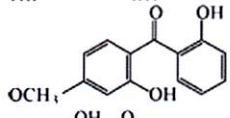
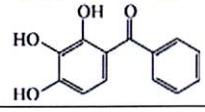
Benzophenone-3 (BP-3, 2-hydroxy-4-methoxybenzophenone, oxybenzone) is one of the most widely used BP type UV filters and has been available as a sunscreen agent for over 40 years. BP-3 can be used at levels of up to 5–6% as an active ingredient in sunscreen in Japan and the U.S.A. (The Society of Japanese Pharmacopoeia, 1985; U.S. FDA Department of Health and Human Services, 2013b), while up to 10% can be used in Europe (EEC Directive, 1983). In Korea, a maximum of 5% BP-3 can be used as a cosmetic ingredient (Korea Food and Drug Administration, 2012). BP-3 is approved by the U.S. Food and Drug Administration also as an indirect food additive (U.S. FDA Department of Health and Human Services, 2013a).

Other BP type UV filters are also commercially used. For example, benzophenone-1 (BP-1, or 2,4-dihydroxybenzophenone) which is a major metabolite of BP-3 in both experimental animals and humans (Kadry et al., 1995; Kunisue et al., 2012; Okereke et al., 1993; Wang and Kannan, 2013), is employed as a UV stabilizer in plastic surface coatings on food packages (Suzuki et al., 2005).

Widespread use of BP-3 has led to the release of this compound and its derivatives into aquatic environment such as lakes and rivers around the world (Balmer et al., 2005; Cuderman and Heath, 2007; Kameda et al., 2011; Loraine and Pettigrove, 2006). Up to 125 ng/L of BP-3 has been detected in surface water (Poiger et al., 2004). Wastewater treatment plant (WWTP) effluents are reported to contain higher levels of BP-3 (Loraine and Pettigrove, 2006). Therefore, the potential impact on aquatic ecosystems is of concern.

BP-3 has been frequently reported for endocrine disruption (Fent et al., 2008; Heneweer et al., 2005; Kunz et al., 2006; Schreurs et al., 2002; Sieratowicz et al., 2011). Experimental animal and in vitro studies have shown that BP-3 influences reproduction and sex hormone signaling (Blair et al., 2000; Kunz et al., 2006; Schlumpf et al., 2001; Schreurs et al., 2005; Schultz et al., 2000; Suzuki et al., 2005). BP-1 is reported to

Table 1
Structure and some physico-chemical properties of benzophenone-3 and its relevant derivatives.

Compound	CAS. number	Abbreviation	Chemical structure	Formula	Molecular weight	Boiling point (°C)	Log Kow[a]	pKa[a]	Vapor pressure[a] (mm Hg) at 25 °C	Bioconcentration factor (BCF) at pH 7, 25 °C
Oxybenzone, 2-hydroxy-4-methoxy benzophenone, benzophenone-3	131–57–7	BP-3 HMB		$C_{14}H_{12}O_3$	228.24	370.3	4.00	7.56	5.26×10^{-6}	502
2,4-Dihydroxy benzophenone, benzophenone-1	131–56–6	BP-1 DHB		$C_{13}H_{10}O_3$	214.22	409.0	3.15	7.53	2.84×10^{-7}	113
Dioxybenzone, 2,2′-dihydroxy-4-methoxybenzophenone, benzophenone-8	131–53–3	BP-8 DHMB		$C_{14}H_{12}O_4$	244.24	375.0	4.31	7.11	3.73×10^{-6}	524
2,3,4-Trihydroxy benzophenone	1143–72–2	THB		$C_{13}H_{10}O_4$	230.22	439.7	1.70	7.51	2.42×10^{-8}	7.75

Kow, octanol–water partition coefficient.
[a] Values obtained from SciFinder Scholar Database, http://www.cas.org.products/sfacad/.

possess even greater estrogen receptor binding affinity compared to BP-3 (Kunz et al., 2006; Molina-Molina et al., 2008). Furthermore, BP-3 and BP-1 are suspected to influence on hormone-dependent diseases, and are associated with the birth outcome of humans (Kunisue et al., 2012; Wolff et al., 2008).

This paper reviews the available literature on various aspects of BP-3 and its major metabolites, including their chemical properties, toxicokinetics, environmental occurrence, endocrine disrupting potential, and ecological risks. Knowledge gaps, environmental health implications, and the future direction of research are identified.

2. Characterization of BP-3 and its major derivatives

2.1. Physico-chemical properties and fates

BP has two benzene rings joined by a carbonyl group. Twelve substituted derivatives of BP, i.e., BP-1 to BP-12, have been used in various commercial products because of their UV absorption properties (Park et al., 2013). Among them, BP-3 is the most well-known compound. The physico-chemical properties of BP-3 and its relevant BP derivatives are shown in Table 1.

As with other organic UV filters, BP-3 is a photostable, lipophilic and potentially bioaccumulative compound. The relatively high log-Kow value of BP-3, i.e., 4.0, suggests its slow biodegradation, tendency to adsorb to suspended solids and sediments, and low volatilization potential from water surfaces. BP-3 has been shown to degrade by about 4% after 28 d in water (Chemicals Inspection and Testing Institute, 1992) indicating its persistence in aquatic environment. During summer, the half-life of BP-3 in surface water was estimated at a few weeks, and the persistence appeared to be 7 to 9 times greater in winter under mid-latitude conditions (Vione et al., 2013). In oxic conditions, BP-3 is reported to produce BP-1 as a biodegradation product (Liu et al., 2012). The biodegradation of BP-3 is favored under anaerobic (4.2 d half-life) compared to aerobic conditions (10.7 d half-life). BP-3 is relatively stable under UV light and artificial sunlight (Gago-Ferrero et al., 2012; Rodil et al., 2009a).

BP-1 has lower log-Kow value (3.15) and bioconcentration factor (BCF) compared to its parent compound. Unlike BP-3, BP-1 is readily photodegraded, and disappears after 24 h under UV radiation (Gago-Ferrero et al., 2012). Another BP derivative is 2,3,4-trihydroxybenzophenone (THB), but this compound has not been commercially used. THB has never been detected in environmental media. BP-8 (2,2′-dihydroxy-4-methoxybenzophenone, dioxybenzone), another metabolic product of BP-3, has similar chemical properties to BP-3 and is often detected in soil or sediments due to its high lipophilicity. Analytical methods for BP-3 and its derivatives are beyond the scope of this review, and related information can be found in Díaz-Cruz et al. (2008) and Giokas et al. (2007).

2.2. Toxicokinetics

BP-3 can be rapidly absorbed after oral, intravenous, or topical skin administration in rats and piglets (El Dareer et al., 1986; Kadry et al., 1995; Kasichayanula et al., 2007; Okereke et al., 1993). In male rats, BP-3, BP-1, and BP-8 were detected in plasma 5 min after gavage feeding (Okereke et al., 1993) and the absorption half-life was 0.71 h (Kadry et al., 1995). Maximum plasma concentration of BP-3 was found at 2 h after the topical skin application of BP-3 contained sunscreen products in piglets (Kasichayanula et al., 2007). In humans, milligrams of BP-3 were absorbed to systemic circulation following a topical application of sunscreen products containing BP-3 (Hayden et al., 1997; Janjua et al., 2004). While a skin permeability coefficient of BP-3 is not available, it was shown that about 1 to 2% of the topically applied amount could be absorbed through skin over 10 h period (Hayden et al., 1997).

Absorbed BP-3 can be hydroxylated to form metabolic byproducts such as BP-1, BP-8, or THB (Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1993). Of these metabolites, BP-1 has been most frequently detected in rats (El Dareer et al., 1986; Okereke et al., 1993). BP-1 is formed via *O*-demethylation of the methoxy side chain on ring A of BP-3, whereas BP-8 is formed via the aromatic hydroxylation of ring B at the ortho position. A small portion of BP-1 can be further converted into THB via the aromatic hydroxylation of ring A at the *meta* position (Fig. 1).

Even though the study on the toxicokinetics of BP-3 tends to be limited to the experiments using rats after oral administration, BP-1 is also believed to be one of the major metabolites of BP-3 in fish. During a 14 d exposure of adult male zebrafish to BP-3, only BP-1 and BP-3 were detected in water and fish samples while BP-2, 4,4′-dihyroxybenzophenone, and 4-hydroxybenzophenone (4-OH-BP) were not found (Bluthgen et al., 2012). In addition, as the nominal concentration of BP-3 in fish decreased, the ratio between the concentrations of BP-1 to BP-3 in fish notably increased. Increased proportion of BP-1 may explain greater endocrine disrupting potential at lower BP-3 concentrations in fish. In contrast to the adult male fish, biotransformation of BP-3 to BP-1 had not occurred in hatched fry fish at 5 days post-hatch (dph). Less developed metabolic functions at the eleuthero-embryo stage may account for this observation.

BP-1 is also the major metabolite in humans. Among women ($n = 625$ from Utah and California, U.S.A.), a positive correlation ($\beta = 0.59$,

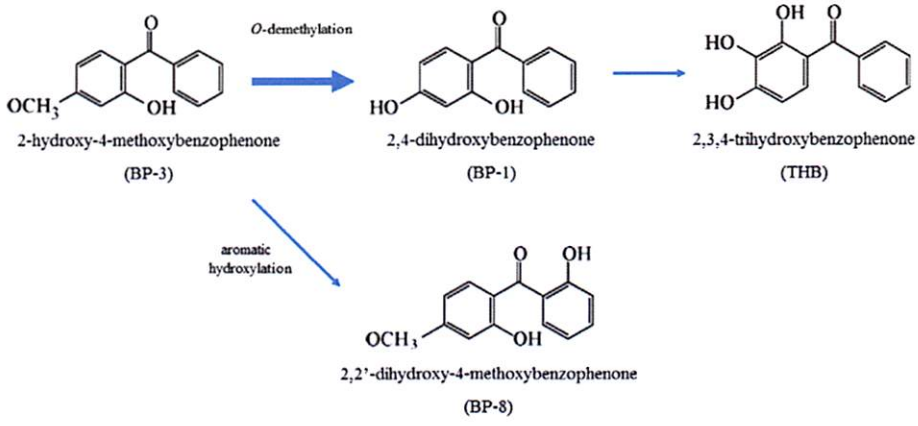


Fig. 1. Metabolic pathways of benzophenone-3 in rats.

r = 0.92) was found between BP-3 and BP-1 concentrations in urine samples with BP-1 levels notably high (Kunisue et al., 2012). The profiles of BP derivatives in human urine suggest that demethylation to BP-1 is the major metabolic pathway (Wang and Kannan, 2013). However, other types of BPs, e.g., BP-2 and 4-OH-BP which were not detected in animal studies, have been found in human urine. Some of the BPs might have originated from the direct use of those compounds but also might be explained by human-specific metabolism of BP-3. In urines collected from the children and adults of the U.S.A., the total concentrations of four BP derivatives including BP-1, BP-2, BP-8, and 4-OH-BP were positively correlated with the concentration of BP-3 (Wang and Kannan, 2013). Interestingly, a recent study (Zhang et al., 2013) reported no significant correlations between urinary BP-3 and 4-OH-BP concentrations, suggesting sources other than BP-3. Sex dependent differences in metabolism have been reported. After repeated topical application of BP-3, blood and urine concentrations of BP-3 in men were higher than in women (Janjua et al., 2008).

Like other ketonic compounds (Westphal, 1986), BP derivatives bind with plasma proteins and are transported through blood vessels. After oral administration of BP-3 in male Sprague–Dawley rats, tissue analysis at 6 h revealed that BP-1 was present in most tissues including the liver, kidney, testes, intestine, spleen and skin (Okereke et al., 1993). Using the same study design, Kadry et al. (1995) demonstrated that liver contained the highest concentrations of total and free BP-3, in the liver, 6.47% and 0.18% of the initial total and free BP-3 doses were detected, followed by the kidney with 0.97% and 0.02%, respectively. A similar distribution pattern was found also for BP-1 (Okereke et al., 1993). High levels of both BP-3 and BP-1 in liver samples imply that the liver is a major organ of BP-3 biotransformation. In hepatocyte suspensions, BP-3 can be converted enzymatically to BP-1 and probably to BP-8 (Nakagawa and Suzuki, 2002). While it is uncertain which enzymes exactly are responsible for the metabolism of BP-3, cytochrome P450 (CYP) enzymes are thought to play a role (Porter and Coon, 1991). In rat liver microsomes, the oxidation to BP-1 was mainly catalyzed by CYP2C6 and to a lesser extent by CYP1A1 (Kamikyouden et al., 2013). However, metabolism of BP-3 in fish may be different, because fish lack CYP2C homologs (Smith et al., 2010). In the brain of male zebrafish, transcripts of *cyp1a1* were significantly up-regulated by exposure to BP-3 (Bluthgen et al., 2012) suggesting the potential role of this CYP isozyme in metabolism of BP-3.

Conjugation with glucuronic acid and expulsion in the urine are one of the major routes of BP-3 excretion (El Dareer et al., 1986; Kadry et al., 1995; Okereke et al., 1993). In rats, the removal of BP-3 from blood was reported to be faster than that of BP-1, and the biological half-life of BP-3 was determined at 4.58 h (Jeon et al., 2008). Following topical application on skin in piglets, the elimination half-lives of BP-3 ranged between 7.14 and 8.04 h (Fediuk et al., 2012; Kasichayanula et al., 2005, 2007). Prolonged absorption phase through skin may explain longer elimination half-life following topical application.

3. Occurrences in environment and biota

3.1. Occurrence in aquatic environment

The concentrations of BP-3 and other UV filters fluctuate significantly by location, levels of public access, season, and sampling conditions such as water depth or flow (Díaz-Cruz et al., 2008; Fent et al., 2010b; Poiger et al., 2004). These compounds can reach the ambient water through both direct and indirect inputs. The direct inputs involve removal from the skin during recreational activities such as swimming or bathing in water bodies. Indirect inputs include releases via WWTPs, which may originate for example from processes such as laundry or showering (Balmer et al., 2005; Hernández Leal et al., 2010; Langford and Thomas, 2008).

In lake Hüttnersee, a recreational lake of Switzerland, notably higher levels of BP-3 were detected compared to other UV filters, such as (3-(4-methylbenzylidene) camphor (4-MBC), ethylhexyl methoxycinnamate (EHMC), or octocrylene (OC) (Balmer et al., 2005). Similarly, in a lake and a swimming pool in Slovenia, BP-3 was present at the highest level and frequency among the six UV filters measured which include 4-MBC, OC, octyl methoxycinnamate (OMC), octyl dimethyl PABA (OD-PABA), and homosalate (Cuderman and Heath, 2007). In ambient freshwater, BP-3 has been detected up to 125 ng/L. This maximum level was reported in lake Hüttnersee, Switzerland during the summer of 1998 where no indirect input other than public recreational access was expected, and the level was consistent with the predicted value based on the survey of sunscreen usage (Poiger et al., 2004). BP-3 was detected in other popular outdoor bathing areas. In outdoor swimming pools (n = 5) and recreational ponds (n = 6) of South Bohemia during the peak summer season of 2011, BP-3 has been found at concentrations as high as 620 and 550 ng/L, respectively (Grabicova et al., 2013). In beach seawaters, high levels of BP-3 have been reported. Up to 577.5 ng/L BP-3 was found in coastal waters of Majorca Island, Spain, during summer 2011 (Tovar-Sanchez et al., 2013). Higher levels of BP-3 were detected in surface nearshore beach waters of semi-enclosed or densely populated resort areas. These levels are several times greater than those reported for rivers or lakes. These observations underline the importance of human recreational activities as a source of BP-3 in water.

Wastewater effluents are another major source of BP-3 into ambient water, although BP-3 is removed efficiently (68 to 96%) in conventional WWTPs (Balmer et al., 2005; Li et al., 2007). BP-3 concentrations in WWTP influents and effluents ranged from <5 to 10,400 ng/L, with the highest concentrations reported in a wastewater influent at San Diego County in the U.S.A. (Loraine and Pettigrove, 2006). Along the River Glatt, Switzerland, BP-3 was first detected downstream of the Dubendorf WWTP, which indicates the contribution of WWTP as an important source of BP-3 release (Fent et al., 2010b; Zenker et al., 2008). In contrast, BP-1 was detected in only one sample (47 ng/L) among 25 river and lake water samples collected during spring of 2003 in South Korea (Jeon et al., 2006). BP-1 concentrations of <0.3–17 ng/L were found in the Rivers Taff and Ely in South Wales regions, UK (Kasprzyk-Hordern et al., 2008). The reason for less frequent detection and lower concentrations of BP-1 in water can be found from its lesser persistence in water compared to BP-3.

Although contamination in sediment or soil samples has received lesser attention compared to that in natural waters, BP-3 and its byproducts are likely to exist at higher levels in sediments or soil due to their lipophilic properties. Schlenk et al. (2005) performed an in vivo vitellogenin assay for sediment samples collected from the Southern California Bight, U.S.A. using male or juvenile fish, and identified the samples with estrogenic potential. From those samples with estrogenic activity, only BP-3 was unequivocally detected among 62 PPCP analytes. In soil or sediments, the levels of BP-3 ranged between <0.5 and 27 ng/g dw. For BP-1, the levels ranged between 0.26 and 0.61 ng/g dw, and for BP-8, the range was 0.13 and 4.17 ng/g dw. Other BP derivatives, such as BP-2 and 4-OH-BP, have also been detected in the soil and sediment of the U.S.A. (Zhang et al., 2011).

3.2. Occurrence in biota

Only few studies are available on BP derivatives in aquatic biota. BP-3 and BP-4 have been reported in fish lipid (Balmer et al., 2005; Fent et al., 2010b; Zenker et al., 2008). In fish from lakes in Switzerland, BP-3 and 4-MBC were most frequently detected among four UV filters (Balmer et al., 2005).

Reports on occurrence of BP-3 and related compounds among biological samples are mostly limited to humans. As shown in Table 3 and Fig. 2, BP-3 was detected in the majority of urine samples. In the 2003–2004 National Health and Nutrition Examination Survey (NHANES), BP-3 was found at >0.4 μg/L in the urine of 96.8% of 2517 participants aged 6 years or older (Calafat et al., 2008). BP-3 was also found in breast milk samples from Germany and Switzerland (Hany and Nagel, 1995; Schlumpf et al., 2010). BP-1 and 4-OH-BP have also

Table 2
Environmental levels of benzophenone-3 and its relevant derivatives.

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
BP-3										
	Water (ng/L)									
	Surface water	River	Slovenia	1/2	–	<54	Nadiza-Soca	114	Kolpa	Cuderman and Heath (2007)
		River	Spain	–	–	<7	Jan. and Jul., 2007/ River Mero	27 ± 3	Mar., 2007/River Mero	Rodil et al. (2008)
		River	Spain	1/2	–	<LOD	May, 2007/River Elsterbecken	30 ± 3	May, 2007/River Parthe	Rodil and Moeder (2008)
		River	Switzerland	–	–	<LOD	Jul., 2006/River Glatt, Upstream WWTP Dübendorf	96 ± 93 (ng/ POCIS)	Jul., 2006/River Glatt, Downstream WWTP Dübendorf	Zenker et al. (2008)
		River	Switzerland	–	–	56	Sep., 2007/River Glatt	68	Oct., 2007/River Glatt	Fent et al. (2010b)
		River	UK	–	–	<0.3	River Taff	17	River Taff	Kasprzyk-Hordern et al. (2008)
		River	Brazil	0/3	–	<2	Oct., 2012 and Mar., 2013	<2	Oct., 2012 and Mar., 2013	Silva et al. (2013)
		Rivers heavily polluted by industrial and domestic wastewaters	Japan	1/6	4[b]	–	Summer, 2008	–	Summer, 2008	Kameda et al. (2011)
		Moderately polluted rivers	Japan	8/12	6	4	Summer, 2008	12	Summer, 2008	Kameda et al. (2011)
		River Tama	Japan, Tokyo	–	14	–	–	–	–	Kawaguchi et al. (2008)
		Rivers where the municipal wastewater is directly discharged	Taiwan	2/2	–	12.3	–	15.4	–	Wu et al. (2013)
		River	South Korea	2/8	2[b]	1.2	–	2.7	–	Kim et al. (2007)
		River	Unknown	1/1	52 ± 5	–	Sep., 2008	–	Sep., 2008	Negreira et al. (2009)
		Streams with direct inputs of domestic wastewater	Japan	2/2	25[b]	16	Summer, 2008	41	Summer, 2008	Kameda et al. (2011)
		Lake Cospuden (Recreational water)	Leipzig, Germany	–	40 ± 3	–	Summer	–	Summer	Rodil et al. (2009b)
		Lake	Slovenia	4/5	45	<28	Bakovci	85	Rakitna	Cuderman and Heath (2007)
		Lake	Spain	1/2	–	<LOD	May, 2007/Lake Cospudener	27 ± 4	Jun., 2007/Lake Bagger	Rodil and Moeder (2008)
		Lake Zurich	Switzerland	–	–	<2	Jul., 1998	4	Jul., 1998	Poiger et al. (2004)
		Lake Hüttnersee	Switzerland	–	–	5	Jul., 1998	125	Jul., 1998	Poiger et al. (2004)
		Lake	Switzerland	–	–	14	Sep., 2002/ Zurichsee (direct and indirect input)	35	Jul., 2002/Hu¨ttnersee (direct input only)	Balmer et al. (2005)
		Swimming pool	Slovenia	2/2	251.5	103	Kdeljevo	400	Portoroz	Cuderman and Heath (2007)
	Influent	–	Leipzig, Germany	–	234 ± 41	–	Summer	–	Summer	Rodil et al. (2009b)
		WWTP influent	Italy	–	–	6	Aug. and Sep., 2011	163	Jul., 2011	Magi et al. (2013)
		Raw waste water	Spain	–	–	<7	Jan., 2007	168 ± 7	Jul., 2007	Rodil et al. (2008)
		WWTP influent	Switzerland	–	–	700	Apr., 2002/Meilen	7800	Jun., 2002/Thalwil	Balmer et al. (2005)
		Wastewater influent	San Diego county, California, U.S.A.	–	6870	5300	Aug. to Nov., 2001	8300	Aug. to Nov., 2001	Loraine and Pettigrove (2006)
		Wastewater influent	San Diego county, California, U.S.A.	–	6240	110	Jan. to Jun., 2002	10,400	Jan. to Jun., 2002	Loraine and Pettigrove (2006)
		Stanley WWTP influent	Hong Kong	–	258	–	–	–	–	Yu et al. (2012)

(continued on next page)

Table 2 (*continued*)

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
		STP influent	Unknown	4/4	–	216 ± 27	Feb., 2008	462 ± 74	Sep., 2008	Negreira et al. (2009)
	Effluent	WWTP effluent	Italy	–	–	5	Apr. and Jun., 2011	28	May., 2011	Magi et al. (2013)
		Treated wastewater	Spain	2/2	–	42 ± 3	May, 2007	54 ± 6	Jul., 2007	Rodil and Moeder (2008)
		WWTP effluent	Switzerland	–	–	<10	Sep., 2003/Kloten-Opfikon	700	Jun., 2002/ Wadenswil	Balmer et al. (2005)
		Sewage treatment plant (STP) effluent	South Australia	–	32.7 ± 1.7	–	–	–	–	Liu et al. (2011)
		STP effluents	Japan	4/4	54	29	–	164	–	Kameda et al. (2011)
		WWTP effluent	Hon Kong	–	323	–	–	–	–	Yu et al. (2012)
		Industrial drainage	South Korea	1/7	–	<5	Apr., 2003	27	Apr., 2003	Jeon et al. (2006)
		WWTP effluent	South Korea	5/7	11	1.0	–	30	–	Kim et al. (2007)
		WWTP effluent	Taiwan	2/2	–	12.5	–	21.4	–	Wu et al. (2013)
		STP effluent	Unknown	2/4	–	13 ± 4	Feb., 2008	44 ± 8	Sep., 2008	Negreira et al. (2009)
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	–	Northeastern Spain	13/20		<LOQ	Dec., 2009	27	Dec., 2009	Gago-Ferrero et al. (2011)
		From Saginaw River (2002) and Detroit River (1998)	Michigan, U.S.A.	6/6	2.34	0.728	1998 and 2002	4.66	1998 and 2002	Zhang et al. (2011)
		From Songhua River	China	6/6	0.380	0.272	2009	0.545	2009	Zhang et al. (2011)
		–	Japan	0/29	–	–	Summer, 2008	–	Summer, 2008	Kameda et al. (2011)
		–	South Korea	0/15	–	<0.5	Apr. to May, 2003	<0.5	Apr. to May, 2003	Jeon et al. (2006)
	Ground soil	–	South Korea	5/33	2.650	0.73	Apr. to May., 2003	3.880	Apr. to May., 2003	Jeon et al. (2006)
	Sludge	From WWTPs serving five large cities	Northeastern China	5/5	12.8	2.05	Jul., 2009	13.3	Jul., 2009	Zhang et al. (2011)
	Fish (ng/g lipid)									
	Andalusian Barbel	Along the Guadalquivir river basin	South Spain	2/2	20.4 ng/g dw	16.5 ng/g dw	2010	24.3 ng/g dw	2010	Gago-Ferrero et al. (2013)
	Roach	–	Switzerland	5/5	–	66	Sep., 2002/ Huttnersee	118	Aug., 2002/ Greifensee	Balmer et al. (2005)
	White fish	–	Switzerland	2/4	–	<54	Sep., 2002/ Pfaafikersee	<240	Jan., 2002/Thunersee	Balmer et al. (2005)
	Brown trout	From River Ergolz	Switzerland	–	–	<LOD,	Sep., 2006	151	Sep., 2006	Fent et al. (2010b)
BP-1										
	Water (ng/L)									
	Surface water	River	UK	–	–	<0.3	River Taff	17	River Taff	Kasprzyk-Hordern et al. (2008)
		River	UK	–	–	<0.3	River Ely	13	River Ely	Kasprzyk-Hordern et al. (2008)
		River	South Korea	1/31	47	–	–	–	–	Jeon et al. (2006)
		Rivers where the municipal wastewater is directly discharged	Taiwan	1/2	6.1	–	–	–	–	Wu et al. (2013)
		River	Unknown	1/1	37 ± 6	–	–	–	–	Negreira et al. (2009)
	Influent	STP influent	Unknown	4/5	–	161 ± 11	–	245 ± 20	–	Negreira et al. (2009)
	Effluent	STP effluent	Unknown	1/4	41 ± 2	–	–	–	–	Negreira et al. (2009)
		WWTP effluent	Taiwan	2/2	–	7.7	–	16.8	–	Wu et al. (2013)

Table 2 (*continued*)

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	From Saginaw River (2002) and Detroit River (1998)	U.S.A., Michigan	4/6	0.454	0.259	1998 and 2002	0.607	1998 and 2002	Zhang et al. (2011)
	Sludge	–	Northeastern China	5/5	32.7	4.41	Jul., 2009	91.6	Jul., 2009	Zhang et al. (2011)
BP-8										
	Water (ng/L)									
	Surface water	Rivers where the municipal wastewater is directly discharged	Taiwan	0/2	–	–	–	–	–	Wu et al. (2013)
	Effluent	WWTP effluent	Taiwan	2/2	–	9.8	–	10.1	–	Wu et al. (2013)
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	From Saginaw River (2002) and Detroit River (1998)	U.S.A., Michigan	4/6	0.424	0.133	1998 and 2002	0.796	1998 and 2002	Zhang et al. (2011)
		–	South Korea	12/15	0.95	0.5	Apr. to May, 2003	2.14	Apr. to May, 2003	Jeon et al. (2006)
	Ground soil	–	South Korea	5/33	1.670	0.5	Apr. to May, 2003	4.170	Apr. to May, 2003	Jeon et al. (2006)

[a] Detection frequency.
[b] Mean value detected.

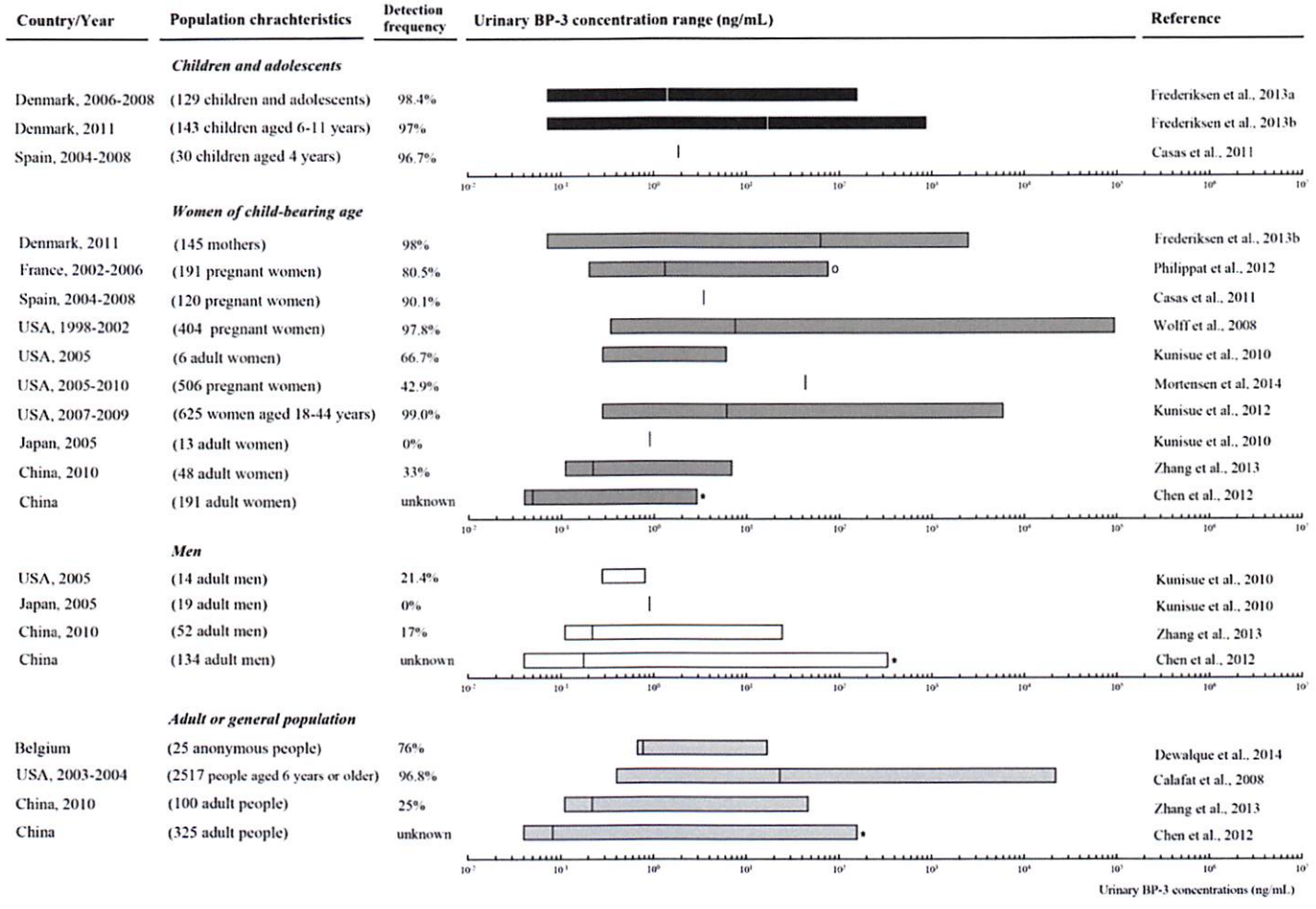


Fig. 2. Urinary benzophenone-3 (BP-3) concentrations (ng/mL) reported worldwide. The left and right edges of the horizontal bar graph indicate the lowest and the highest levels of detection, respectively. The vertical line shown within the box represents the median concentrations. If the highest level of detection is not available in the literature, the 95th (open circle) or 99th (star) percentile was shown.

Table 3
Levels of human exposure to benzophenone-3 and its relevant derivatives.

Sample type	Country/region	Year	Test subjects	M/F[d]	Compounds	Detect. freq. [a] (%)	Median conc. (ng/mL)	Range (ng/mL)	Note	Reference
Urine										
	Belgium	–	25 anonymous Belgian donors	–	BP-3	76	0.77	<0.67–16.8	–	Dewalque et al. (2014)
	Denmark	2006–2008	104 children aged 6–16 years and 25 adolescents aged 17–21 years	65/64	BP-3	98.4	1.41	<0.07–162	Children were recruited from Copenhagen puberty study and adolescents were recruited among pupils in a high school from the Northern Copenhagen area.	Frederiksen et al. (2013a)
	Denmark	2011	145 mothers 143 children aged 6–11 years	0/145 –	BP-3	98 97	62[c] 17[c]	<0.07–2442 <0.07–885	Children and their mothers were recruited from schools in Gentofte (urban area, n = 70) and Viby Sj. (rural area, n = 75)	Frederiksen et al. (2013b)
	France	2002–2006	191 pregnant women	0/191	BP-3	80.5	1.3	5th: 0.2 95th: 74.5	EDEN Mother–Child cohort; recruited before gestational week 28	Philippat et al. (2012)
	Greece, Athens	2012, Mar. to Apr.	100 people aged 2.5–87 years	50/50	BP-1 BP-8 BP-2 4-OH-BP	78 24 40 23	1.8 1.0 1.1 1.6	<1–1117 <2–24.7 <1–54.3 <0.7–46.8	–	Asimakopoulos et al. (2014)
	Spain	2004–2008	120 pregnant women 30 children aged 4 years	0/120 30/0	BP-3	90.1 96.7	3.4 1.9	– –	INMA (INfancia y Medio Ambiente [Environment and Childhood]) Spanish project	Casas et al. (2011)
	U.S.A., Albany, New York	1998–2002	404 pregnant women	0/404	BP-3	97.8	7.5	<0.34–92,700	The Children's Environmental Health Study: a prospective ethnically diverse birth cohort of 404 mother–infant pairs	Wolff et al. (2008)
	U.S.A.	2003–2004	2517 people aged 6 or older	1229/1288	BP-3	96.8	22.9[c]	0.4–21,700	National Health and Nutrition Examination Survey (NHANES) Study	Calafat et al. (2008)
	U.S.A., Albany, New York	2005, Feb. to Mar.	6 women	0/6	BP-3 BP-1 4-OH-BP	66.7 100 33.3	– – –	<0.28–6.0 0.9–9.0 <0.28–0.8	–	Kunisue et al. (2010)
			14 men	14/0	BP-3 BP-1 4-OH-BP	21.4 42.6 7.1	– – –	<0.9–5.0 <0.28–6.0 0.61–0.8		
	U.S.A.	2005–2010	506 pregnant women	0/506	BP-3	100	42.9 59.5[c]	–	National Children's Study (NCS) Vanguard Study	Mortensen et al. (2014)
	U.S.A., Utah and California	2007–2009	625 women aged 18–44 years	0/625	BP-3 BP-1 BP-8	99.0 93.3 2.6	6.1 6.1 –	<0.28–5900 <0.082–3200 –	ENDO (Endometriosis, Natural history, Diagnosis, and Outcomes) Study	Kunisue et al. (2012)
	Japan, Matsuyama	2005, Feb. to Mar.	13 women	0/13	BP-3 BP-1 4-OH-BP	0 15.4 0	– – –	<0.9 <0.28–1.0 ND[b]	–	Kunisue et al. (2010)
			19 men	19/0	BP-3 BP-1 4-OH-BP	0 0 0	– – –	<0.9 <0.28 ND[b]		
	China	2010, Feb. to May	100 adults	52/48	BP-3 BP-1 4-OH-BP	25 57 61	<0.22 0.14 0.14	<0.11–46.1 <0.07–14.6 <0.06–5.14	General adult population in Tianjin (n = 50), Shanghai (n = 26), and Qiqihar (n = 25)	Zhang et al. (2013)

			48 adult women	0/48	BP-3	33	<0.22	<0.11–6.91		
					BP-1	65	<0.33	<0.07–14.3		
					4-OH-BP	69	<0.33	<0.03–8.11		
			52 adult men	52/0	BP-3	17	<0.22	<0.11–24.3		
					BP-1	50	0.11	<0.07–20.3		
					4-OH-BP	54	0.25	<0.06–2.82		
	China	–	325 adults	191/134	BP-3	–	0.082	95th: 2.179 99th: 157.820	Non-occupational Chinese adults were recruited from affiliated hospitals of Nanjing Medical University	Chen et al. (2012)
			191 adult women	0/191		–	0.049	95th: 1.013 99th: 2.881		
			134 adult men	134/0		–	0.175	95th: 5.070 99th: 332.923		
Serum										
	U.S.A.	2001–2002	936 children aged 3–11 years	–	BP-3	63	–	<0.5–8.7 (free conc.)	NHANES Study	Ye et al. (2012)
Whole blood										
	China	2010, Feb. to May	10 children aged 1–5 years	5/5	BP-3	30	<0.52	<0.52–2.20	Whole blood samples were collected from children in Nanchang; matched maternal blood and fetal cord blood samples were collected from women who were delivering in Tianjin; paired blood and urines samples were collected from adults in Tianjin.	Zhang et al. (2013)
					BP-1	10	<0.06	<0.06–0.09		
					4-OH-BP	100	0.32	0.23–0.40		
			20 pregnant women	0./20	BP-3	35	<0.41	<0.41–2.30		
					BP-1	0	<0.12	<0.12		
					4-OH-BP	100	0.58	0.32–1.78		
			23 adults	12/11	BP-3	83	2.09	<0.52–3.38		
					BP-1	4	<0.06	<0.06–0.15		
					4-OH-BP	100	0.35	0.26–1.29		
Cord blood										
	China	2010, Feb. to May	22 fetuses at the time of delivery	11/11	BP-3	55	0.59	<0.41–2.55		
					BP-1	0	<0.12	<0.12		
					4-OH-BP	100	0.41	0.26–0.51		
Human breast milk										
	Switzerland	2004–2006	54 mothers	0/54	BP-3	12.96	26.70 ng/g lipid	7.30–121.40 ng/g lipid	Cohorts; mothers who gave birth to a single child at the University, Women's Hospital Basel	Schlumpf et al. (2010)
Placental tissue										
	Spain	–	16 pregnant women	0/16	BP-3	0	ND[b]	ND[b]	Placenta samples were collected during different deliveries in the Maternity Unit of San Cecilio University Hospital of Granda	Vela-Soria et al. (2011)
					BP-1:	87.5	2.6 ng/g	0.5–9.8 ng/g		

[a] Detection frequency; percentage of samples containing BP derivatives above analytical detection limit.
[b] Not detected.
[c] Geometric mean.
[d] Sex ratio; male/female.

Table 4
Endocrine disrupting activities of benzophenone-3 and its relevant derivatives in vitro.

Compounds	Hormonal activity	Cell line	Assay	Endpoints	Conc. (μM)	Reference
BP-3	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation EC50	3.73	Schlumpf et al. (2001)
			MCF-7 cell proliferation assay	6 d, cell proliferation LOEC	>100	Nakagawa and Suzuki (2002)
			pS2 protein assay	pS2 protein secretion, LOEC	10	Schlumpf et al. (2001)
			ERE-luciferase reporter assay	Estrogenic EC50	19.5	Suzuki et al. (2005)
		Recombinant yeast	Recombinant yeast assay	Agonism toward rtER, EC50	21.9	Kunz et al. (2006)
			Recombinant yeast assay	Agonism toward hERα, EC50	18.6	Kunz and Fent (2006b)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	20.315	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation of hERα, NOEC	10	Gomez et al. (2005)
			Luciferase assay	Transactivation of hERβ, NOEC	10	Gomez et al. (2005)
			Luciferase assay	Transactivation for hERα, EC50	>30	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	18.426	Molina-Molina et al. (2008)
		HEK293 cells	Gene expression assay	Transactivation for hERα, EC50	2.9	Schreurs et al. (2005)
			Gene expression assay	Transactivation for hERβ, EC50	25	Schreurs et al. (2005)
			Gene expression assay	Transactivation for hERα, LOEC	10	Schreurs et al. (2002)
			Gene expression assay	Transactivation for hERβ, LOEC	10	Schreurs et al. (2002)
	Antiestrogenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hERα, IC50	17.8	Kunz and Fent (2006b)
			Recombinant yeast assay	Antagonism toward hERα, IC50	3.68	Kunz and Fent (2006b)
		HELN cells	Luciferase assay	Antagonism toward hERα, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward hERβ, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward rtERα, NOEC	0.01	Molina-Molina et al. (2008)
	Antiprogestagenic	U2-OS cells	Gene expression assay	Transrepression for hPR, IC50	5.2	Schreurs et al. (2005)
	Antiandrogenic	U2-OS cells	Gene expression assay	Transrepression for hAR, IC50	2	Schreurs et al. (2005)
		NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)
		HEK293 cells	Gene expression assay	Transrepression for hAR, IC50	3.1	Nashev et al. (2010)
BP-1	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation, LOEC	0.01	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	1.26	Suzuki et al. (2005)
		Recombinant yeast	Recombinant yeast assay	Agonism toward rtER, EC50	0.799	Kunz et al. (2006)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	9.192	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation for hERα, EC50	8.513	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, EC50	3.965	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	3.48	Molina-Molina et al. (2008)
	Antiestrogenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hERα, EC50	1.15	Kunz and Fent (2006b)
		HELN cells	Luciferase assay	Antagonism toward hERα, IC50	3.19	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward hERβ, IC50	1.59	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward rtERα, IC50	2.45	Molina-Molina et al. (2008)
	Antiprogestagenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hAR, IC50	0.692	Kunz and Fent (2006b)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	10	Suzuki et al. (2005)
		HEK293 cells	Gene expression assay	Transrepression for hAR, IC50	5.1	Nashev et al. (2010)
BP-8	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation LOEC	0.1	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	>100	Suzuki et al. (2005)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)
THB	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation, LOEC	1	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	11.8	Suzuki et al. (2005)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	4.012	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation for hERα, EC50	2.272	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, EC50	0.827	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	0.578	Molina-Molina et al. (2008)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)

been detected in human samples. Kunisue et al. (2012) reported BP-3 in 99.0%, BP-1 in 93.3%, and 4-OH-BP in 83.8% of urine samples (n = 625 U.S.A. women). In placental tissue samples of pregnant women, BP-1 was present in 87.5% (n = 16) (Vela-Soria et al., 2011). Frequent detection of BP-3 and BP-1 in breast milk or placental tissues implies potential transfer to fetus and breastfed infant. Trans-placental transfer ratios of BP-3, i.e., 0.48 (geometric mean), were also suggested by Zhang et al. (2013) employing whole blood samples.

Urinary levels of BP-3 appeared to be affected by several factors. The urinary concentrations of BP-3 are reported to be higher in females than in males of the U.S.A. (Calafat et al., 2008; Ye et al., 2012) while no gender differences were found in urine of Danish children and adolescents (Frederiksen et al., 2013a). Furthermore, urinary or serum concentrations of BP-3 tend to be higher in urban mothers and in individuals of higher socioeconomic status (SES) (Frederiksen et al., 2013b; Tyrrell et al., 2013), probably reflecting their greater frequency of usage. Differences by sampling countries also have been reported. Detection frequencies of BP-3 appeared to be much higher in the U.S.A. and Europe (80.5–99.0%, Table 3) than in Asian countries such as Japan (0%, n = 32) and China (25%, n = 100) (Kunisue et al., 2010; Zhang et al., 2013). These results might be attributable to different life styles or to the different pattern of UV filter use among countries. For example, both concentrations and detection rates of BP-3 in personal care products of the U.S.A. (1200 ng/g product weight with 99.1% of detection frequency) were reported higher than those of China (20.1 ng/g product weight, 64.1%) (Liao and Kannan, 2014).

Recent studies indicate that BP-3 exposure may occur from sources other than sunscreens or PCPs (Krause et al., 2012). Frederiksen et al. (2013b) reported BP-3 in almost all urine samples (97–98%) that were collected during seasons when sunscreens are not generally used. In addition, in the river or lakes near WWTP, levels of BP-3 in water were generally higher during spring or fall than in summer (Fent et al., 2010b; Rodil et al., 2008). BP-3 was detected at a similar frequency as adults in the urines of young children aged 6–16 years (Frederiksen et al., 2013a, 2013b). These observations indicate the presence of BP-3 sources other than sunscreen or cosmetics.

Dust ingestion is thought to be one of major sources of BP-3 exposure. Five types of BPs were detected in indoor dust samples from the

U.S.A., China, Japan, and Korea, and the detection rates of BP-3 and BP-1 were 100% (Wang et al., 2013). Concentrations of BP-3 in dust were 64.5–1190 ng/g in the U.S.A. and 9.72–1690 ng/g in Korea.

4. Adverse effects of BP-3 in vitro and in vivo

4.1. Endocrine disruption potentials in vitro

A number of in vitro studies indicate that UV filters such as BP-3, 4-MBC, OMC, and OD-PABA have endocrine-disrupting capacities (Gomez et al., 2005; Kunz and Fent, 2006b; Morohoshi et al., 2005; Schlumpf et al., 2001; Schreurs et al., 2005). Table 4 summarizes endocrine disrupting activities of BP-3 and its metabolites reported from in vitro studies.

BP-3 was determined to be slightly estrogenic in an MCF-7 human breast cancer cell proliferation assay and pS2 protein assay; the maximal MCF-7 cell count increase was 95.09% of 17β-estradiol (E2) and the maximal weight increase was 7.60% of 17α-ethinylestradiol (EE2) (Schlumpf et al., 2001). Weak estrogenicity of BP-3 was also found in a yeast bioassay transfected with estrogen receptor (ER), estrogen responsive elements (ERE), and a *lacZ* reporter gene (Miller et al., 2001). BP-1, a major metabolite of BP-3, was found to possess stronger estrogenicity than its parent compound. Molina-Molina et al. (2008) examined four types of BP derivatives for the transcription activation toward ERs. BP-1 and other BP derivatives such as BP2 and THB had considerably lower EC50 values than those of BP-3 in MELN (MCF-7-ERE-Luciferase-Neo) and HELN (HeLa-ERE-Luciferase-Neo) cell lines. In a similar human breast cell line, MCF-7 cells, BP-1 induced cell proliferation at concentrations of 0.01–1 μM, whereas BP-3 did not induce the proliferation even at 100 μM (Nakagawa and Suzuki, 2002). In a yeast two-hybrid assay, Kawamura et al. (2003) reported that estrogenic activities of BP-3 and its metabolites were in the order of BP-1 > THB ≫ BP-3 > BP-8. Relative to E2, estrogenic activity of BP-1 was calculated at 1/5000 fold, while that of BP-3 was 1/45,000 fold in an in vitro yeast sex hormone receptor transactivation study (Kunz and Fent, 2006b). For BP-8 and THB, significant MCF-7 cell proliferation was found (Nakagawa and Suzuki, 2002) although estrogenic activity of BP-8 was not evident in a yeast bioassay (Kawamura et al., 2003; Miller et al., 2001). The ER binding affinity of BPs could be explained by the hydroxyl group at the *para*-position of BP (Kawamura et al., 2003; Miller et al., 2001; Schultz et al., 2000).

BP-3 can also elicit both antiestrogenic (Molina-Molina et al., 2008; Schreurs et al., 2002) and antiandrogenic activities (Ma et al., 2003; Nashev et al., 2010; Schreurs et al., 2005; Suzuki et al., 2005) in vitro. Antiestrogenic activity of BP-3 was calculated at 1/45 fold relative to that of 4-hydroxytamoxifen, and antiandrogenic activity relative to flutamide was calculated at 1/1.3 fold in an in vitro yeast sex hormone receptor transactivation assay (Kunz and Fent, 2006b). However, conflicting results have often been published regarding the antiestrogenic potential of BP-3: BP-3 showed no antagonistic effects at both human ERα (hERα) and hERβ transfected in human embryonal kidney (HEK293) cell line (Schreurs et al., 2002). Different binding affinities to ERs by species may explain such inconsistency (Krause et al., 2012). For BP-1, strong estrogenic and antiandrogenic activities were detected.

The binding affinity of BP-3 appears to also vary depending on the ER subtype, e.g., ERα and ERβ (Molina-Molina et al., 2008; Schreurs et al., 2002, 2005). In HEK293 cells, the EC50 value of transactivation for hERα was one order of magnitude lower than for hERβ (Schreurs et al., 2005). Estrogenic activity of BP-1 is likely to be ERα mediated rather than ERβ (Park et al., 2013). ERα activation is related to the cell proliferation while ERβ is reported to play an important role in cell differentiation (Förster et al., 2002; Helguero et al., 2005).

A few in vitro studies have been performed to understand the estrogenicity of BP-3 in fish. Based on a recombinant yeast assay expressing rainbow trout ERα (rtERα) as well as hERα (Kunz et al., 2006b; Molina-Molina et al., 2008), BP-1 displayed the highest binding affinity to both fish and human receptors among 23 UV filters. The binding activity of BP-3 was relatively higher in rtERα than in hERα (Kunz et al., 2006).

Toxicity interactions of UV filter mixtures are of concern because UV filters are generally used in combination and are present as such in the aquatic environment (Heneweer et al., 2005; Kunz and Fent, 2006a). Estrogenic compounds are expected to interact additively if they possess similar modes of action (Kortenkamp, 2007). Binary mixtures of BP-3 and BP-1, and a multi-component mixture of BP-1, BP-3, OMC, and 4-MBC showed additivity in ER binding in MCF-7 cells (Heneweer et al., 2005). While these observations suggest similar modes of endocrine disruption among BP based UV filters, other types of mixture interactions have also been reported. A tertiary mixture of BP-1, BP-2 and 3-benzylidene camphor (3BC) displayed additivity at high concentrations but antagonism at lower concentrations, in terms of fish vitellogenesis (Kunz and Fent, 2009). Contrary to these reports, synergistic estrogenic activities were detected for mixtures of four or eight UV filters in a recombinant yeast assay with hERα, indicating significant interactions among UV filters (Kunz and Fent, 2006a). Further investigation on the modes of action of BP-3 in mixture is warranted.

Table 5
Endocrine disrupting activities of benzophenone-3 and its relevant derivatives in vivo.

Compounds	Hormonal activity	Assay	Experimental animals	Exposure route/duration	Endpoints	Conc.	Reference
BP-3	Estrogenic	Uterotrophic assay	Immature Long-Evans rats	Oral, 4 d	Increase of uterine weights, ED50	1000–1500 mg/kg/day	Schlumpf et al. (2001)
		Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, NOEC	500 mg/kg/day	Suzuki et al. (2005)
		Uterotrophic assay	Female Sprague–Dawley rats	Oral, 5 d	Increase of uterine weights, NOEC	250 mg/kg/day	Schlecht et al. (2004)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	$>1.00 \times 10^{-4}$ M	Blair et al. (2000)
BP-1	Estrogenic	Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, LOEC	500 mg/kg/day	Suzuki et al. (2005)
		Uterotrophic assay	Female Crj:CD rats	Sc, 3 d	Increase of uterine weights, LOEL	625 mg/kg/day	Koda et al. (2005)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	3.65×10^{-5} M	Blair et al. (2000)
BP-8	Estrogenic	Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, LOEC	300 mg/kg/day	Suzuki et al. (2005)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	$>1.00 \times 10^{-4}$ M	Blair et al. (2000)
	Androgenic	Hershberger assay	F344 male rats	Sc, 10 d	Increase of prostate gland and seminal vesicle	300 mg/kg/day	Suzuki et al. (2005)

Table 6
Acute and chronic effects of benzophenone-3 in aquatic organisms.

Compounds	Taxonomic group	Species	Test duration/endpoint	Conc. (mg/L)	Reference
BP-3	Algae	*Scenedesmus vacuolatus*	24 h, reproduction IC50	0.36	Rodil et al. (2009a)
		Desmodesmus subspicatus	72 h, growth IC10	0.61	Sieratowicz et al. (2011)
		Desmodesmus subspicatus	72 h, growth IC50	0.96	Sieratowicz et al. (2011)
	Invertebrate	*Daphnia magna*	48 h, immobilization EC50	1.67	Sieratowicz et al. (2011)
		Daphnia magna	48 h, immobilization EC50	1.9	Fent et al. (2010a)
		Dugesia japonica	48 h, immobilization EC50	0.9	Li (2012)
		Daphnia magna	21 d, reproduction NOEC	0.5	Sieratowicz et al. (2011)
	Fish	*Danio rerio*	14 d, vitellogenin induction NOEC	0.312	Bluthgen et al. (2012)
		Oncorhynchus mykiss	14 d, vitellogenin induction LOEC	0.749	Coronado et al. (2008)
		Oncorhynchus mykiss	14 d, vitellogenin induction NOEC	0.132	Coronado et al. (2008)
		Oryzias latipes	7 d, reproduction LOEC	0.62	Coronado et al. (2008)
		Oryzias latipes	7 d, reproduction NOEC	0.132	Coronado et al. (2008)
		Oryzias latipes	F1 hatchability LOEC following 21 d F0 exposure	0.62	Coronado et al. (2008)
		Oryzias latipes	F1 hatchability NOEC following 21 d F0 exposure	0.132	Coronado et al. (2008)
		Oryzias latipes	21 d, vitellogenin induction LOEC	0.62	Coronado et al. (2008)
		Oryzias latipes	21 d, vitellogenin induction NOEC	0.132	Coronado et al. (2008)
		Pimephales promelas	14 d, vitellogenin induction NOEC	3.9	Kunz et al. (2006)
BP-1	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	2.8	Li (2012)
	Fish	*Pimephales promelas*	14 d, vitellogenin induction LOEC	4.919	Kunz et al. (2006)
BP-8	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	4.4	Li (2012)
THB	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	34.6	Li (2012)

4.2. Endocrine disruption and other adverse effects in vivo

Relatively limited information is available on endocrine disruption of BPs in experimental animals (Table 5). Some hydroxylated BPs have produced both estrogen agonistic and antagonistic activities in an immature rat uterotrophic assay, but no antiandrogenic activity was observed in a Hershberger assay (Yamasaki et al., 2003). Compared to 4-MBC and OMC, uterotrophic effects of BP-3 in an ovariectomized rat are negligible (Schlecht et al., 2004; Schlumpf et al., 2001; Suzuki et al., 2005). BP-3 was also reported for significant down-regulation of *erα* mRNA in pituitary, and reduced *erβ* expression in uterus of female Sprague–Dawley rats (Schlecht et al., 2004). However, BP-1 appeared to have significant uterotrophic effects (Koda et al., 2005). BP-1 exposure stimulated the growth of BG-1 human ovarian cancer cells in the absence of endogenous ovarian estrogen in xenograft mouse models (Park et al., 2013). Based on an ER competitive-binding assay in Sprague–Dawley rats, estrogenic potential of BP-1 is also evident while those for BP-3 and BP-8 were not observed (Blair et al., 2000). These reports are generally consistent with the results of the in vitro studies.

For aquatic organisms, several studies have reported the adverse effects of BP-3 on reproduction and development (Table 6). Adverse acute and chronic effects on algae (*Scenedesmus vacuolatus* and *Desmodesmus subspicatus*), the freshwater flea (*Daphnia magna*) and planarian (*Dugesia japonica*) have been reported for BP-3 (Fent et al., 2010a; Li, 2012; Rodil et al., 2009a; Sieratowicz et al., 2011). BP-3 can alter the expression of an endocrine signaling gene, *ultraspiracle* (*USP*) in the aquatic invertebrate, *Chironomus riparius* (Ozaez et al., 2013).

A 21 d exposure to 620 μg/L BP-3 led to a decrease of reproduction and hatching success in Japanese medaka (*Oryzias latipes*). The exposure to a positive chemical, i.e., 0.05 μg/L E2, also led to the similar reproduction damages (Coronado et al., 2008). VTG induction by BP-3 varies by fish species. VTG concentrations in plasma samples from male Japanese medaka and rainbow trout (*Oncorhynchus mykiss*) increased by 21 d exposure to 620 μg/L and 14 d exposure to 749 μg/L, respectively (Coronado et al., 2008). In contrast, no significant alterations of plasma or whole body VTG were observed in male zebrafish (*Danio rerio*) and juvenile fathead minnow (*Pimephales promelas*) at concentrations of BP-3 as high as 312 and 3900 μg/L, respectively (Bluthgen et al., 2012; Kunz et al., 2006), while exposure to 0.1 μg/L E2 induced VTG in the same fish (Kunz et al., 2006). The interspecies differences in VTG synthesis by chemical exposure have already been reported by Van den Belt et al. (2003). The variation among species might be also explained in part by differences in the metabolic rate of BP-3 into BP-1. BP-1 induced VTG synthesis in juvenile fathead minnows at concentrations of 4919 μg/L (Kunz et al., 2006). The estrogenic activity of BP-3 such as VTG induction reported in certain aquatic species, could therefore be at least in part attributed to its metabolic product, i.e., BP-1, rather than by BP-3 alone which has shown only weak estrogenic activities in many in vitro studies. Among other BP derivatives, BP-2 has been reported to cause VTG induction and adverse reproduction effects in adult fathead minnows at high level of exposure ranging between 1200 and 9700 μg/L (Weisbrod et al., 2007).

Low level BP-3 can also inhibit steroidogenesis and affect hormonal pathways in male zebrafish (Bluthgen et al., 2012). Upon exposure to 84 μg/L BP-3, steroidogenic enzymes including *17β-hsd* were down-regulated, implying both antiestrogenic and antiandrogenic activities of BP-3 in the zebrafish. BP-1 is also believed to inhibit human *17β-HSD3*, and therefore could possibly reduce testosterone production (Nashev et al., 2010). A similarly designed study showed that BP-4

Table 7
Derivation of predicted no effect concentration (PNEC) for benzophenone-3 in fresh water environment.

Toxicity	Taxonomic group	EC50 or NOEC	Conc. (μg/L)	Reference	Assessment factor	PNEC (μg/L)
Acute	Algae	72 h, EC50	960	Sieratowicz et al. (2011)	100[a]	1.32
	Invertebrate	48 h, EC50	1670	Sieratowicz et al. (2011)		
Subchronic	Fish	21 d, NOEC	132[b]	Coronado et al. (2008)		
Chronic	Algae	72 h, EC10[c]	610	Sieratowicz et al. (2011)		
	Invertebrate	21 d, NOEC	500	Sieratowicz et al. (2011)		

[a] An assessment factor of 100 can be used in cases where the acutely most sensitive species has a lower toxicity than the two chronic toxicity data from two trophic levels (European Commission, 2003).

[b] Based on the lowest *Oryzias latipes* F1 hatchability NOEC after 21 days of parental exposure. The test duration of 21 days for fish was considered as acute with conservative perspectives.

[c] If no NOEC value is available for a long-term test, EC10 obtained by extrapolation using appropriate statistics can be considered as a NOEC (European Commission, 2003).

Table 8
Measured environmental concentrations (MECs) of benzophenone-3 in water environment and their hazard quotients (HQs).

Country	Source	Type	MEC (μg/L)	HQ	Reference
Unidentified	Surface water	Mean	0.052	0.04	Negreira et al. (2009)
Switzerland		Max	0.125	0.09	Poiger et al. (2004)
U.S.A.	Influent	Median	6.87	5.20	Loraine and Pettigrove (2006)
U.S.A.		Max	10.4	7.88	Loraine and Pettigrove (2006)

could also alter the expression of the genes involved in steroidogenesis in zebrafish (Zucchi et al., 2011).

5. Ecological risk assessment

To estimate the potential risk of BP-3 in aquatic environment, we performed an ecological risk assessment based on the available information, following European Commission (2003). In order to derive the predicted no-effect concentration (PNEC), only ecotoxicological data that are related to ecologically meaningful endpoints, e.g., survival, growth, or reproduction, were selected from the available toxicological information (Table 6), and were listed in Table 7. Considering the amount of available toxicological information, a factor application method which employs an assessment factor, was used to derive PNEC. As relevant toxicological information currently available includes only two chronic toxicity values from algae and water flea (Table 7), an assessment factor of 100 was chosen based on European Commission (2003). While results of 21 d fish exposure studies are often considered as chronic toxicity value (e.g., Fent et al., 2010a), we did not consider the results of the 21 d adult fish exposure test (Coronado et al., 2008) as "chronic". This was because a chronic toxicity test should include at least 10% of the organism's life span as an exposure duration (Suter and Barnthouse, 1993), or should include early life stage of the organism (Stephen et al., 1985).

Based on a hatchability no-observed-effect concentration (NOEC) of 132 μg/L in *O. latipes* following 21 d exposure of F0 (Coronado et al., 2008) and an assessment factor of 100, the PNEC was determined at 1.32 μg/L. This PNEC, however, should be used with caution, because this value reflects only the current knowledge of ecotoxicological information of BP-3, and is likely to be refined in the future with forthcoming toxicological data.

Hazard quotient (HQ) derived based on the measured environmental concentrations (MECs, Table 2) implies that direct impact of BP-3 would be negligible at the current levels in ambient waters (Table 8). However, in hotspots, e.g., wastewater influents of the U.S.A. and Switzerland, the HQ values for BP-3 often exceeded unity. For example, in wastewater influent in San Diego County, U.S.A., BP-3 was detected at as high as 10.4 μg/L (Loraine and Pettigrove, 2006), of which HQ is close to 8. Since the level of this compound varies by season and by human activities, and relatively limited information is available for environmental occurrences of this compound, systematic monitoring program and thorough toxicological studies are warranted to better understand risks of BP-3 in the aquatic environment.

6. Conclusion and future research

Widespread use of BP-3 in various personal care products including sunscreens and cosmetics, and its frequent detection in both water environment and biota have raised concerns on ecological risks of this compound. BP-3 and other BP derivatives have shown multiple endocrine disrupting activities. Animal studies have shown that BP-3 could be biotransformed into its hydroxylated forms, and some of which could exhibit greater estrogenic affinity both in vivo and in vitro. Based on the available in vivo toxicity studies, and the levels occurring in ambient water, BP-3 likely poses a negligible risk to freshwater ecosystems except for some hotspot areas. However, temporal and spatial pattern of environmental occurrences of BP-3 and its derivatives are not well understood. In addition, ecotoxicological information on long-term exposure to BP-3 and related derivatives is limited. Considering continuous release of this group of compounds into the water as well as their bioaccumulative potential, consequences following long-term exposure of aquatic organisms warrant further investigation.

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The relationship between prenatal exposure to BP-3 and Hirschsprung's disease

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HIGHLIGHTS

- We examined the association between maternal BP-3 exposure and the offspring's HSCR risks.
- BP-3 suppressed cell migration and regulated RET, miR-218, PLAG1, SLIT2 and ROBO1 expressions.
- SLIT2/ROBO1-miR-218-RET/PLAG1 pathway was involved in the pathogenesis of HSCR induced by BP-3.

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ABSTRACT

Hirschsprung's disease (HSCR) is neonatal intestinal abnormality which derived from the faliure of enteric neural crest cells migration to hindgut during embryogenesis from 5 to 12 weeks. Currenly, the knowledge of environmental factors contributing to HSCR is still scarce. Benzophenone-3 (BP-3) is one of the most widely used UV filters, and has weak estrogen and strong anti-androgenic effects. In order to examine the effect of maternal BP-3 exposure on development of offspring and explore the potential mechanism, we conducted case and control study and in vitro study. In this work, BP-3 concertrations in maternal urine was detected by ultra-high performance liquid chromatography. Besides, we investigated the cytotoxicity and receptor tyrosine kinase (RET) expression in cells exposed to BP-3. The results showed that maternal BP-3 exposure was associated with offspring's HSCR in the population as well as inhibited migration of 293T and SH-SY5Y cells. What's more, we discovered dose-response relationship between RET expression and BP-3 exposure dose, and miR-218 and some other genes involved in SLIT2/ROBO1-miR-218-RET/PLAG1 pathway were also related to BP-3 exposure. Therefore, we deduced that BP-3 influenced cell migration via SLIT2/ROBO1-miR-218-RET/PLAG1 pathway. Our study firstly revealed the relationship between maternal BP-3 exposure and HSCR as well as its potential mechanism.

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1. Introduction

Hirschsprung's disease (HSCR) is a complex congenital disease caused by gene–environemnt interaction and can lead to intestinal obstruction and chronic constipation (Goldberg, 1984; Amiel et al., 2008). The incidence of HSCR is 1:2000–1:5000 in live births while males are 4 times more susceptible than females (Parisi et al., 2002). HSCR results from enteric neural crest cells' failing in migrating to certain segments of colon during embronic development from 5 to 12 weeks (Nishiyama et al., 2012; McKeown et al., 2013; Takahashi et al., 2013). Genetic studies have found that various gene mutations are associated with HSCR, such as receptor tyrosine kinase (RET), EDNRB, GDNF and SOX10. In particular, RET, which encodes a tyrosine-kinase receptor, is the most frequently mutated gene (Angrist et al., 1996; Paratore et al., 2002; Iwashita et al., 2003; Miao et al., 2010). However, the knowledge of environmental factors contributing to HSCR is still quite limited (Gershon, 2010; Lake and Heuckeroth, 2013).

Abbreviations: HSCR, Hirschsprung's disease; BP-3, Benzophenone-3; RET, receptor tyrosine kinase; DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate; DEPC, diethylpyrocarbonate; DMEM, Dulbecco minimal essential medium; FBS, fetal bovine serum; CCK-8, cell counting kit-8; LODs, limits of detection; CR, creatinine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; BMI, body mass index; ORs, odds ratios; CIs, confidence intervals.

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The toxic potential of UV filters that used in a variety of sunscreen and personal care products to attenuate the effects of harmful UV radiation on skin and hair, has attracted public concerns (Okereke et al., 1995). Benzophenone-3 (BP-3) occurs naturally in flower pigments and is synthesized for use in sunscreens, various cosmetic products, and plastic surface coatings and polymers. The application of some personal care products that contain UV filters on the skin can increase the systemic absorption (Gustavsson Gonzalez et al., 2002; Liao and Kannan, 2014). In some cases, as much as 10% of the applied dermal dose was absorbed into the systemic circulation (Janjua et al., 2004). 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, at concentrations ranging from 0.4 to 21,700 ng/mL (Calafat et al., 2008). It is also worth noting that exposure to BP-3 in women is much higher than men (Chen et al., 2012).

BP-3 is a bioactive chemical which is established to be weakly estrogenic and exhibits both intensely anti-estrogenic and anti-androgenic activities in vitro (Schlumpf et al., 2001; Schreurs et al., 2002, 2005). It is reported that BP-3 altered embryo development in insects (Ozaez et al., 2014). Previous study have found prenatal BP-3 exposure is associated with adverse birth outcomes (Wolff et al., 2008; Tang et al., 2013). Therefore, the effects of BP-3 on *fetal development* need particular concerns.

miR-218 has been extensively studied in pathologies. And it can markedly suppresses cell motility, invasion, and proliferation (Tu et al., 2013; Heckmann et al., 2014). According to a previous report, miR-218 suppresses tumor cell migration through SLIT2-ROBO1 pathway (Alajez et al., 2011). In this study, we conducted a population study to explore the potential effects of BP-3 exposure on HSCR and underlying mechanism in relation to miR-218-mediated pathways was further studied in vitro.

2. Materials and methods

2.1. Study population

We recruited 101 HSCR patients' mothers and 322 mothers as controls in affiliated hospitals of Nanjing Medical University from October 2009 to May 2014 (Nanjing Medical University Birth Cohort, NJMU Birth Cohort). The Institutional Review Board of Nanjing Medical University approved the protocols. After detailed explanation of the study procedures and clarification of questions raised, the total 423 women signed informed consent forms for the questionnaire interview and sample collections. A complete physical examination was performed on them and questionnaires were used to collect information including personal background, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and reproduction status, medical history and physical activity. As far as we were aware, all participants in our study had not changed their eating habits, life styles or environments for several months prior to the sample collection. Spot urines samples of HSCR mothers as well as 322 controls were collected for BP-3 detection and were frozen at −20 °C until analysis. Besides, 101 HSCR patients were diagnosed by pathological analysis after surgery. All the HSCR patients were diagnosed by barium enema and anorectal manometry evaluation before surgical procedures. From the surgical pathology perspective, HSCR is confirmed none ganglion cells in colorectum which is identified by the submucosa of a distal rectal biopsy. In the meantime, the 322 controls' children were divided into two groups, i.e. 103 infants received surgery because of intussusception or incarcerated and strangulated inguinal hernia without ischemia or necrosis as controls which got rid of HSCR or other congenital malformations, and the other 219 children who received non-surgical treatment. Finally, 101 HSCR and 103 control colon tissues were obtained. The tissues were immediately frozen and stored at −80 °C after surgery. All the studies involving human subjects were done under full compliance with government policies and the Helsinki Declaration.

2.2. Chemicals and reagents

BP-3 (CAS NO. 131-57-7, 99.5% purity) was purchased from Dr. Ehrenstorfer GmbH (Bürgermeister-Schlosser, Germany). β-glucuronidase/sulfatase type H-1 from Helix pomatia, dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS), diethylpyrocarbonate (DEPC) were obtained from Sigma–Aldrich (St. Louis, USA). BP-3 was first dissolved in DMSO and added to the Dulbecco minimal essential medium (DMEM) to final concentrations of 100 μM which was further diluted to lower concentrations for testing. The cells treated with 0.1% DMSO served as controls.

2.3. Measurement of urinary phenols

We measured total (free and conjugated) urinary concentrations of phenols using a sensitive method as previously described (Chen et al., 2012). Briefly, urine samples were incubated in 1 M ammonium acetate buffer solution (pH = 5.0) for hydrolyzation with β-glucuronidase/sulfatase (20,000 units mL^{-1}) overnight. After hydrolysis, the phenols were extracted and preconcentrated with solid phase extraction (500 mg/3 mL, Supelclean, USA), and further detected using ultra-high performance liquid chromatography (Waters, USA)–electrospray ionization tandem mass spectrometry (Waters, USA). The detection was done in the negative ion mode by multiple reaction monitoring. The limits of detection (LODs) were 0.04 ng mL^{-1}. Strict quality control was conducted during the analysis. CR data were collected using an automated chemistry analyzer (7020 Hitachi, Japan), which were obtained for correcting the phenol concentrations caused by urine concentration and dilution.

2.4. Cell culture and BP-3 treatment

Human 293T and SH-SY5Y cells were widely used as cell models in research on mechanisms of HSCR (Kawamoto et al., 2003; Vargiolu et al., 2009). Human 293T cells and SH-SY5Y cells were obtained from American Type Culture Collection (USA). These cells were cultivated in complete growth medium DMEM (Hyclone, USA), supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C, 5% CO_2. For chemical treatment, 293T cells and SH-SY5Y cells were plated in 6-well, 24-well or 96-well plates and then treated with BP-3 (0.1 μM, 1 μM, 10 μM, and 100 μM) dissolved in DMSO for 24 h. As a negative control, cells were also exposed to 0.1% DMSO alone. 293T cells and SH-SY5Y cells were collected and used for gene and protein expression analysis on d 2.

2.5. Cell proliferation assays

Cell viability was assessed by cell counting kit-8 (CCK-8 kit, Beyotime Institute of Biotechnology, China). 293T cells or SH-SY5Y cells were seeded at 5000 cells/well in 96-well plates. After 24 h incubation, the cells were treated with BP-3 at concentrations of 0, 0.1, 1, 10, 100 and 1000 μM. After 24 h, 100 μL medium solution (content 10% CCK-8) was added and incubated at 37 °C for 1 h. Finally, absorbance was measured on DU-800 Nucleic Acid and Protein Analyzer (Beckman, USA) at 450 nm. The experiment was repeated three times.

2.6. Cell cycle and apoptosis analysis

To estimate if BP-3 could affect the cell cycle and induce apoptosis in 293T cells and SH-SY5Y cells, flow cytometric analysis was used to determine the state of cell cycle and the DNA fragmentation. 293T cells and SH-SY5Y cells were seeded on 6-well plates at a density of about 1×10^6 cells per well. Cells were incubated overnight and subsequently exposed to BP-3 (0.1 μM, 1 μM, 10 μM and 100 μM) and control medium containing 0.1% DMSO. After 24 h, cells were washed with PBS and harvested with trypsin. Cells were fixed in 70% ethanol for 2 h or washed in cold PBS, then stained with propidium iodide and annexin V for 30 min protected from light. The fixed/stained cells were analyzed by FACS Calibur Flow Cytometry (BD Biosciences, USA) to quantify cell cycle or cell apoptosis.

2.7. Cell transwell assays

For those cells treated with BP-3 (0, 0.1, 1, 10 and 100 μM), after 24 h, cells (1×10^4) were seeded in the upper chamber with serum-free medium. DMEM containing 10% FBS was added to the lower chamber and followed by incubation for 1 d. Cells in upper chamber were stained with crystal violet staining solution (Beyotime Institute of Biotechnology, China), and then counted and photographed under 40 × magnification (five views per well). Migrated cells were counted using Image-pro Plus 6.0 while cell numbers of normal control group were normalized to 1. All experiments were performed in triplicate.

2.8. RNA isolation and quantitative real-time PCR assay

Total RNA was isolated using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA pellets were dissolved in ddH_2O containing 0.1% DEPC, and quantified by measuring the absorbency at 260 nm by DU-800 Nucleic Acid and Protein Analyzer (Beckman, USA). We manipulated all real-time PCR reactions on ABI7900 Fast Real-Time System (Applied Biosystems, USA) according to the manufacturer's instructions for quantification of gene expression. cDNA synthesis for coding genes was performed with 1 μg of total RNA according the manufacturer's instructions (Takara, Japan). TaqMan®MicroRNA Assays (Applied Biosystems, USA) were used as the probe for hsa-miR-218 and U6 which acted as an internal control. mRNA levels of RET and other genes were measured using SYBR PCR Master Mix reagent kits (Takara, Japan). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. All primer sequences are given in Table S1. The experiments were carried out three times.

2.9. Protein analysis

Total proteins were isolated from cultured cells using RIPA buffer containing protease inhibitors complete, ULTRA, Mini, ethylenediaminetetraacetic acid-free, EASY pack (Roche, Switzerland), while the membrane proteins were extracted from tissues by Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Thermo Scientific, USA). The protein samples (100 μg from each group) were separated with 7.5%/12.5% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane. Primary polyclonal antibodies included RET antibody (SC167, Santa Cruz, USA), PLAG1 antibody (BP11647a, Abgent, USA), SLIT2 antibody (ab134166, Abcam, UK) and ROBO1 antibody (MAB7118, R&D, USA). The secondary antibodies with horse radish peroxidase were anti-rabbit, anti-goat and anti-mouse HRP-linked (Beyotime Institute of Biotechnology, China). The bands were visualized using ECL reagent (Millpore, USA). Equal amount of protein loading in each lane was confirmed using GAPDH antibody. The experiment was replicated thrice for each protein.

2.10. Statistical analysis

The data analysis was performed using Stata 9.2 statistical software package (Stata Corp, LP). T test was used to compare the mean age, body mass index (BMI) between case and control groups; if the variances were far from equal, the Wilcoxon signed rank test was used. The chi-squared test was used to evaluate the differences in smoking status and drinking status between case and control groups. All urine samples were divided into three-level variable for statistical analysis. The samples with concentrations <LOD were assigned to the low exposure group, and the remaining samples were divided into 2 groups approximately as the median exposure group and the high exposure group. The samples between detectable concentrations and 0.10 ng mL^{-1} were assigned to the median exposure group. The remaining samples were assigned to the high exposure group. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for the estimation of the relationship between HSCR and urinary BP-3 levels by unconditional logistic regression analysis adjusted by BMI which was significantly different between case and control groups. Additionally, we employed urinary creatinine (CR) as a continuous variable to adjust for the urinary dilution.

Statistically significant differences between the treatments and the control were determined by one-way analysis of variance, followed by Dunnett's multiple comparison test. All tests of statistical significance were two-sided, and the statistical significance was set at $p < 0.05$.

3. Result

3.1. The association between BP-3 and HSCR in the populations

All of the 423 women participants were ethnic Han-Chinese, including 101 cases and 322 controls. Age, weight and BMI of mother were possible confounders which were related with birth outcome (Fraser et al., 1995; Cnattingius et al., 1998; Kiel et al., 2007). As shown in Table 1, there were significant differences in weight and BMI between cases and controls, which could be due to higher weight of the controls in pregnancy. While no significant differences were identified in smoking status and alcohol consumption. Therefore, we matched age in mothers of HSCR and controls in our study. Weight and BMI were significant in the case and control groups and BMI was adjusted to calculate ORs and CIs between the case and control groups. The distributions of urinary concentrations of BP-3 in the 423 participants are presented in Table 2. BP-3 were detectable in over 50% samples and adjusted ORs for the relationship between phenol exposure levels and HSCR are presented in Table 2. The maximum detection concentration was 400.72 ng mL^{-1} and percentiles at 50th, 75th, 90th, 95th were 0.08, 0.17, 0.54, 1.10 ng mL^{-1}. Compared with women in the lowest exposure group, women in both the median and high BP-3 exposure groups were more likely to give birth of HSCR children, [for BP-3: adjusted ORs for increasing exposure levels = 2.39 (95% CI, 1.10–5.21), 2.61 (95% CI, 1.15–5.92), p-value for trend <0.05] which indicated that maternal BP-3 exposures were associated with the offspring's HSCR.

The age of 101 HSCR and 103 control groups were 3.62 ± 0.21 and 3.67 ± 0.25 months old, respectively. The body weight were 5.13 ± 0.14 and 5.35 ± 0.12 kg, respectively. There was no statistical difference between cases and controls in age and body weight. The gender rate (Male/Female) of HSCR and control was 79/22 and 83/20, respectively, which matched the common gender rate of this disease in human beings.

Table 1
Characteristics of participants in this study.

	HSCR	Control	P
Mother characteristics (n = 423)			
Maternal age (year, mean ± SD)	28.88 ± 4.30	27.48 ± 4.65	0.07[a]
Parity (%)			
0	65.28	68.96	0.54[b]
≥1	34.72	31.04	
Education (year, %)			
≤9	67.60	69.25	0.38
10–12	32.40	30.75	
Maternal height (cm, mean ± SD)	160.41 ± 3.71	161.19 ± 4.03	0.23
Maternal weight (kg, mean ± SD)	56.27 ± 8.19[c]	71.32 ± 10.17[d]	0.01*
BMI	21.86 ± 3.01	28.57 ± 4.80	0.01*
Smoking in pregnancy (%)	0	0	
Drinking in pregnancy (%)	6.90	6.73	0.56
Children characteristics (n = 204)			
Age (month, mean, SD)	3.62 ± 0.21	3.67 ± 0.25	0.47
Sex (%)			
Male	79 (78.22)	83 (80.58)	0.58
Female	22 (21.78)	20 (19.42)	
Weight (kg, mean, SD)	5.13 ± 0.14	5.35 ± 0.12	0.39

There was significant difference both at maternal weight and BMI variables between HSCR mothers and the controls' mother. In this case our results of BP-3 detection in urine was corrected by BMI.
*P < 0.05 indicates significant difference compared with the control group.
[a] Student t-test.
[b] Two-sided x^2 test.
[c] Values are usual weight (kg) divided by the height (m) squared.
[d] Values are end-of-pregnancy weight (kg) divided by the height (m) squared.

3.2. The cytobiology change after BP-3 exposure in 293T and SH-SY5Y cell lines

To examine the effect of BP-3 on cell viability and morphology, 293T cells and SH-SY5Y cells were exposed to various concentrations of BP-3 for 24 h. After that, a significant decrease in viability was observed at the dose of 1000 μM (Fig. S1A). Since cytotoxic effects were not observed during 0.1 μM, 1 μM, 10 μM, 100 μM of treatment, cells were treated with BP-3 at these concentrations in all of the following experiments.

As abnormal migration is the major cause of HSCR, the migration of 293T and SH-SY5Y cell lines after BP-3 treatment were tested with transwell assay. A suppressive effect was observed in both 293T and SH-SY5Y cell lines at the dose of 100 μM (Fig. 1A). The number of migrated cells were significantly lower in 100 μM of BP-3 treatment group (Fig. 1B), which indicates a causal association between BP-3 exposure and HSCR in the population study. We also examined the effect of BP-3 on the cell cycle and apoptosis after 24 h exposure using flow cytometery, but no significant differences were observed in both cell apoptosis and cell cycle (Fig. S1B and C).

3.3. The expression of RET was inhibited in BP3 contaminated cell lines

The RET proto-oncogene is considered the major disease-causing locus in HSCR, mutations of which are identified in 15–35% sporadic cases (Brooks et al., 2005; de Groot et al., 2006). To evaluate the effect of BP-3 exposure on RET expression, we conducted RT-PCR to detect mRNA expression with BP-3 treatment from 0 to 100 μM doses. As show in Fig. 2A, similar to HSCR stenosis tissues as reported, RET mRNA expressions were decreased in BP-3 treated 293T and SH-SY5Y cells. Meanwhile, BP-3 reduced RET protein expressions significantly in western blot experiments (Fig. 2B).

3.4. Effects of BP-3 on miR-218-RET pathway

miR-218 is predicted to target RET as well as PLAG1 by bioinformatics methods in previous research (Tang et al., 2015). To explore the potential role of miR-218 and PLAG1 in BP-3 induced effect, both gene expression levels were tested by RT-PCR in BP-3 infected 293T and SH-SY5Y cells. The results showed that miR-218 was up-regulated while PLAG1 was down regulated, which were consistent with protein levels (Fig. 2A and B). Therefore, BP-3 treatment might suppress RET and PLAG1 expression through increasing miR-218 expression. As a consequence, SLIT2, the host gene of miR-218, was assessed at mRNA level. The mRNA and protein levels of SLIT2, and its receptor ROBO1 were dose-dependently increased by BP-3 exposure (Fig. 2).

3.4.1. The validation of the miR-218-RET pathway in HSCR colon tissues

To determine whether BP-3 was involved in the pathogenesis of HSCR through miR-218-RET pathway, we detected those gene expressions in colon tissues of HSCR children and controls' at mRNA levels. As shown in Fig. 3, we confirmed the mRNA expression levels of miR-218, SLIT2 and ROBO1 in HSCR were significantly higher than those in controls ($P = 7.13 \times 10^{-8}$, $P = 5.03 \times 10^{-8}$ and 4.12×10^{-6}, respectively), while those of RET and PLAG1 were extreme lower ($P = 5.47 \times 10^{-9}$, $P = 1.02 \times 10^{-6}$).

4. Discussion

As far as we know, this is the first study to comprehensively explore the effects of BP-3 exposure (at concentrations that do not induce cytotoxic effects) on HSCR, and the possible underlying mechanism was revealed. We found maternal BP-3 exposure was associated with HSCR. BP-3 suppressed migration in 293T and SH-SY5Y cells. It also decreased RET and PLAG1 and increased miR-218, SLIT2 and ROBO1 both at the mRNA and protein levels.

HSCR is a partially penetrant oligogenic birth defect that occurs when ENS precursors fail to colonize the distal bowel during early pregnancy (Lake et al., 2013). Ganglion migration failure during 5–12 weeks of gestionis is one of the leading causes of the disease. Maternal exposure to chemicals with endocrine disrupting effects can alter the development of progeny, inducing birth defects (Ngalame et al., 2013; Veiga-Lopez et al., 2013). Population study manifests that phthalate exposure of pregnant women during critical window contributes to preterm births (Ferguson et al., 2014). Nevertheless, we got barely research on mammals or primates about BP-3 toxicity in spite of its extensive exposure and potential deleterious effect (Vela-Soria et al., 2011). In our study,

Table 2
Adjusted ORs (95% CIs) for HSCR by exposure level of BP-3 in maternal urines (n = 423)[a].

Chemicals		Control/case (NO.)	Adjusted-OR[a] (95% CI)[BMI]	p
BP-3	low	129/37	1	
	medium	97/33	2.39 (1.10–5.21)	0.03*
	high	96/31	2.61 (1.15–5.92)	0.02*

The tables showed significances in both medium and high BP-3 exposure than low exposure in maternal urines.
*P < 0.05 compared with the low BP-3 exposure level.
[a] ORs are adjusted for maternal BMI and CR level.

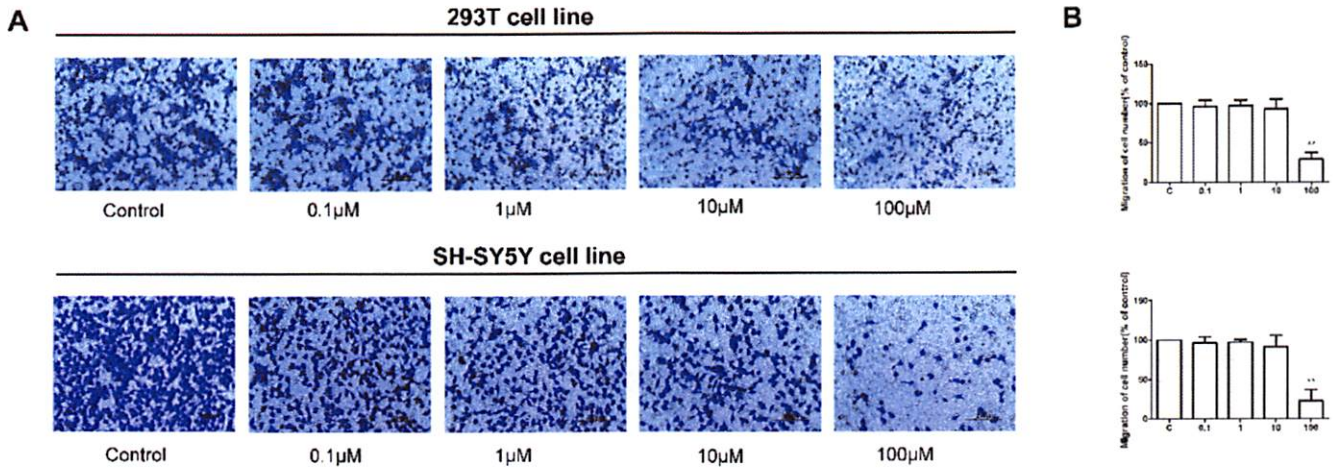

Fig. 1. Cytotoxicity measurement after BP-3 exposure. (A) The representative images of metastasis cells at the bottom of the membrane stained with crystal violet were visualized as shown in 293T and SH-SY5Y cells. (B) The quantifications of cell migration were presented as percentage migrated cell numbers, in 293T and SH-SH5Y cells respectively. ***P < 0.001, $n = 5$. All tests were performed for three times and presented as mean ± SEM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

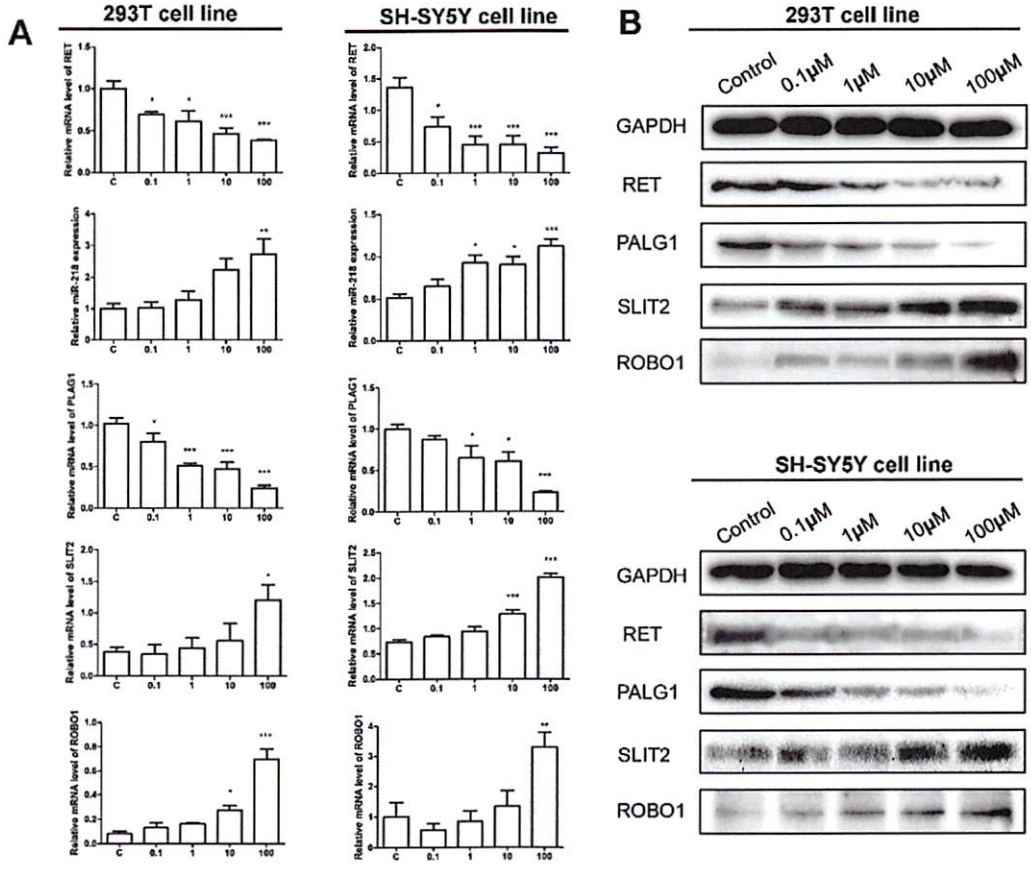

Fig. 2. mRNA and post-transcriptional expression levels of miR-218 pathway. (A) The mRNA expression level of gene of BP-3 exposure at 0, 0.1, 1, 10, 100 μM concentrations in 293T and SH-SY5Y cells.*P < 0.05, **P < 0.01, ***P < 0.001, n = 3. (B) GAPDH, RET, PLAG1, SLIT2, ROBO1 protein levels of BP-3 treatment in 293T and SH-SY5Y cells. GAPDH is used as internal control. All tests were performed for three times and presented as mean ± SE.

maternal BP-3 exposure levels were extremely consistent with disease risk of offspring's HSCR. Therefore, maternal BP-3 exposure during critic windows of fetal neural development may affect the development of offspring and induce HSCR.

It has been demonstrated that miRNAs not only regulate multiple protein coding genes, but also control all biological processes (Lin et al., 2012; Liu et al., 2012). It is universally aknowledged that miRNAs are susceptible to environmental exposures, such as bisphenol A and nicotine. miRNA dysfunction may be a mechanism through which toxicants can exert both developmental and carcinogenic effects (Jirtle and Skinner, 2007; Izzotti et al., 2009; Avissar-Whiting et al., 2010; Hou et al., 2012). Studies showed that prenatal chemical exposure could modify the epigenome of specific genes of the offspring and social behavior in the childhood, which

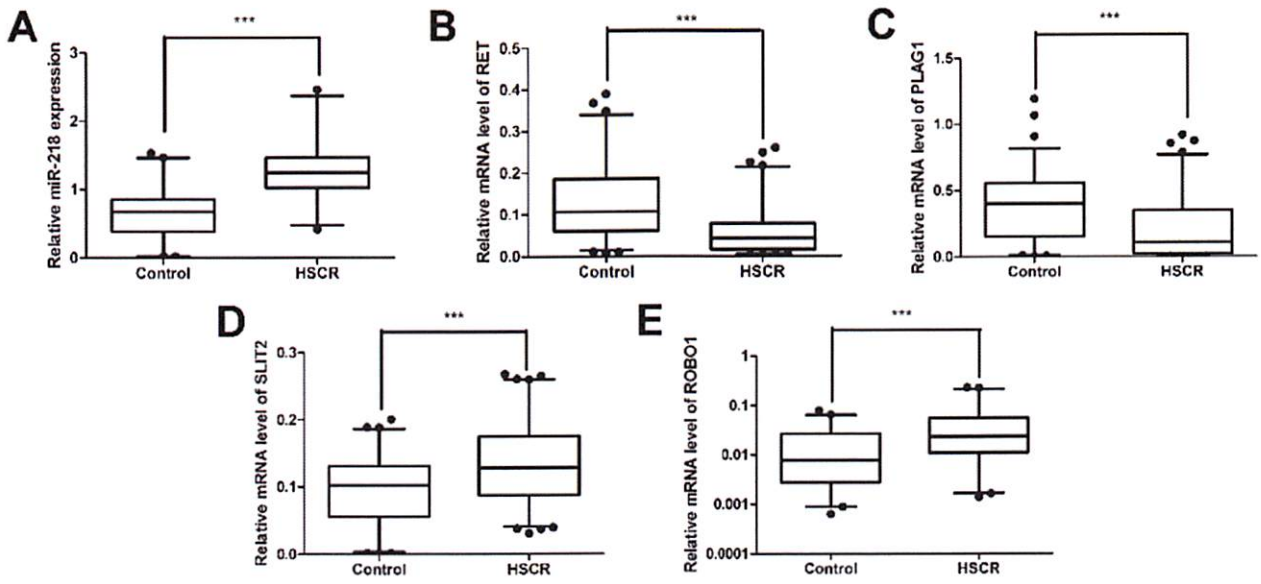

Fig. 3. Validation of population. (A)–(E) The mRNA expression levels of miR-218, RET, PLAG1, SLIT2, ROBO1, in the control and HSCR tissues. ***P = 0.0005, n = 103 controls/101 HSCR. All tests were performed for three times.

gave us clues that prenatal BP-3 exposure might alter gene expressions through epigenetic level (Furlong et al., 2014; Vilahur et al., 2014). Our former study has clarified that increased expression of miR-218 supressed cell migration and proliferation in 293T and SH-SY5Y cells. Therefore, modified expression level of miR-218 caused by BP-3 exposure may play roles in the pathogenesis of HSCR.

Some evidences confirmed that RET was upregulated by miR-218 in acute myeloid leukemia (Diaz-Beya et al., 2013). Early researches have confirmed that miR-218 inhibits cell migration and proliferation by targeting RET and PLAG1 (Tang et al., 2015). In this study, we firstly demonstrated that BP-3 inhibited cell migration through RET and PLAG1 at the concertraion of 100 μM. To ensure the down-regulating of RET and PLAG1 were caused by the enhanced miR-218, we then exanmined the expression level of miR-218 in both cell lines after the exposure of BP-3. As expected, miR-218 was up-regulated. Considering the relationship between SLIT2 and miR-218 as well as ROBO1, we performed experiments to test the expression levels of both SLIT2 and ROBO1. Our study manifested BP-3 exposure led to higher expression quantities of SLIT2, and conversly lower expression quantities of ROBO1 which acted as its receptor in central nervous. Based on all the cell function experiments, we demonstrated that BP-3 contributed to the pathopoiesis of HSCR via regulating genes signal transduction.

A limitation of the present study was the spot urine determination to assess exposure. However, as far as we were aware, all participants in our study had not changed their eating habits, life styles or environments for several months prior to the sample collection. Therefore, their urinary levels of BP-3 exposure are expected to be relatively stable over time. In addition, although the risk of misclassification may still exist, since this would be most likely non-differential, one would expect mainly attenuation of observed exposure–effect associations. Second, this study was the case–control design that limited our strength in making a causal conclusion. Third, although we clarify the fact that maternal BP-3 exposure brought high risk probability to offspring's HSCR, cell lines are limited in elucidating the mechanism in the present study and chemical exposure in enteric neural crest cells is needed for further investigation. What's more, other environment factors which may take part in the process of pathogenesis of HSCR are still unknown.

5. Conclusions

In conclusion, our study firstly pointed out that maternal BP-3 exposure may cause offspring's HSCR through SLIT2/ROBO1-miR-218-RET/PLAG1 pathway. These findings enhanced our understanding of BP-3 effects on embryonic development.

Conflicts of interest

The authors declare no conflict of interest with the study or preparation of the manuscript.

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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.chemosphere.2015.09.019.

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BENZOPHENONE

1. Exposure Data

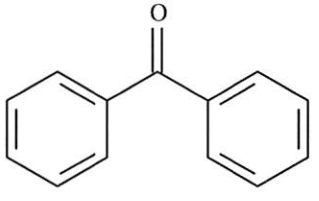
1.1 Chemical and physical data

From IUCLID (2000), IPCS-CEC (2005), NTP (2006), GESTIS (2010), and Repertoire Toxicologique (2010), unless otherwise specified

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 119-61-9
Chem. Abstr. Name: Benzene, benzoyl-; benzoylbenzene, phenyl ketone; diphenylketone; diphenyl ketone; diphenylmethanone; ketone, diphenyl; methanone, diphenyl-; α-oxodiphenylmethane; α-oxoditane
RTECS No.: DI9950000
EINECS No.: 204-337-6

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{13}H_{10}O$
Relative molecular mass: 182.22

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless crystalline solid with geranium- or rose-like odour
Boiling-point: 305.4 °C
Melting-point: 48.5 °C (α form) and 26 °C (β form)
Density: 1.111 at 18 °C
Vapour pressure: 1.93×10^{-3} mm Hg at 25 °C
Refractive index: 1.6077 at 19 °C
Solubility: Practically insoluble in water, but soluble in organic solvents such as alcohol, acetone, ether, acetic acid, chloroform and benzene.
Flash-point: > 110 °C
Stability: Decomposes on heating to produce toxic gases; reacts with strong oxidants.
Octanol/water partition coefficient: log K_{ow}, 3.18 (LOGKOW, 2010)
Henry's law constant: 1.9×10^{-6} atm.m^3/mol at 25 °C

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

(a) Air

One method has been reported by the Occupational Safety and Health Administration of the United States of America (OSHA PV2130) regarding the possibility of measuring benzophenone in air using a tube filled with chromosorb 106 (100/50-mg sections, 60/80 mesh) at a recommended maximum volume of 48 L and a maximum flow rate of 0.2 L/min. An analytical solvent (99:1 carbon disulfide:*N*,*N*-dimethylformamide) is used to desorb the chromosorb, and the substance is then measured by gas chromatography with flame ionization detection.

(b) Food

Analysis of benzophenone in breakfast cereals has been reported using ultrasonic extraction in combination with gas chromotography-tandem mass spectrometry (Van Hoeck *et al.*, 2010).

1.2 Production and use

1.2.1 Production

A 66% yield of benzophenone can be obtained by Friedel-Crafts acylation of benzoyl chloride with an excess of benzene in the presence of anhydrous aluminium chloride (NTP, 2006). Benzophenone is also produced by atmospheric oxidation of diphenylmethane in the presence of metal catalysts, such as copper naphthenate (HSDB, 2010).

According to the US Environmental Protection Agency, it was classified in 2003 as a high volume chemical, with an annual production exceeding 1 million pounds [453 000 kg], in the USA (NTP, 2006).

1.2.2 Use

Benzophenone is used as a flavour ingredient, a fragrance enhancer, a perfume fixative and an additive for plastics, coatings and adhesive formulations; it is also used in the manufacture of insecticides, agricultural chemicals, hypnotic drugs, antihistamines and other pharmaceuticals (HSDB, 2010). Benzophenone is used as an ultraviolet (UV)-curing agent in sunglasses, and to prevent UV light from damaging scents and colours in products such as perfumes and soaps. Moreover, it can be added to plastic packaging as a UV blocker, which allows manufacturers to package their products in clear glass or plastic rather than opaque or dark packaging. It is also used in laundry and household cleaning products (NTP, 2006; HSDB, 2010).

Benzophenone is widely used as a photoinitiator for inks and varnishes that are cured with UV light. In addition to being a drying catalyst, benzophenone is an excellent wetting agent for pigments; it can also be used in printing to improve the rheological properties and increase the flow of inks by acting as a reactive solvent.

[No data were available to the Working Group on the use of benzophenone in sunscreens, whereas data were available on the use of one of its derivatives (3-benzophenone, 2-hydroxy-4-methoxybenzophenone) in such products.]

1.3 Occurrence

1.3.1 Natural occurrence

Benzophenone has been reported to occur naturally in food (see Section 1.3.3).

1.3.2 Occupational exposure

Benzophenone can be absorbed into the body by inhalation, through the skin and by ingestion (IPCS-CEC, 2005).

Industrial sectors that entail risks of occupational exposure are painting (paints,

varnishes and lacquers), the manufacture of plastic composites and the manufacture and use of glues and adhesives. The National Institute for Occupational Safety and Health conducted the National Occupational Exposure Survey in 1981–1983, which estimated that, among the 4490 establishments surveyed in the USA (522 industry types, employing approximately 1 800 000 workers), 41 516 workers (18 162 women) were potentially exposed to benzophenone (NIOSH, 1990).

1.3.3 Occurrence in food and dietary exposure

Dietary sources of exposure to benzophenone include its natural occurrence in food, its presence as a contaminant in drinking-water, its migration from food packaging and its addition to food as a flavouring.

(a) Food

Benzophenone was reported to occur naturally in wine grapes (*Vitis vinifera* L.) at concentrations of 0.08–0.13 ppm [mg/kg] (TNO, 2010). According to the Council of Europe (2000), it mainly occurs in muscat grapes. Benzophenone has been detected quantitatively in passiflora species at 0.045 ppm (TNO, 2010) and qualitatively in black tea, cherimoya (*Annona cherimola*), mountain papaya (*Carica pubescens*) and soursop (*Annona muricata* L.) (Burdock, 2005). Concentrations in mountain papaya (*C. pubescens* and *C. candamarcensis*) were reported to be lower than 0.01 ppm (TNO, 2010).

Based on its concentration in muscat grapes, the Working Group estimated that consumption of 200 g grapes would result in exposure to approximately 20 µg benzophenone, i.e. 0.3 µg/kg body weight (bw) for a 60-kg adult.

(b) Drinking-water

The data on benzophenone in drinking-water are limited. Levels of 8.8 ppb [µg/L] were found in tap-water in Japan (Shinohara *et al.*, 1981) and 0.26 µg/L in finished drinking-water in a water filtration plant in the USA in 2001–02 (Loraine & Pettigrove, 2006, see Section 1.3.4).

To assess exposure to contaminants through drinking-water, the WHO uses a default consumption value of 2 L of drinking-water per capita per day for a typical adult of 60-kg bw (WHO, 2008), based on the assumption that total water consumption is 3 L per capita per day, including water present in food, which represents a conservative estimate (WHO, 2003). However, such a default assumption is not appropriate for all populations and climates. Reference hydration values under average conditions are 0.75 L in infants (5 kg), but, for physically active persons in areas with higher temperatures, could reach 4.5 L for men, women and children, 4.8 L for pregnant women and 5.5 L for lactating women (WHO, 2003).

The available data on concentrations of benzophenone in drinking-water were used by the Working Group to assess dietary exposure in adults and infants (60-kg and 5-kg bw, respectively), assuming a consumption of 2 and 0.75 L of drinking-water, respectively, i.e. 33 and 150 mL/kg bw, respectively. The infant scenario (in mL/kg bw) would correspond to a consumption of 9 L of drinking-water per day in a 60-kg adult and would therefore encompass any possible scenario of physically active persons in high-temperature areas. Hence, the estimated dietary exposure to benzophenone through the drinking-water of a standard 60-kg adult would range from 0.52 to 17.6 µg per day, i.e. 9–290 ng/kg bw per day, and that of a 5-kg infant would range from 0.2 to 6.6 µg per day, i.e. 40–1320 ng/kg bw per day.

(c) Migration from food packaging

The main source of exposure to benzophenone through food packaging is related to its wide use as photo-initiator in UV-cured inks on the external face of paperboard packaging. Benzophenone is neither totally exhausted during the printing process nor removed thereafter, and

is nor irreversibly bound into the print film layer (Koivikko *et al.*, 2010). It may thus migrate to food from paperboard, either by direct contact or through the vapour phase. Substances present on the external face of the packaging may contaminate the internal face when the carton is rolled and compressed, which is a common practice in the food packaging industry, and thus contaminate food through direct contact. Benzophenone may also contaminate food through the vapour phase, even from the secondary packaging. Internal plastic bags that are used as a barrier against moisture are not always effective (EFSA, 2009). Benzophenone is known to migrate easily through polypropylene film, whereas aluminium and multilayer materials inhibit migration efficiently (Nerín & Asensio, 2007; Pastorelli *et al.*, 2008).

Under low-temperature conditions (-20 °C), benzophenone migrates from cartonboard to food during frozen storage, even when there is no direct contact between the packaging and the food or when the packaging is polyethylene-coated (Johns *et al.*, 2000). Moreover, the most commonly used raw material for paperboard is recycled, and the product therefore often contains photo-initiators, including benzophenone. Recycled board is commonly used in direct contact with dry foodstuffs, such as flour and pasta, but also with fast-food items, i.e. foodstuffs with a short duration of contact, such as pizzas. Normally, a functional barrier, e.g. plastic or aluminium foil, is used between fatty or aqueous foodstuffs and the recycled material to avoid direct contact.

Analytical data are available on the concentrations of benzophenone in food packagings and in foods. In particular, in a comprehensive survey performed by the United Kingdom Food Standards Agency (UK FSA, 2006), benzophenone was detected in four of 115 samples of foodstuff packaged in printed plastic (maximum concentration, 0.15 mg/kg), in 60/296 samples packaged directly or indirectly in printed paper or board that contained 0.05–3.3 mg benzophenone/dm^2 at a concentration of 0.035–4.5 mg/kg (mean concentration, 0.9 mg/kg) and in one of 54 foodstuffs to which a printed sticky label had been attached (at 0.029 mg/kg). In this survey, a high percentage of products tested positive for benzophenone among the categories of frozen foods (18/35), 'jelly' (3/5) and 'savoury snacks' (15/40). A lower percentage of products tested positive in the categories of 'sweets, chocolate biscuits and crisps' (5/35), 'bakery products' (8/35) and 'cereals' (4/25). Only one 'ready meal' of 20 and none of 10 'desserts' tested positive.

According to the United Kingdom Food Standards Agency (UK FSA, 2006), potential dietary exposure to benzophenone in high-level consumers is 1.2–1.5 μg/kg bw for adults. These estimates were calculated by combining a high level of consumption of foods that may contain benzophenone (449 g/day, the 97.5th percentile in the United Kingdom national survey of adults) with two average levels of its occurrence therein (160 and 200 μg/kg), depending on different assumptions of the values below the limit of quantification (45 μg/kg), for a 60-kg bw adult.

More recent but limited data on benzophenone concentrations in food products are available in other countries.

Samples of milk packaged in cartons on the market in the People's Republic of China were tested: skimmed milk, whole milk and partially skimmed milk. Benzophenone was detected in the packaging of all products at a concentration of 0.94–1.37 μg/dm^2, and in five of six of the milk products. Higher levels were found in milk with a higher fat content and ranged from 2.84 to 18.35 μg/kg (Shen *et al.*, 2009).

The migration of benzophenone into five selected dry foods (cake, bread, cereals, rice and pasta) sampled in a supermarket in Spain was assessed by Rodríguez-Bernaldo de Quirós *et al.* (2009). The highest concentration of benzophenone was found in cake (12 mg/kg). Migration levels were positively correlated with both

porosity and fat content. These results correlated well with those reported by Anderson & Castle (2003), who analysed 71 food samples selected at random from a total of 143 items packaged in printed cartonboard, in which benzophenone had been detected. The highest value (7.3 mg/kg) was found in a high-fat chocolate packaged in direct contact with cartonboard.

In a study by Sanches-Silva *et al.* (2008), samples of 36 commercial beverages (fruit and vegetable juices, wine and soft drinks) were collected in Italy, Portugal and Spain in 2005 and 2006, all of which were packaged in multi-material multilayer boxes or aluminium cans. Benzophenone was detected in four samples of packaging (one under the limit of quantification of 1.7 μg/dm^2 and three ranging from 3.6 to 12.3 μg/dm^2), and samples of the beverages contained therein were analysed. None of the extracts yielded positive results. However, according to the authors, although fruit juices contain low amounts of fat, photo-initiators can migrate and be adsorbed by juice fibres (fibre content, 0.2%) and thus contaminate the beverage.

In a study conducted by Koivikko *et al.* (2010) in the European Union (EU), samples of printed board used for secondary packaging were collected from supermarkets, together with the food contained therein (n = 22), and some were acquired from industrial production lines before the introduction of foodstuffs (n = 24). In addition, samples were taken of recycled paperboard collected from a supplier to evaluate the background level of benzophenone and other derivatives therein (n = 19). The most abundant photo-initiator found in the non-recycled products was benzophenone, which was detected in 61% of samples. Traces of the compound were also found in 42% of the samples of recycled unprinted board. The content of benzophenone in these samples varied from 0.57 to 3.99 mg/m^2.

Benzophenone migrated into 95% ethanol from recycled paperboard used for contact with food in Japan, but not from virgin paper. Migration ranged from 1.0 to 18.9 ng/mL in eight of the 21 samples of recycled paperboard collected (Ozaki *et al.*, 2006).

In 2009, high levels of 4-methylbenzophenone (another photo-initiator) detected in some breakfast cereal products (chocolate crunch muesli) were notified under the EU Rapid Alert System for Food and Feed (RASFF) (European Commission, 2009). Further analysis performed by the producer demonstrated high concentrations of this substance and up to 4210 μg/kg benzophenone in these products (CS AFSCA Belgium, 2009).

(d) Addition to foods as a flavouring

In the USA, the average reported levels of use of benzophenone as an additive range from 0.57 ppm [mg/kg] in non-alcoholic beverages to 1.57 ppm in baked goods, and maximum reported levels range from 1.28 ppm in non-alcoholic beverages to 3.27 ppm in frozen dairy products. Other reported uses are in soft candy, gelatins and puddings (Burdock, 2005).

Maximum levels reported by the Council of Europe are 0.5 mg/kg in beverages and 2 mg/kg in foods in general, with no exception (Council of Europe, 2000). Benzophenone is listed in the EU register of chemically defined flavourings. In the European Food Safety Authority (EFSA) Flavouring Group Evaluation 69 (EFSA, 2008), dietary exposure to benzophenone in the EU, based on poundage data provided by industry, was estimated to be 23 μg per capita per day, assuming that consumers represent 10% of the population. On the same basis, dietary exposure is estimated to be 11 μg per capita per day in the USA (EFSA, 2008).

As a flavouring of threshold of toxicological concern class III, evaluated by the EFSA on the basis of a Joint FAO/WHO Expert Committee on Food Additives evaluation, refined, surveyed levels of additive use were provided by industry to the European Commission (IOFI-DG SANCO,

2008). The single portion exposure technique was developed by the Joint FAO/WHO Expert Committee on Food Additives to estimate dietary exposure from the consumption of one standard portion per day of flavoured food or beverages containing the flavouring substance at its average level of use (Leclercq *et al.*, 2009). Using this technique, the Working Group calculated that estimated exposure to benzophenone is 6 μg per person per day when applied to IOFI-DG SANCO (2008) data, 40 μg per person per day when applied to data from the Council of Europe (2000) and 170 μg per person per day when applied to data from the USA reported by Burdock (2005).

1.3.4 Environmental occurrence

Benzophenone is harmful to aquatic organisms (IPCS-CEC, 2005). Benzophenones in general have the environmentally critical properties of high lipophilicity and persistence, and are known to have adverse effects on the reproduction and hormonal functions of fish (Parks, 2009). According to Brooks *et al.* (2009), benzophenone is persistent, bioaccumulative and toxic (PBT).

Because of its high octanol:water partition coefficient and its insolubility in water, benzophenone partitions in soil and sediment (US EPA, 1984, cited by NTP, 2006), and its adsorption to soil is proportional to the organic content therein (OHMTADS, 1991, cited by NTP, 2006).

(a) Water and sediments

Benzophenone is among the pharmaceuticals and personal care products that are known to occur in drinking-water and in reclaimed wastewater when water sources are impacted by sewage treatment plant effluent (Loraine & Pettigrove, 2006). The removal of these compounds during wastewater-treatment processes is not fully effective, and effluent-dominated streams represent 'worse-case scenarios' for studying personal care products and other organic wastewater contaminants. In these streams, even compounds with relatively short environmental half-lives, such as benzophenone, may act as 'pseudo-persistent' compounds. Due to their continuous introduction from wastewater-treatment plants, these compounds are continuously released into the environment. As a result, aquatic organisms are exposed over their entire life cycle (Pedrouzo *et al.*, 2010).

Another route by which benzophenone enters the aquatic environment is from municipal solid-waste landfill leachates. In a study by Pitarch *et al.* (2010), benzophenone was qualitatively identified in wastewater samples from the municipal solid-waste treatment plant at Reciplasa (Castellón province, Spain) between March 2007 and February 2009. Samples of water were collected before and after reverse osmosis treatment, which is performed before the release of water into the environment. Benzophenone was detected in 38% of treated samples and in 55% of raw leachates. In a study by Trzcinski and Stuckey (2010), submerged anaerobic membrane bioreactors were fed a simulated feedstock of the organic fraction of municipal solid waste, and benzophenone was found among contaminants in the permeate of the leachate.

In a study by Yoon *et al.* (2010) of surface waters from sampling sites on the river and in effluent-dominated creeks along the Han River (Seoul, Republic of Korea), benzophenone was detected (limit of detection, 50 ng/L) in two of four river samples (mean, 52 ng/L; maximum, 59 ng/L) and in all four effluent-dominated creek samples (mean, 102 ng/L; maximum, 130 ng/L) as a result of wastewater outfall. Benzophenone was detected in surface water at Ozark Plateau of northeastern Oklahoma (USA) at a site downstream from the outfall of a municipal wastewater-treatment plant and in a hydrologically linked cave (Bidwell *et al.*, 2010). It was present in wastewater effluent from the main sewer of the city of Zagreb (Croatia), which received no

treatment at the time of the survey and comprised a mixture of effluent from domestic and industrial sources (Grung *et al.*, 2007).

Benzophenone was detected qualitatively in water from the Baltic Sea (Ehrhardt *et al.*, 1982) and from Hamilton harbour, Bermuda (Ehrhardt, 1987), and was determined in two water samples from the Tama river in Japan at concentrations of 21.0 and 22.8 ng/L (Kawaguchi *et al.*, 2006). It has been detected at concentrations of < 2.6–1040 ng/L in water samples from Venice lagoon and San Francisco estuary (Oros *et al.*, 2003, Pojana *et al.*, 2004; Pojana *et al.*, 2007) and of 14–200 µg/kg in sediment samples (Burkhardt *et al.*, 2005; Pojana *et al.*, 2007).

Benzophenone was detected in all 11 samples of bluegill fish collected from a regional effluent-dominated stream, i.e. about 650 m downstream from the effluent discharge of the Pecan Creek Water Reclamation Plant, in Denton County, TX, USA, at a mean concentration of 57 ng/g wet weight (standard deviation, 18 ng/g) and a range of 37–90 ng/g. The mean concentration in three samples of bluegill fish in Clear Creek (Denton County, TX, USA), a stream that experiences limited, if any, anthropogenic influence, was 24 ng/g (Mottaleb *et al.*, 2009). A survey of water, sediment and biota (Mediterranean mussel, *Mytilus galloprovincialis*) in the Venice lagoon, a highly urbanized coastal water ecosystem that receives both industrial and municipal wastewater effluents, detected concentrations of benzophenone in lagoon sediments of 14–110 µg/kg (Pojana *et al.*, 2007).

Benzophenone was detected by gas chromatography–mass spectrometry at a level of 8.8 ppb [µg/L] in tap-water from the Kitakyushu Municipal Institute in Japan (Shinohara *et al.*, 1981). A survey of raw and treated drinking-water from four water filtration plants in San Diego County (CA, USA) conducted in 2001–02 showed large seasonal variations in benzophenone concentrations, with higher levels in the summer than in the winter, probably because sunscreens are used more frequently during the summer months (Loraine & Pettigrove, 2006). Benzophenone was detected in one of 15 samples of finished drinking-water at a concentration of 0.26 µg/L, and in four of six samples of reclaimed wastewater at a concentration of 0.99 µg/L (range, 0.56–1.35 µg/L).

Benzophenone has been used as a model hydrophobic contaminant (Brooks *et al.*, 2009). Due to their hydrophobic nature, PBT contaminants move out of the water phase and become associated with sediments. Animals that reside in or on these sediments are therefore at risk of bioaccumulating PBT compounds, and acting as vectors in their transfer to predators that may otherwise have limited direct contact with contaminated sediments. Predator species accumulate benzophenone from their prey, and exposure to narcotic organic contaminants, such as benzophenone, results in hypoactivity which may alter their ability to capture such animals successfully (Brooks *et al.*, 2009).

Benzophenone was identified in surface sediment samples from the Havel and Spree Rivers (Germany), which are characterized by high inputs of anthropogenic contaminants into their eutrophic to hypertrophic riverine system with very slow flowing conditions. In the sedimentary records from 1979/80 up to 1995, benzophenone was detected and quantified in 10 out of 11 samples at concentrations ranging from 0.5 to 4 ng/g dry matter (Ricking *et al.*, 2003).

(b) Air

Benzophenone was identified qualitatively in the atmosphere of a 45-year-old spruce forest located in North Rhine-Westfalia (Germany) in 1988 at a height of 1 m, where severe forest damage had been observed (Helmig *et al.*, 1989). Leary *et al.* (1987) found that benzophenone was a component of emissions from a standard residential oil burner. 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).

Within an indoor-air monitoring survey conducted by the Japanese Ministry of Environment, benzophenone was detected in 67/68 samples analysed (The Japanese Ministry of Environment, 2006). Human exposure through inhalation should therefore be taken into account.

[The Working Group noted that there is no consensus in relation to the potential for bioaccumulation of benzophenone in the environment, nor for its persistence or pseudo-persistence.]

1.3.5 Other occurrence

Because of its use as an additive in fragrances, cosmetics, toiletries, pharmaceuticals, insecticides, and laundry and household cleaning products, exposure to benzophenone through dermal contact may be significant. The percutaneous absorption of benzophenone was determined *in vivo* in monkeys, and was approximately 70% of the dose applied to occluded skin within 24 hours. Under unoccluded conditions, skin penetration was reduced to 44%, presumably because of evaporation from the site of application (Bronaugh *et al.*, 1990).

Many dentures are commonly prepared through a polymerization reaction that uses benzoyl peroxide as the initiator, of which benzophenone is a decomposition product that was found to be eluted in artificial saliva from four commercial soft denture liners (two plasticized acrylates and two silicone elastomers) (Brożek *et al.*, 2008).

1.3.6 Total human exposure

Benzophenone ingested by humans is excreted in the urine as metabolites, such as benzhydrol (Kawaguchi *et al.*, 2009), and the measurement of its derivatives in urine may therefore provide an indication of overall human exposure to benzophenone. In a study conducted by Ito *et al.* (2009) in 14 healthy volunteers, benzophenone derivatives were detected in all urine samples. The concentration of benzhydrol ranged from 0.27 to 10.0 ng/mL, but the parent compound was not detected in any sample.

1.4 Regulations and guidelines

The current American Industrial Hygiene Association workplace environmental exposure level for benzophenone is 0.5 mg/m^3 (AIHA, 2009)

The EU Standing Committee for the Food Chain and Animal Health endorsed a limit of 0.6 mg/kg for the sum of benzophenone and 4-methylbenzophenone (European Commission, 2009). In its conclusions, the Committee stated that the European Printing Ink Association, as well as the European Carton Board Manufacturers, advised their members that printing inks containing 4-methylbenzophenone and benzophenone are not suitable for printing of food packaging unless a functional barrier is present that blocks their transfer into food and also via the gas phase. Examples of functional barriers are aluminium, poly(ethylene terephthalate)/silicon oxide or an equivalent layer.

According to the EU Directive 2002/72/EC, benzophenone may be used in the EU as an additive in plastics materials, with a specific migration limit of 0.6 mg/kg (European Commission, 2002).

Benzophenone has been listed by the Council of Europe in category B (flavouring substances for which further information is required before the Committee of Experts is able to offer a firm opinion on their safety in use; these substances can be used provisionally in foodstuff) (Council of Europe, 2000).

The United Kingdom authorities have so far judged benzophenone as a 'class B volatile

organic compound' within the context of integrated pollution control (IUCLID, 2000).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

The results of carcinogenicity studies of oral administration of benzophenone are summarized in Table 3.1.

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 50 male and 50 female B6C3F$_1$ mice, 8 weeks of age, were fed diets containing 0, 312, 625 or 1250 ppm benzophenone (> 99.5% pure; equivalent to average daily doses of approximately 40, 80 or 160 and 35, 70 or 150 mg/kg bw for males and females, respectively) for 105 weeks. Feed consumption of exposed males and females was similar to that of controls, but 1250-ppm females weighed 14% less than controls at the end of the study. A positive trend in the incidence of hepatocellular adenoma was observed in males; the incidence in the 625- and 1250-ppm groups was significantly greater than that in controls and exceeded the historical control ranges (12–30%) for feed studies. Hepatoblastomas were also observed in treated males, and, although the incidence in the 1250-ppm group (3/50, 6%) was not statistically significant, it exceeded the historical control range for feed studies (0–2%). The incidence of hepatocellular adenoma in 625- and 1250-ppm female mice was increased, but the difference from controls was not significant. A positive trend in the incidence of histiocytic sarcoma of the liver, lung, ovary, uterus, spleen, adrenal gland, kidney, urinary bladder and multiple lymph nodes was observed in female mice; the incidence in the 625-ppm group was significantly increased, and that in the 625- and 1250-ppm groups exceeded the historical control range for feed studies (0–2%) (NTP, 2006; Rhodes *et al.*, 2007). [The Working Group noted that hepatoblastomas and histiocytic sarcomas are rare neoplasms in mice.]

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344/N rats, 6 weeks of age, were fed diets containing 0, 312, 625 or 1250 ppm benzophenone (> 99.5% pure; equivalent to average daily doses of approximately 15, 30 or 60 and 15, 30 and 65 mg/kg bw for males and females, respectively) for 105 weeks. Feed consumption of 1250-ppm males was lower than that of controls after week 70, and that of 1250-ppm females was generally lower than that of controls throughout the study. Survival of 1250-ppm males was significantly shorter than that of control group, which was attributed to the increased severity of chronic progressive nephropathy in the kidney. In the standard (single sections) and extended (step-sections) evaluations of the kidney, the incidence of renal tubule adenoma was increased in male rats exposed to 625 or 1250 ppm, and the combined incidence (single and step-sections) of renal tubule adenoma in males was also increased in these groups; the incidence in the 1250-ppm group was significantly greater than that in controls. A renal tubule carcinoma and a transitional epithelium carcinoma of the renal pelvis also occurred in 625-ppm males. Male rats exposed to 312 or 625 ppm had a significantly increased incidence of mononuclear-cell leukaemia, whereas the incidence in 1250-ppm males was slightly decreased compared with controls. This incidence and that in all groups of treated females exceeded the range for historical controls from feed studies (30–68% for males,

Table3.1 Carcinogenicity studies of exposure to benzophenone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 105 wk NTP (2006), Rhodes *et al.* (2007)	Oral (feed) 0, 312, 625 or 1 250 ppm (M, F) 50 animals/group	Liver (hepatocellular adenoma): M[a]–11/50 (22%), 15/50 (30%), 23/50 (46%), 23/50 (46%) F[b]–5/50 (10%), 4/50 (8%), 10/50 (20%), 8/50 (16%)	$P = 0.01$ (mid- and high-dose M) $P = 0.006$ (trend in M)	The incidence of non-neoplastic hepatocellular lesions was significantly increased including hepatocyte necrosis, cystic degeneration, centrilobular, hypertrophy, multinucleated hepatocytes and chronic active inflammation in male mice and centrilobular, hypertrophy in female mice; > 99.5% pure
		Liver (hepatocellular carcinoma): M–8/50 (16%), 5/50 (10%), 6/50 (12%), 6/50 (12%) F–0/50, 0/50, 1/50 (2%), 0/50		
		Liver (hepatocellular adenoma or carcinoma): M–18/50 (36%), 20/50 (40%), 25/50 (50%), 27/50 (54%) F–5/50 (10%), 5/50 (10%), 10/50 (20%), 9/50 (18%)	$P = 0.027$ (high-dose M) $P = 0.013$ (trend in M)	
		Liver (hepatoblastoma, multiple): M[c]–0/50, 1/50 (2%), 1/50 (2%), 3/50 (6%)		
		All organs (histiocytic sarcoma) F[d]–0/50, 0/50, 5/50 (10%), 3/50 (6%)	$P = 0.03$ (mid-dose) $P = 0.032$ (trend)	
Rat, F344 (M, F) 105 wk NTP (2006), Rhodes *et al.* (2007)	Oral (feed) 0, 312, 625 or 1 250 ppm (M, F) 50 animals/group	Kidney (renal tubule adenoma, standard evaluation): M[e]– /50 (2%), 1/50 (2%), 2/50 (4%), 4/50 (8%) F[f]–0/50, 0/50, 2/50 (4%), 1/50 (2%)	$P = 0.046$ (trend in M)	Survival of the 1 250-ppm males was significantly lower than that of controls ($P < 0.001$). The incidence of renal tubule hyperplasia was significantly increased ($P \leq 0.01$) in all treated groups. The incidence of renal pelvis transitional epithelial hyperplasia was significantly increased ($P \leq 0.01$) in treated males. In male and female rats, the severity of chronic nephropathy increased significantly ($P \leq 0.05$) with increasing exposure concentration; > 99.5% pure.
		Kidney (renal tubule carcinoma, standard evaluation): M–0/50, 1/50 (2%), 0/50, 0/50		
		Kidney (renal tubule adenoma, extended evaluation): M–1/50 (2%), 1/50 (2%), 5/50 (10%), 4/50 (8%) F–3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%)	$P = 0.034$ (trend in M)	
		Kidney (renal tubule carcinoma, extended evaluation): M–0/50, 1/50 (2%), 0/50, 0/50		
		Kidney (renal tubule adenoma, standard + extended evaluations): M[e]–2/50 (4%), 2/50 (4%), 7/50 (14%), 8/50 (16%) F[f]–3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%)	$P \leq 0.017$ (high-dose M) $P = 0.006$ (trend in M)	
		Kidney (renal tubule carcinoma, standard + extended evaluations): M–0/50, 1/50 (2%), 0/50, 0/50		

Table3.1 Carcinogenicity (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rat, F344 (M, F) (contd)		Kidney (transitional epithelial carcinoma of the renal pelvis): M–0/50, 0/50, 0/50, 1/50 (2%) (M)		
		Haematopoietic (mononuclear-cell leukaemia): M[g]–27/50 (54%), 41/50 (82%), 39/50 (78%), 24/50 (48%) F[h]–19/50 (38%), 25/50 (50%), 30/50 (60%), 29/50 (58%)	$P = 0.003$ (low-dose M) $P = 0.005$ (mid-dose M) $P = 0.048$ (mid-dose F)	
		All organs (histiocytic sarcoma): F[i]–0/50, 0/50, 1/50 (2%), 2/50 (4%)		
Mouse, Swiss (F) 120 wk Stenbäck & Shubik (1974)	0% (vehicle), 5%, 25% or 50% in acetone Dermal application twice/wk for 120 wk 50 animals/group	Skin (squamous-cell papilloma): 2/50 (4%), 2/50 (4%), 0/50, 0/50	NS	Squamous-cell papillomas in the 5% group occurred at the site of application; the squamous-cell carcinoma in the 25% group occurred on the lip; papillomas in the control occurred on the tail and ear; purity not specified.
		Skin (squamous-cell carcinoma): 0/50, 0/50, 1/50 (2%), 0/50		
		Lung (adenomas): 9/50 (18%), 3/50 (6%), 3/50 (6%), 6/50 (12%)		
		Liver (haemangioma): 2/50 (4%), 1/50 (2%), 1/50 (2%), 2/50 (4%)		
		Haematopoietic (lymphoma): 12/50 (24%), 15/50 (30%), 11/50 (22%), 6/50 (12%)		
		Haematopoietic (thymoma): 0/50, 1/50 (2%), 1/50 (2%), 0/50		

[a] Historical incidence (mean ± SD) for 2-year feed studies in mice: 90/460 (20.0 ± 7.1%), range 12–30%
[b] Historical incidence (mean ± SD) for 2-year feed studies in mice: 40/457 (9.6 ± 2.4%), range 6–12%
[c] Historical incidence (mean ± SD) for 2-year feed studies in mice: 1/460 (0.2 ± 0.6%), range 0–2%
[d] Historical incidence (mean ± SD) for 2-year feed studies in mice: 2/459 (0.3 ± 0.8%), range 0–2%
[e] Historical incidence (mean ± SD) for 2-year feed studies in rats: 1/459 (0.3 ± 0.8%), range 0–2%
[f] Historical incidence (mean ± SD) for 2-year feed studies in rats: 1/460 (0.1 ± 0.4%), range 0–1%
[g] Historical incidence (mean ± SD) for 2-year feed studies in rats: 231/460 (49.1 ± 11.9%), range 30–68%
[h] Historical incidence (mean ± SD) for 2-year feed studies in rats: 112/460 (24.6 ± 9.5%), range 12–38%
[i] Historical incidence for 2-year feed studies in rats: 0/460
F, female; M, male; NS, not significant; SD, standard deviation; wk, week or weeks

12–38% for females). A low incidence of histiocytic sarcoma occurred in both 625- and 1250-ppm female rats (1/50 and 2/50, respectively). Histiocytic sarcomas have not been observed in historical controls in feed studies and in only 1/1209 historical controls in studies by all routes of administration during the time these studies were conducted (NTP, 2006; Rhodes *et al.*, 2007).

3.2 Dermal application

3.2.1 Mouse

Groups of 50 female Swiss mice received dermal applications of 0, 5, 25 or 50% benzophenone [purity unspecified] dissolved in acetone twice a week for 120 weeks. Dermal application of benzophenone was not carcinogenic in the skin of mice (Stenbäck & Shubik, 1974).

4. Other Relevant Data

4.1 Absorption and metabolism

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

After dermal application of [^{14}C]benzophenone, approximately 70% was absorbed in rhesus monkeys within 24 hours (Bronaugh *et al.*, 1990). Benzophenone was rapidly absorbed from the gastrointestinal tract of Sprague-Dawley rats that were administered a single dose (100 mg/kg bw) by gavage in corn oil (Jeon *et al.*, 2008).

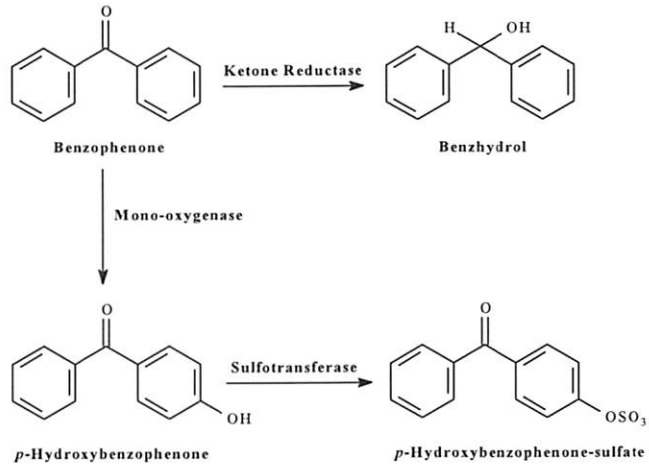
The metabolism of benzophenone in rabbits was originally shown to involve reduction of the keto group to produce benzhydrol, which was excreted in the urine as a glucuronide conjugate (Robinson, 1958). In a subsequent study, 4-hydroxybenzophenone was isolated from the urine of Sprague-Dawley rats that had been administered benzophenone in corn oil by gavage (Stocklinski *et al.*, 1980), and accounted for about 1% of the administered dose. It was isolated after treatment of the urine samples with a β-glucuronidase/aryl sulfatase preparation. A schema for the metabolism of benzophenone is shown in Fig. 4.1.

Twenty-four hour plasma time courses for benzophenone, benzhydrol and 4-hydroxybenzophenone were determined in Sprague-Dawley rats administered benzophenone by gavage in corn oil (Jeon *et al.*, 2008). 4-Hydroxybenzophenone, a product of aromatic hydroxylation, was identified after hydrolysis of the isolated metabolite with sulfatase. No dihydroxybenzophenone metabolites were identified in this study. Peak levels of benzophenone and its metabolites were reached approximately 4 hours after dosing, and the elimination half-life of the parent compound was approximately 19 hours. In toxicokinetic studies, the plasma elimination half-life of benzophenone in F344 rats was approximately 4 hours after intravenous injection and 8 hours after administration by gavage in corn oil; the plasma elimination half-life in B6C3F$_1$ mice was approximately 1 hour after intravenous injection and 1.5 hours after gavage in corn oil (NTP, 2006).

Benzophenone was metabolized to 4-hydroxybenzophenone, its sulfate conjugate, and benzhydrol in isolated F344 rat hepatocytes. Pretreatment of the hepatocyte suspension with 2,6-dichloro-4-nitrophenol, a sulfotransferase inhibitor, resulted in increased concentrations of free 4-hydroxybenzophenone (Nakagawa *et al.*, 2000).

Exposure of an aqueous solution of benzophenone to UV or sunlight irradiation produced two-ring hydroxylated derivatives — 3-hydroxybenzophenone and 4-hydroxybenzophenone — with concomitant generation of hydrogen peroxide, and the formation of 4-hydroxybenzophenone by UV irradiation was enhanced by the addition of hydrogen peroxide. The authors

Fig. 4.1 Proposed metabolism of benzophenone



Adapted from Nakagawa & Tayama (2001)

suggested that benzophenone might act as a photosensitizer that generates a reactive oxygen species which can cause aromatic ring hydroxylation (Hayashi *et al.*, 2006).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Benzophenone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537 in the presence or absence of metabolic activation systems. It did not increase the frequency of micronucleated polychromatic erythrocytes in samples of bone marrow obtained from male B6C3F$_1$ mice administered three intraperitoneal injections of benzophenone (200 to 500 mg/kg bw), or the frequency of micronucleated normochromatic erythrocytes in the peripheral blood of male or female B6C3F$_1$ mice administered benzophenone (1250 to 20 000 ppm) in the diet (estimated daily dose range, 200–4200 mg/kg bw) for 14 weeks (NTP, 2006).

Neither benzophenone nor its metabolites — benzhydrol or 4-hydroxybenzophenone — induced *umu* gene expression in *S. typhimurium* strain TA1535 in the presence or absence of rat or mouse liver microsomes. However, *umu* gene expression, which can be caused by DNA damaging agents, was elicited when *Escherichia coli* membranes expressing recombinant human cytochrome P450 (CYP) 2A6, 1A1, 1A2 or 1B1 were added to the incubation medium of *Salmonella*. The metabolite(s) responsible for this genotoxic effect were not identified (Takemoto *et al.*, 2002).

4.3 Toxic effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In a 2-year feed study, treatment with benzophenone increased the severity of chronic nephropathy and the incidence of renal tubule hyperplasia and hepatocellular hypertrophy in rats, and the incidence of nephropathy, metaplasia of the olfactory epithelium and hyperplasia

of splenic lymphoid follicles in mice (NTP, 2006). [The animals were 6 weeks (rats) or 8 weeks (mice) of age when treatment began and, consequently, any potential endocrine-related effects associated with perinatal exposure were not captured in these studies.]

4.4 Endocrine-disrupting effects

4.4.1 In-vitro effects

The benzophenone metabolite, 4-hydroxybenzophenone, induced proliferation of MCF-7 cells (an estrogen-responsive human breast cancer cell line) when cultured in estradiol-free medium; this effect was also produced by 17β-estradiol, but not by benzophenone or benzhydrol (Nakagawa *et al.*, 2000).

4-Hydroxybenzophenone competed with 17β-estradiol to bind to human recombinant estrogen receptor α (ERα) coated on 96-well plates (50% inhibitory concentration, ~5×10^{-5} M), but neither benzophenone nor benzhydrol demonstrated such competition (Nakagawa & Tayama, 2001). The competitive potency of 4-hydroxybenzophenone was approximately three orders of magnitude lower than that of diethylstilbestrol.

The two-ring hydroxylated compounds (3- and 4-hydroxybenzophenone) that are produced during exposure of benzophenone to sunlight competitively inhibited the binding of 17β-estradiol to human recombinant ERα and elicited ER-mediated transcriptional activity in yeast cells (Hayashi *et al.*, 2006).

Certain derivatives of benzophenone that have been widely used as UV screens also have estrogenic activity: benzophenone-2 (2,2′,4,4′-tetrahydroxybenzophenone) also competed with 17β-estradiol to bind to ERα and ERβ (Seidlová-Wuttke *et al.*, 2004).

Moreover, benzophenone-3 (2-hydroxy-4-methoxybenzophenone) elicited anti-androgenic activity in a human breast carcinoma cell line (MDA-kb2) by inhibiting dihydrotestosterone-induced activation of androgen receptor, but showed no evidence of agonistic activity for this nuclear receptor (Ma *et al.*, 2003). It transcriptionally activated human ERα and ERβ in transfected human embryonic kidney cells (HEK293) and was antagonistic to the transcriptional activation of the androgen receptor by dihydrotestosterone and the progesterone receptor by a synthetic progestin (ORG 2058) in a transfected human osteosarcoma cell line (U2-OS) (Schreurs *et al.*, 2005).

Benzophenone and its metabolite, 4-hydroxybenzophenone, elicited estrogenic activity in MCF-7 cells and anti-androgenic activity in transfected rat fibroblast NIH3T3 cells. In both assays, 4-hydroxybenzophenone was more potent than benzophenone but less potent than benzophenone-2 (Suzuki *et al.*, 2005).

4.4.2 In-vivo effects

The in-vivo estrogenic activity of benzophenone was confirmed in the uterotrophic assay. Subcutaneous injection of 4-hydroxybenzophenone (once a day for 3 days at doses of 100, 200 or 400 mg/kg bw) to immature (21-day-old) female Sprague-Dawley rats produced a dose-related increase in absolute and relative uterine weights (Nakagawa & Tayama, 2001). Morphological evaluation showed that the treatment increased the luminal epithelial height and the thickness of the stromal layer of the uterus due to proliferation of uterine luminal epithelial cells, and increased the thickness and induced cornification of the vaginal epithelium. The same uterotrophic effects were observed in ovariectomized Sprague-Dawley rats administered benzophenone at doses of 100 and 400 mg/kg bw for 3 consecutive days by gavage in corn oil (Nakagawa & Tayama, 2002). Uterine weights were also increased in ovariectomized female F344 rats that received intraperitoneal injections of benzophenone (300 mg/kg bw) for 3 days

(Suzuki *et al.*, 2005). [The estrogen-like effects of benzophenone in the female reproductive tract appear to be due to metabolism to 4-hydroxybenzophenone, which binds to ERα.]

3- and 4-Hydroxybenzophenone induced increases in uterine weights in immature female Sprague-Dawley rats exposed subcutaneously for 3 consecutive days (Hayashi *et al.*, 2006). The effect on uterine weight was suppressed by pretreatment with the anti-estrogen ICI 182 780 (Faslodex). Thus, estrogenic products of benzophenone can also be generated by photochemical activation. [This observation is important because benzophenone has been used as a UV filter in cosmetics.]

The same uterotrophic effects as those described for 4-hydroxybenzophenone were observed in ovariectomized Sprague-Dawley rats fed benzophenone-2 in the diet for 3 months (Seidlová-Wuttke *et al.*, 2004). In addition to uterotrophic effects, the expression of ER-related receptor 1 in the uterus and ERβ expression in the thyroid was increased and ERα expression in the uterus was decreased in ovariectomized Sprague-Dawley rats administered benzophenone-2 by gavage in olive oil for 5 days (Schlecht *et al.*, 2004). At similar exposures, benzophenone-3 did not increase uterine weight, but did decrease ERα expression in the pituitary and ERβ expression in the uterus. Apart from the induction of estrogen-like effects by benzophenone-2 in multiple organs (including increased expression of insulin growth factor 1 in the vagina, decreased expression of insulin growth factor 1 in the liver, reduced luteinizing hormone synthesis by the pituitary gland, and a reduction of serum cholesterol high- and low- density lipoproteins), the 5-day treatment caused a reduction in serum thyroxine and triiodothyronine levels through a non-ER-mediated process (Jarry *et al.*, 2004). The latter effect of benzophenone-2 appears to be due to interference of thyroid hormone biosynthesis by inhibiting or inactivating thyroid peroxidase (Schmutzler *et al.*, 2007).

Among 17 benzophenone derivatives that were evaluated for anti-androgenic activity *in vitro*, the most potent (2,4,4′-trihydroxybenzophenone) also significantly suppressed the effect of testosterone on the weight gains of prostate and seminal vesicle in castrated male F344 rats (Hershberger assay), confirming the in-vivo anti-androgenic effect of this chemical (Suzuki *et al.*, 2005). Benzophenone-2 — an estrogenic chemical — also induced hypospadias in male C57BL/6 mice that were treated by gavage from gestational day 12 through to gestational day 17 (Hsieh *et al.*, 2007). The authors concluded that this effect was dependent on ER signalling because co-administration with an ER antagonist (EM-800) prevented the induction of hypospadias by benzophenone-2.

Benzophenone was also shown to induce an interaction between the pregnane X receptor and the steroid receptor coactivator 1 *in vitro*, and to induce the expression of *CYP2B1/2, –2C11* and *-3A1* genes in the liver of male Sprague-Dawley rats that had been administered intraperitoneal doses of 50, 100 or 250 mg/kg bw per day for 3 days (Mikamo *et al.*, 2003). [The increased expression of *CYP2B1* suggests the involvement of the constitutive androstane receptor.] Thus, benzophenone can also disrupt normal endocrine function by transcriptionally activating the pregnane X receptor and upregulating the expression of genes that code for enzymes involved in the metabolism of endogenous steroid hormones.

The above studies indicate that endocrine-active, benzophenone-derived chemicals may alter normal development and affect endocrine regulation in multiple organs by multiple mechanisms.

4.5 Mechanisms of carcinogenesis

Although the mechanisms of tumour induction by benzophenone are not fully known, they may be complex. The effects may include the generation of reactive oxygen species, or

endocrine disruption through multiple receptors, which include the induction of estrogen-like effects as a result of binding of benzophenone to ERα, alteration of the metabolism of endogenous steroid hormones, antagonism of transcriptional activation of the androgen receptor, and possible activation of nuclear constitutive androstane and pregnane X receptors. [The Working Group noted that aromatic hydroxylation of benzophenone to a transcriptionally active metabolite is likely to occur in humans.]

5. Summary of Data Reported

5.1 Exposure data

Benzophenone is produced by the acylation of benzoyl chloride with an excess of benzene. It may also be formed by atmospheric oxidation of diphenylmethane. Benzophenone is used as an ultraviolet curing agent, flavour ingredient, fragrance enhancer and perfume fixative, and as an additive for plastics, coatings and adhesive formulations. Benzophenone is also used as a screen to prevent ultraviolet light-induced damage to cosmetics. It is used in laundry and household cleaning products, and in the manufacture of pharmaceuticals, insecticides and agricultural chemicals. Benzophenone enters the environment after having been washed from skin and clothes through wastewater and from municipal solid-waste landfill leachates, and is ubiquitous in water, sediment and biota.

Occupational exposure may occur through inhalation and dermal contact in the manufacture of products that contain benzophenone. Dietary exposure to benzophenone occurs as a result of its natural occurrence in food or addition to food as a flavouring agent, its presence in drinking-water as a contaminant, and through its migration from food packaging, printing inks or recycled paperboard. Exposure may also occur through the inhalation of fragrances used in indoor air and dermal contact with household cleaning and personal care products.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Benzophenone was tested for carcinogenicity by oral administration in the diet in one study in mice and rats and by dermal application in one study in mice. Oral administration of benzophenone significantly increased the incidence of hepatocellular adenoma, and hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma (combined) in male mice and histiocytic sarcoma in female mice. It increased the incidence of mononuclear-cell leukaemia in male and female rats (not statistically significant in females), renal tubule adenoma in male rats and histiocytic sarcoma in female rats (not statistically significant). Dermal application of benzophenone did not induce tumours in mice.

Tumours of the kidney, histiocytic sarcomas and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available on the toxicokinetics of benzophenone in humans. Benzophenone is absorbed in monkeys after dermal application, and is rapidly absorbed from the gastrointestinal tract of rodents. It is metabolized by reduction to benzhydrol or by oxidation to 4-hydroxybenzophenone. The latter compound can also be formed by ultraviolet or sunlight irradiation of benzophenone.

Benzophenone was not mutagenic in *Salmonella* and did not induce micronuclei in mice. Benzophenone and its metabolites induced *umu* gene expression, an indication of

DNA damage, in *Salmonella* in the presence of *Escherichia coli* membranes expressing recombinant human cytochrome P450s.

The benzophenone metabolite, 4-hydroxybenzophenone, elicits estrogenic activity and anti-androgenic activity *in vitro*, and the in-vivo estrogenic activity of benzophenone has been confirmed in multiple uterotrophic assays. Benzophenone may alter endocrine signalling through multiple effects on receptors.

The mechanistic evidence for tumour induction by benzophenone is weak, but the relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of benzophenone.

6.3 Overall evaluation

Benzophenone is *possibly carcinogenic to humans (Group 2B).*

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