Food and Chemical Toxicology 56 (2013) 443–449

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Dietary exposure of juvenile female mice to polyhalogenated seafood contaminants (HBCD, BDE-47, PCB-153, TCDD): Comparative assessment of effects in potential target tissues

F. Maranghi [a,*], R. Tassinari [a,1], G. Moracci [a,2], I. Altieri [b,3], J.D. Rasinger [c], T.S. Carroll [d], C. Hogstrand [d], A.-K. Lundebye [c], A. Mantovani [a,4]

[a] Food and Veterinary Toxicology Unit, Veterinary Public Health and Food Safety Dept., Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[b] Food Chemical Safety and Human Health Unit, Veterinary Public Health and Food Safety Dept., Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[c] National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway, PO Box 2029, Nordnes, 5817 Bergen, Norway
[d] Diabetes and Nutritional Sciences Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK

ARTICLE INFO

Article history:
Received 13 September 2012
Accepted 28 February 2013
Available online 14 March 2013

Keywords:
Thyroid
Thymus
Feed
Steroid hormones
Liver

ABSTRACT

Fish represents source of nutrients and major dietary vehicle of lipophilic persistent contaminants. The study compared the effects of two legacy and two emerging fish pollutants (Hexabromocyclododecane HBCD; 2,2′,4,4′-Tetrabromodiphenyl ether BDE-47; 2,2′,4,4′,5,5′-Hexachlorobiphenyl PCB-153; 2,3,7,8-Tetrachlorodibenzo-p-doxin TCDD) in juvenile female mice exposed through a salmon based rodent diet for 28 days (dietary doses: HBCD 199 mg/kg bw/day; BDE-47 450 μg/kg bw/day; PCB-153 195 μg/kg bw/day; TCDD 90 ng/kg bw/day). Dose levels were comparable to previously reported developmental Lowest Observed Adverse Effect Levels. None of the treatments elicited signs of overt toxicity, but HBCD increased relative liver weight. All compounds caused changes in liver, thymus and thyroid; spleen was affected by BDE-47 and PCB-153; no effects were seen in uterus and adrenals. Strongest effects in thyroid follicles were elicited by PCB-153, in thymus and liver by BDE-47. HBCD and BDE-47 induced liver fatty changes, but appeared to be less potent in the other tissues. HBCD, BDE-47 and TCDD increased serum testosterone levels and the testosterone/estradiol ratio, suggesting a potential involvement of pathways related to sex steroid biosynthesis and/or metabolism. The results support the role of toxicological studies on juvenile rodents in the hazard characterization of chemicals, due to endocrine and/or immune effects.

1. Introduction

In many countries fish represents an important source of nutrients (FAO/WHO, 2011; EFSA, 2005). Due to the high lipid content in comparison to other species, both wild and farmed oily fish (e.g. herring, mackerel, tuna and salmon) are – at the same time – a dietary source of long chain *n*-3 polyunsaturated fatty acids and tend also to accumulate lipophilic contaminants such as persistent organic pollutants (POPs); therefore they represent a significant source of human exposure (EFSA, 2012). POPs include compounds regularly monitored in feeds and foods, such as dioxins and polychlorinated biphenyls (PCBs) as well as compounds that have still to be included in routine food control programmes, such as some brominated flame retardants (BFRs).

BFR include hexabromocyclododecane (HBCD) (EFSA, 2011a) and polybrominated diphenyl ethers (PBDEs) (EFSA, 2011b). The 2,2′,4,4′ Tetrabromodiphenyl ether (BDE-47) congener is a major contributor to dietary intake and human body burden (EPA, 2008); main targets of PBDE and HBCD toxicity are the liver (e.g. hepatic enzyme induction), thyroid (reduced hormone levels and gland hyperplasia) as well as the reproductive, nervous and immune system (EFSA, 2011b).

Dioxins, including 2,3,7,8 Tetrachlorodibenzo-p-doxin (TCDD) and dioxin-like PCBs are thoroughly investigated POPs, due to their widespread distribution and high toxicity; main targets are the

Abbreviations: BDE-47, 2,2′4,4′ Tetrabromodiphenyl ether; BFR, brominated flame retardants; Bw, body weight; DMSO, dimethylsulfoxide; E2, 17-beta estradiol; EU, European Union; HBCD, hexabromocyclododecane; LOAEL, Lowest Observed Adverse Effect Level; NDL, non dioxin-like; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PCB-153, 2,2′4,4′5,5′ Hexachlorobiphenyl; PM, post-mortem; POPs, persistent organic pollutants; SD, Standard Deviation; T, testosterone; TCDD, 2,3,7,8 Tetrachlorodibenzo-p-doxin.

* Corresponding author. Tel.: +39 06 49902527; fax: +39 06 49902658.
E-mail addresses: francesca.maranghi@iss.it (F. Maranghi), roberta.tassinari@iss.it (R. Tassinari), gabriele.moracci@iss.it (G. Moracci), ilaria.altieri@iss.it (I. Altieri), jra@nifes.no (J.D. Rasinger), thomas.carroll@kcl.ac.uk (T.S. Carroll), christer.hogstrand@kcl.ac.uk (C. Hogstrand), aha@nifes.no (A.-K. Lundebye), alberto.mantovani@iss.it (A. Mantovani).
[1] Tel.: +39 06 49902528; fax: +30 06 49387099.
[2] Tel.: +39 06 49902540; fax: +30 06 49387099.
[3] Tel.: +39 06 49902650; fax: +39 06 49903686.
[4] Tel.: +39 06 49902815; fax: +39 06 49902658.

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.02.056

reproductive and immune systems (Yoshioka et al., 2011). Tolerable weekly intake of 14 pg/kg body weight (bw) per week (expressed in toxic equivalents) has been established (EU, Scientific Committee on Food, 2001). However, in fish the bulk of PCB exposure is made up by the non dioxin-like PCB (NDL-PCB), that include the most widespread and persistent congener 2,2′,4,4′,5,5′ Hexachlorobiphenyl (PCB-153) (EFSA, 2012). PCB-153 has been associated with a number of effects (thyroid, reproductive system, liver and brain biochemistry, immunotoxicity, oestrogenicity, and neurodevelopmental impairment upon *in utero* exposure): indeed, health guidance values still have to be developed (EFSA, 2005).

The developing organism is considered most susceptible to these POPs, as indicated by critical endocrine and immune effects, and uncertainties do exist about the risk characterization for children. Indeed, the post-natal juvenile developmental phase is increasingly pointed out as a window specifically vulnerable to endocrine-disrupting chemicals (Maranghi and Mantovani, 2012).

An innovative approach has been introduced in the present study performed within the project AQUAMAX (http://www.aquamaxip.eu) of the EU 6th Framework Programme. The study aimed at comparing the toxicity in the juvenile life-stage of four POPs, relevant to human exposure through fish consumption, namely: HBCD, BDE-47, PCB-153 and TCDD. Juvenile female mice were selected as a model for a highly vulnerable sub-group of fish consumers, namely young women in the peri-pubertal phase, at the start of fertile age. Particular attention was paid to possible histopathological effects on liver, uterus, thyroid, adrenals, thymus and spleen as indicators of specific toxicological effects upon exposure during the juvenile life-stage. (Maranghi et al., 2009, 2010b). Mice were exposed to dietary levels of the four POPs at the respective, potentially relevant Lowest Observed Adverse Effect Level (LOAEL) (see below Section 2). The design of the study was developed with the consideration that human exposure to these contaminants occurs to a large extent through consumption of fish; the POPs were therefore administered to mice through fish-based diets.

2. Materials and methods

2.1. Animals

All procedures were carried out in accordance with the project license guidelines (PPL70/6407) granted under the UK Home Office Animals (Scientific Procedures) Act, 1986. Experimental study was performed at the Diabetes and Nutritional Sciences Division, King's College London.

Fifty-five 22-day old female BALB/c mice), 10 mice/treatment group and 15 mice in control group, were purchased from Charles River (UK). Mice were housed individually (one animal *per* cage) in Utemp1284 mouse cages (Tecniplast, UK) with wood chips bedding (Aspen-wood chips, B and K Ltd.) in rooms controlled for temperature (20–22 °C), relative humidity (45–65%), and lighting (12 h light/dark cycle). Mice had access to water *ad libitum*. To avoid overfeeding, the feed intake of the mice was restricted to 15% (w/w) a day. At the start of the experiment mice were fed 2.25 g feed/mouse/day, and the amount was increased twice a week in relation to increase in bw. After an acclimation period of 3 days, mice were allocated at random into experimental diet groups.

2.2. Experimental diets

Animals – 10 mice/treatment group and 15 mice in control group – were exposed to POPs via a fish-based diet to mimic human exposure: experimental diets were produced in house as described in Haave et al. (2011). In brief, diets were produced in accordance with the standard rodent diet formulation AIN-93 G as described in Reeves et al. (1993), with the exception that freeze-dried Atlantic salmon (*Salmon salar*) was used as the diet's main protein and fat source (concentration of approximately 17% w/w and 7% w/w, respectively). The salmon used in mouse feed was experimentally raised on plant protein and oil substituting fish feed during the AQUAMAX project, which yielded very low background levels of contaminants in the fish fillets (Berntssen et al., 2010).

Due to the addition of freeze-dried salmon to the diets a ratios of marine omega-6 to omega-3 fatty acids of approximately 1.35 was obtained.

The diet produced was split into five equal batches: four batches were spiked with the four POPs at the measured concentrations 1.3 mg HBCD/g feed, 3.0 μg BDE-47/g feed, 1.3 PCB-153 μg/g feed and 0.6 ng 2,3,7,8-TCDD/g feed (nominal concentration), one batch was used as control.

Each POP was dissolved and diluted in 100% dimethylsulfoxide (DMSO) with final DMSO content of 0.4 mL/kg feed as described in Haave et al. (2011). Dietary concentration was determined prior to the study start with the exception of HBCD. Contaminant analyses were performed in triplicate samples, as described in Haave et al. (2011). The quantity of HBCD used to prepare the experimental feed was more than three orders of magnitude higher than the other POPs investigated. Hence the HBCD concentrations were determined in the feeds administered to the three other experimental groups, in order to identify any cross contamination.

2.3. Rationale for dose selection

Based on: (i) concentrations in the diets, (ii) a daily ratio of 15% (w/w) and (iii) expected growth rate of Balb/c juvenile female mice, the target dose levels were as follows: HBCD 199 mg/kg bw/day; BDE-47 450 μg/kg bw/day; PCB 153 195 μg/kg bw/day; TCDD 90 ng/kg bw/day. For each test compound the target dose was comparable with the available LOAELs that could be potentially relevant to juvenile mice, namely:

- *HBCD*: no data are available in mice. The target dose level of 199 mg/kg bw/day is comparable to the LOAEL of 213 mg/kg bw/day defined in weanling rats exposed once a week by gavage from gestation day 10 until weaning (Saegusa et al., 2009).
- *BDE-47*: the target dose level of 450 μg/kg bw/day is in the range of the lowest effective dose (700 μg/kg bw/day) and benchmark dose 10% (338 μg/kg bw/day) identified for BDE-47 in neonatal mice by the European Food Safety Authority (EFSA, 2011b).
- *PCB-153*: a postnatal LOAEL in the mouse is unavailable. The target dose level of 195 μg/kg bw/day is comparable to the 250 μg/kg bw/day LOAEL for reduced fetal weight in mice (Morrissey et al., 1992).
- *TCDD*: three mouse reproductive studies using TCDD, administered once a week by gavage reported LOAEL (reproductive parameters and offspring survival) in the range of 0.5–1 μg/kg bw/week (Public Agency for Toxic Substances and Disease Registry, 1998). Thus, the target dose level of 0.09 μg/kg bw every day for 28 days, resulting in a weekly dose of 0.63 μg/kg bw, falls within the range of mouse reproductive LOAELs.

2.4. Feeding experiment

Throughout the feeding experiment, animals were controlled daily for general health conditions; body weight was recorded weekly.

After 28 days of treatment, mice were anaesthetised for 5 min with Isoflurane (5% mix with oxygen) before placed under an anaesthetic delivery mask. Blood was collected by cardiac puncture; then the anaesthetised animals were sacrificed by cervical dislocation.

2.5. Tissue sampling and processing

Liver, uterus, thyroid, adrenals, thymus and spleen were sampled; liver, spleen, thymus and uterus were weighed immediately after dissection. Thyroid and adrenals were not weighed due to both their small size and to avoid post-mortem (PM) changes which they are highly vulnerable to. Indeed, the specific aim of the study was to evaluate tissue effects and priority has been given to minimizing the chance of PM changes. Subsequently, sampled tissues were rinsed in PBS solution, blotted on tissue-paper, fixed in 10% buffered formalin at room temperature for 24 h, stored in 80% ethyl alcohol and embedded in paraffin for histological and histomorphometrical analyses.

2.6. Steroid hormone serum levels

Blood was left to coagulate at room temperature for 1 h and then centrifuged for 15 min at 2000 rotations per minute in a cooled bench-top centrifuge (Microlite Microfuge, Thermo Electron Corporation). After centrifugation the serum was snap frozen in liquid nitrogen and stored at −80 °C.

Testosterone (T) and 17-beta estradiol (E2) serum levels were determined using radioimmunoassay kits (DPC-coat-a-count total testosterone kit and DSL-4400 estradiol RIA kit). The concentrations were expressed as pg/ml and ng/ml, respectively.

2.7. Histological and histomorphometrical analysis

Paraffin embedded tissues were cut into 5 μm sections and stained with haematoxylin and eosin for light microscopic microscopy (Nikon Microphot FX) with different objectives.