EFFECTS OF LACTATIONAL EXPOSURE TO NON-PLANAR PCB-155 AND PLANAR PCB-169 ON BODY WEIGHT GAIN AND CRANIOFACIAL GROWTH IN RAT OFFSPRING

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Summary: The adverse effects of two polychlorinated biphenyls (PCBs), non-planar PCB-155 and planar PCB-169, individually and in combination, on body weight gain and craniofacial growth in rat offspring lactationally exposed to PCBs during the early postnatal period were investigated. Lactating adult Wistar rats (n=15) were intraperitoneally administered a total of 12 mg/kg b.w. PCB-155 (group 1, n=4), 3 mg/kg b.w. PCB-169 (i.e., 90 mg toxic equivalents (TEQ)/kg b.w.) (group 2, n=4), or PCB-169 and PCB-155 together (group 3, n=3). The fourth group (n=4) served as a control. Offspring were sacrificed on postnatal days (PNDs) 9 and 22. Body weights and craniofacial dimensions were recorded. On PND 9, all of the exposed offspring weighed less (p≤0.001) than the control group, and group 2 (p≤0.001) and 3 (p≤0.01) weighed less than group 1. On PND 22, only groups 2 and 3 weighed less than the control group (p≤0.001). Narrower neurocranium was observed on PND 9 in groups 1 (p≤0.01), 2 (p≤0.05) and 3 (p≤0.001). Skulls were shorter in groups 2 (p≤0.001) and 3 (p≤0.01), and this reduction persisted until PND 22 (p≤0.001). On PND 22, rounder skulls were observed in groups 2 (p≤0.001) and 3 (p≤0.05), and mandibular length was reduced (p≤0.001). The data suggest that PCB-155 may have an additive effect with PCB-169 on growth reduction. In conclusion, lactational exposure to PCB-155 and PCB-169 negatively affected body weight gain and craniofacial growth in rat offspring until PND 9, while the adverse effects of PCB-169 were more potent and persisted until PND 22.

Key words: polychlorinated biphenyls; lactational; body weight; craniofacial growth; rat

Introduction

Polyhalogenated aromatic hydrocarbons (i.e., polychlorinated-biphenyls (PCBs), -dioxins) are persistent and widespread environmental pollutants. Being lipophilic, they accumulate in animal and human tissues, where they can cause a wide range of biological and toxicological effects (1). Planar dioxin-like PCBs exhibit physicochemical properties, environmental distributions, and toxicity profiles that are different from their non-planar homologues (2). The effects of planar PCBs are mediated through the aryl hydrocarbon receptor (AhR), a cytosolic receptor protein with high affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (3). Predominant exposure to PCBs is via ingestion of food, and they also cross the placenta and are excreted in milk (2). Concern has been raised about breast-fed infants, whose daily intake of polyhalogenated aromatic hydrocarbons on a body weight basis may be one or two orders of magnitude higher than in adults (4). Importantly, organisms are not fully developed at birth, and thus, a process of maturation continues postnatally for an extended *period*. Tissues and organs are most sensitive during this critical period of intensive growth, and therefore, an

insult/interference posed to suckling offspring in early postnatal life may influence their postnatal growth and development (5).

Evidence for PCB-induced deficits in growth and development includes studies on exposed human populations that show reduced growth rates in children (1, 6-8) and skull abnormalities with irregular calcifications and wide open sutures and fontanelles (9). Other evidence suggests that PCBs may increase the susceptibility to weight gain and obesity (10). Alterations in bone growth and composition have previously been described in experimental animals exposed to PCBs (11-17) as well as in wild animals environmentally exposed to polyhalogenated aromatic hydrocarbons (18-23). Experimental exposure to TCDD caused growth suppression (24), impaired skull growth in adult rats (25), and osteolysis in maxilla and mandibles in minks (26); similar adverse effects were induced with planar PCB-126 (14). In developmental studies, fish exhibited dysmorphogenesis in craniofacial structures (27) and decreased growth with more juvenile cranial characteristics (17) in response to TCDD and PCB-126, respectively. In rodents, offspring that were perinatally exposed to PCB mixtures showed growth suppression, although in some groups, only transient (28, 29) and facial malformations were observed (28). Alterations were mainly driven by the dioxin-like PCBs, although the contribution of the non-planar PCBs to the exposure outcome could not be excluded (30). Perinatal exposure to TCDD decreased body weights at higher doses (31), caused smaller and less mineralised skulls (32), and affected the shape and size of the mandible (33). Considering the potential toxicity of PCBs and the limited understanding of factors that govern their adverse effects, it is imperative to pursue these studies further.

In our previous studies, different lactational transfer of non-planar PCB-155 and planar PCB-169 to lactationally exposed lambs (34) and their enrichment in lamb mandibular bone (35) were observed. The objective of this study was to therefore examine the adverse effects of two different PCBs on body weight gain and craniofacial growth in rat offspring that were lactationally exposed during early postnatal life, which in rats can be divided into the following periods: presuckling (first 6 hours after birth), suckling (until PND 17) and weaning (from PND 17 until 28). The last starts when offspring ingest food

other than maternal milk (5). We administered two hexa-chlorobiphenyls, non-planar PCB-155 and planar PCB-169, to actively lactating rats. For dioxin-like PCB-169, a toxic equivalency factor (TEF) of 0.03 is proposed (36), representing its toxic potency evaluated in comparison to that of TCDD. To explore possible interactions between individual PCBs, concomitant exposure was also performed. Offspring were sacrificed on PNDs 9 and 22 so that the effects of PCBs on different periods of early postnatal development, i.e., the suckling and weaning periods, could be studied. On PND 9, there is the first peak of daily weight increments, while a decrease is reported around PND 16. Milk consumption, which peaks between PND 17 and 19, decreases with the intake of water and solid food. Maternal milk composition also changes substantially as the fat and protein content is three- and two-fold lower on PND 20 (weaning period) compared to PND 10 (suckling period) (5).

Materials and methods

Animal care and PCB administration. Sexually mature 8-10 week old adult female Wistar rats (n=15), weighing between 230 and 250 g, were obtained from Lek d.d. (Ljubljana, Slovenia). They were housed under standardised conditions at the Veterinary Faculty in Ljubljana. They received standard pellet feed (M-K 02) and tap water *ad libitum.* After mating and delivery, the lactating mothers were randomly assigned to four groups. The first group (n=4) was administered a single dose of 6 mg/kg b.w. PCB-155 (2,2',4,4',6,6'-hexachlorobiphenyl) in olive oil after delivery via an intraperitoneal injection, followed by three maintenance doses of 2 mg/kg b.w. PCB-155 on days 6, 12, and 17 day after delivery; the total amount administered was 12 mg/kg b.w. PCB-155. The second group (n=4) was administered a single dose of 2 mg/kg b.w. PCB-169 (3,3',4,4',5,5'-hexachlorobiphenyl) in olive oil after delivery via an intraperitoneal injection, followed by two maintenance doses of 0.5 mg/kg b.w. PCB-169 on days 6 and 14 day after delivery; the total amount administered was 3 mg/kg b.w. PCB-169. The combined regime of the PCB-155 and PCB-169 administrations was used in the third group (n=3). On the day of delivery, mothers received a single dose of 2 mg/kg b.w.

PCB-169 and 6 mg/kg b.w. PCB-155, followed by maintenance doses as described above for the first and second groups. The fourth group (n=4) served as a vehicle control; lactating mothers were administered 0.5 mL olive oil after delivery by an intraperitoneal injection, and on days 6, 12, 14, and 17, an additional 0.15 mL olive oil. Offspring were exposed to PCB-155 and/or to PCB-169 via the mother's milk.

To achieve equal concentrations of both PCBs in milk, the maintenance doses of PCB-155 and PCB-169 were determined according to the results of our previous study (34). The excreted amount of the PCB-169 in mothers' milk was more than three times higher than the amount of PCB-155; therefore, the administered loading and maintenance doses of PCB-155 were higher and more frequent. Standards of PCBs were purchased from Promochem (Wesel, Germany). IUPAC numbers were used for assigning the PCB congeners (37). All procedures involving the experimental rats complied with the Prevention of Cruelty to Animals law that is consistent with the European Community Directive 86/609/EC and were approved by the Slovenian Veterinary Administration and its Ethics committee (Permits No. 323-02-206/00 and 3440-165/2006).

PCB analysis, measurement of body weights and craniofacial dimensions. From each group, the same number of male and female offspring per litter was sacrificed on PNDs 9 and 22. On PND 9, 19 offspring were sacrificed from the PCB-155 group, the same number was sacrificed from the PCB-169 group, 14 offspring were from the PCB-155+169 group, and 15 from the control group. On PND 22, 17 offspring were sacrificed from the PCB-

155 group, 19 from the PCB-169 group, 14 from the PCB-155+169 group, and 14 from the control group. All offspring were weighed (to the nearest 0.01 g) on PND 9, and those not sacrificed on PND 9 were weighed also on PND 22. At the end of the experimental period, offspring were sacrificed under deep general inhalation anesthesia induced by CO_2 followed by exsanguination. Blood samples for PCB analysis were collected from the ophthalmic plexus into tubes containing lithium heparin as an anticoagulant and stored at −20°C until analysis. PCB residues were determined using the solid phase microextraction technique and gas chromatography with electron capture detection (GC-ECD) as previously described (34). Number of animals used for each chemical analysis was smaller than for the assessment of body weights and craniofacial dimensions, as insufficient blood volume samples were not analysed. Skulls and mandibles were dissected, the muscle and soft tissue were removed, and the linear craniofacial dimensions were measured under a stereomicroscope using a Vernier sliding caliper (to the nearest 0.05 mm) by one inspector who was blinded to the treatment. The skull length was measured as the distance between the occipitointerparietal suture and the anterior margin of the nasal bone, and the neurocranium width was measured immediately caudal to the zygomatic arches; the mandibular length was measured as the distance between the foramen mentale and condylar processus as schematically shown in Fig. 1.

Statistical analysis. Data were expressed as the mean±SD. Analysis of variance (ANOVA) with the Tukey post-test was used to explore differences in
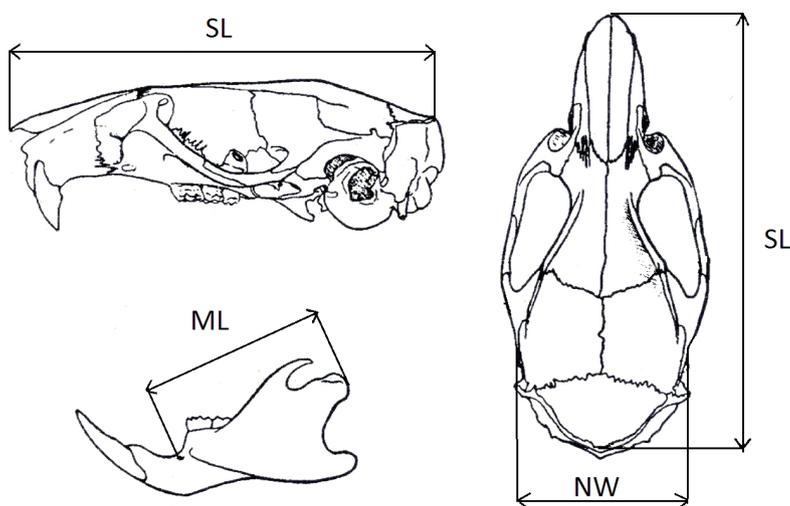
Figure 1: Schematic presentation of rat skull and mandible showing the measurements used to assess craniofacial growth.
SL - skull length; NW - neurocranium width; ML - mandibular length

Table 1: Blood PCB concentrations (mean±SD) of Wistar rat offspring on PNDs 9 and 22 in differently exposed groups

	PCB concentrations (ng/mL)			
	PND 9		PND 22	
Treatment	PCB-155	PCB-169	PCB-155	PCB-169
PCB-155	8.0±5.4 (5)	/	10.3±3.1 (10)	/
PCB-169	/	1.3±0.1 (5)	/	3.2±0.1[a] (11)
PCB-155+169	8.2±2.6 (5)	1.7±0.3 (5)	14.8±3.6[b] (3)	1.9±0.1[c] (3)

Number of animals used for each analysis is given in parentheses.
[a] - significantly different from PND 9 (p≤0.001)
[b] - significantly different from PCB-155 group on PND 22 (p≤0.001)
[c] - significantly different from PCB-169 group on PND 22 (p≤0.001)

Table 2: Body weights and craniofacial dimensions (mean±SD) of Wistar rat offspring at PNDs 9 and 22 in differently exposed groups

	Treatment			
Variable	Control	PCB-155	PCB-169	PCB-155+169
Body weight (g)				
PND 9	21.12±2.73[155,169,155+169] (29)	18.93±1.25[c,169,155+169] (36)	17.14±1.49[c,155] (38)	16.49±3.30[c,155] (28)
PND 22	61.07±3.61[169, 155+169] (14)	58.91±3.16[169,155+169] (17)	48.47±3.75[c,155] (19)	48.25±9.84[c,155] (14)
Skull length (mm)				
PND 9	24.79±0.65[169,155+169] (15)	24.27±0.55 (19)	23.85±0.45[c] (19)	23.52±0.91[c] (14)
PND 22	33.28±0.71[169, 155+169] (14)	33.24±0.39[169,155+169] (17)	30.87±2.44[c,155] (19)	31.07±1.24[c,155] (14)
Neurocranium width (mm)				
PND 9	13.57±0.43[155,169,155+169] (15)	13.03±0.21[c] (19)	13.18±0.25[c] (19)	12.97±0.31[c] (14)
PND 22	15.76±0.42[155+169] (14)	15.74±0.45[155+169] (17)	15.65±0.26[155+169] (19)	15.34±0.33[c,155,169] (14)
Skull length / neurocranium width				
PND 9	1.82±0.04 (15)	1.86±0.03[169,155+169] (19)	1.80±0.01[155] (19)	1.81±0.03[155] (14)
PND 22	2.11±0.05[169,155+169] (14)	2.11±0.05[169,155+169] (17)	1.97±0.15[c,155] (19)	2.01±0.05[c,155] (14)
Mandibular length (mm)				
PND 9	10.93±0.41[155+169] (15)	10.69±0.21 (19)	10.62±0.23 (19)	10.21±0.75[c] (14)
PND 22	14.93±0.30[169,155+169] (14)	15.04±0.19[169,155+169] (17)	13.88±0.19[c,155] (19)	14.05±0.55[c,155] (14)
Skull length / body weight (mm/g)				
PND 9	1.16±0.13[155,169,155+169] (15)	1.29±0.07[c,169] (19)	1.35±0.07[c,155] (19)	1.46±0.27[c] (14)
PND 22	0.54±0.03[169,155+169] (14)	0.56±0.03[169,155+169] (17)	0.64±0.06[c,155] (19)	0.67±0.12[c,155] (14)
Neurocranium width / body weight (mm/g)				
PND 9	0.63±0.07[169,155+169] (15)	0.69±0.04[169] (19)	0.75±0.04[c,155] (19)	0.81±0.16[c] (14)
PND 22	0.26±0.01[169,155+169] (14)	0.26±0.01[169,155+169] (17)	0.32±0.02[c,155] (19)	0.33±0.07[c,155] (14)
Mandibular length / body weight (mm/g)				
PND 9	0.51±0.06[169,155+169] (15)	0.57±0.04[169] (19)	0.60±0.03[c,155] (19)	0.63±0.09[c] (14)
PND 22	0.24±0.01[169,155+169] (14)	0.25±0.01[169,155+169] (17)	0.29±0.02[c,155] (19)	0.31±0.06[c,155] (14)

Number of animals used for each measurement is given in parentheses.
[c] – significant differences in the parameters measured between the control group and differently exposed groups (p≤0.05); [155] – significant differences between the PCB-155 group and differently exposed groups (p≤0.05); [169] – significant differences between the PCB-169 group and differently exposed groups (p≤0.05); [155+169] – significant differences between the PCB-169+155 group and differently exposed groups (p≤0.05) as evaluated by one-way analysis of variance (ANOVA)

body weights and linear craniofacial dimensions among different groups. Two-tailed independent-samples t-test was used to compare PCB blood concentrations and treatment effects between PND 9 and PND 22. A p-value of ≤0.05 was considered statistically significant. The data were analysed using the SPSS 12.0 statistical software package for Windows (SPSS Inc., Chicago, Ill, USA).

Results

Exposure of offspring. To confirm offspring PCB exposure via mothers' milk, PCB blood concentrations on PNDs 9 and 22 were determined (Table 1). The data show that on PND 22, the concentrations of PCB-169 in the blood samples were higher (p≤0.001) than on PND 9. The concentrations of PCB-155 were also higher on PND 22, but not significantly. On PND 22, the concentrations of PCB-155 were lower (p≤0.001), and those of PCB-169 were higher (p≤0.001) in offspring exposed to single PCB compared to offspring exposed to the combination of PCB-155 and PCB-169.

Body weight gain. The body weights of differently exposed offspring are summarised in Table 2. The mean body weight of Wistar offspring on PND 1 (n=142) was 6.83±0.64 g. On PND 9, the body weights of exposed offspring were significantly (p≤0.001) lower compared to the non-exposed (control) offspring. The PCB-155 group gained more weight than the PCB-169 group (84.7% and 72.1% of that of the control group, respectively; p≤0.001) or than the group exposed to the combination of both PCBs (67.6%; p≤0.01). On PND 22, the PCB-169 group and the group exposed to the combination weighed less (p≤0.01) than the control and PCB-155 groups. The offspring of both sexes showed similar patterns of response to PCB treatment.

Craniofacial dimensions. The craniofacial dimensions of the offspring on PNDs 9 and 22 are presented in Table 2. Data from male and female offspring were combined for the statistical evaluation, as no significant sex-by-treatment interactions were observed. On PND 9, the PCB-169 group had significantly shorter skulls (p≤0.001) and narrower neurocranium (p≤0.05) than the control group. This reduction was observed in the more mature stages of PND 22 for skulls and mandibles (p≤0.001), but not for neurocranium. Therefore, on PND 22, PCB-169 exposed offspring had smaller ratios of skull length/neurocranium width (i.e.,

rounder skull) (p≤0.001) compared to the control group. On PND 9, the PCB-155 group had narrower neurocranium (p≤0.01) than the control group, while no significant treatment-related differences were observed for other craniofacial dimensions, and no differences were observed on PND 22. The group exposed to the combination of both PCBs had shorter skulls (p≤0.01), narrower neurocranium (p≤0.001), and shorter mandibles (p≤0.05) than the control group on PND 9. On PND 22, the reduction was still observed in the dimensions of skull and mandible length (p≤0.001) and neurocranium width (p≤0.01), and the skulls were rounder (p≤0.05) than in the control group. Compared to the PCB-155 group, on PND 9, only rounder skulls (p≤0.001) were detected in the PCB-169 group, but on PND 22, the PCB-169 group had shorter and rounder skulls and shorter mandibles (p≤0.001).

On PNDs 9 and 22, significantly longer skulls, wider neurocranium, and longer mandibles relative to body weight than in the control group were observed for the PCB-169 group (p≤0.001) and the group exposed to the combination of both PCBs (p≤0.01). The skull length/body weight ratio was higher (p≤0.01) in PCB-155 exposed offspring on PND 9, but this increase was not observed on PND 22.

Discussion

In the present study, we examined the adverse effects of two hexachlorobiphenyls corresponding to the non-planar (PCB-155) and planar (PCB-169) structure, individually or in combination, on the body weight gain and craniofacial growth of lactationally exposed rat offspring. PCB-169 adversely affected body weight gain and craniofacial growth until weaning on PND 22, alone and in combination with PCB-155 (Fig. 1 and Table 2). On PND 22, skulls and mandibles of the offspring exposed to PCB-169 and to the combination of both PCBs were shorter. In addition, their skulls were rounder, mimicking features expected in less-developed or younger animals. This result of the adverse effects of the dioxin-like PCB-169 on body weight gain and craniofacial growth is similar to results reported in previous studies where TCDD was administered (25, 31-33). TCDD treatment dose-dependently decreased body weight, mandibular length (31), and produced smaller skulls in rat offspring (32).

Likewise, TCDD exposure affected the shape and reduced the size of mandible in mouse offspring (33). In the study of Alaluusua and coworkers (25), TCDD was shown to impair growth resulting in shorter and narrower skulls in young adult rats.

Until PND 9, the PCB-155 exposed group gained less weight, although the weight gain was more than in the PCB-169 group (Table 2), and individual PCB-155 exposure decreased neurocranium width. No adverse effects of PCB-155 on body weight gain and craniofacial growth were observed from PND 9 to PND 22, and until PND 22, the PCB-155 group already gained 96.0% of the control group weight.

On PND 9, the group exposed to the combination of both PCBs gained less weight than the group exposed to PCB-169 alone, and craniofacial dimensions were reduced more when the offspring were exposed to both PCBs (Table 2). This trend suggests that PCB-155 in combination with PCB-169 may have an additive negative effect on growth rate, but was not sufficient to generate significance. This observation is consistent with results reported by Chu et al. (24), who found that growth suppression of adult rats was more pronounced in the group receiving TCDD in combination with a mixture of PCBs than with the TCDD alone.

The observed effect of PCBs on craniofacial bone growth is in agreement with several previous studies where evidence for PCB induced bone effects was found. After accidental exposure of human infants to PCBs and polychlorinated dibenzofurans (PCDFs), growth retardation and skull abnormalities were reported (9). In wildlife studies, Baltic seals exposed to polyhalogenated aromatic hydrocarbons such as PCBs suffered from severe skull bone loss (19). Furthermore, exposed adult herring gulls from heavily contaminated Great Lakes had shorter femurs, with lower mineral content and density (22). Similarly, chicks of tern collected from that area showed growth retardation, and cases of weak ossification and shortened mandibles, even lack of jaw or skull bones were reported (18). Embryos and chicks from eggs laid by hens that consumed a diet containing PCB contaminated carp had malformed brain cases and poorly ossified skull bones, as well as feet and leg deformities (12). Additionally, chronic exposure of Baltic seals (20) and deer mice to PCBs (23) was associated with reduced bone mineral density. In another study on East Greenland polar bears (21), subcutaneous

adipose tissue residues of polyhalogenated aromatic hydrocarbons, including total PCBs, were negatively associated with skull bone mineral density. Furthermore, Hodgson and coworkers (38) found a negative association between serum planar PCB-118 concentration and bone mineral density in Swedish men. On the other hand, the sum of the three most abundant non-planar PCBs was positively associated with bone mineral density.

In the environment, PCBs are always present as mixtures. It is not known if it is the planar or non-planar components in the mixture that cause adverse effects or whether there are synergistic or antagonistic effects of the simultaneous exposure to different compounds (30). Maternal exposure to commercial technical PCB mixture Aroclor 1254, where PCBs are present as mixtures of planar and non-planar congeners, among other organochlorines, resulted in growth suppression in rat offspring (28) and induced shorter, thinner and weaker bones in perinatally exposed rats (30). However, different adverse effects were produced after exposure to a complex mixture of 14 PCBs and 11 organochlorine pesticides, based on blood levels reported in exposed humans, where facial malformations characterized by a rounded skull and underdeveloped snout and lower jaw were observed (28). Thus, different kinds of PCB mixtures may have dissimilar effect on growth/bone, even though it was suggested that effects are mainly driven by the planar congeners (30).

In the present study, adverse effects of individual hexachlorobiphenyls were investigated, with non-planar (PCB-155) and planar (PCB-169) structure. Fewer studies have tested the effects of individual PCB congeners on bone growth and development. Exposure to planar PCB-126 was shown to result in decreased bone growth in nestling American kestrels (11), shorter bone length, impaired bone strength, and increased organic content in experimental rats (13). In addition, juvenile diamondback terrapins exposed to PCB-126 were smaller, with more juvenile cranial characteristics, their skulls had a higher organic content, and their femora had reduced bone mineral density (17). Decreased growth and differences in cranial form are similar to those found in the present study. But adverse bone effects for different types of PCBs are not yet well described. As an example, perinatal exposure to the planar PCB-118, as well as to the non-

planar PCB-153 has been reported to reduce bone length and affect bone composition in lambs (16). Another study demonstrated that perinatal exposure to PCB-153 resulted in altered bone composition in goat offspring (15). In contrast, planar PCB-126 did not induce marked bone effects (15). In the present study, adverse effects were mainly observed after exposure to the planar congener PCB-169. They were also elicited by the non-planar PCB-155, but less effectively (Table 2).

In the current study, lactating dams were exposed from PND 1. The total amount of administered PCB-169 was 3 mg/kg b.w. The corresponding concentration of toxic equivalents (TEQs), an estimate of the total TCDD-like activity (36) calculated by the concentration of PCB-169 multiplied by its TEF, was 90 μg TEQ/kg b.w. In a study with a comparable design, a similar dose of 50 μg TCDD/kg b.w. administered on PND 1 to lactating dams caused smaller and less mineralised skulls on PND 22 (32). In another study, in which TCDD was administered to dams on gestation day 9, just 0.5 μg TCDD/kg b.w. produced a significant decrease in mandible size and altered mandible shape in their offspring (33). However, an extremely high single dose of 1000 μg TCDD/kg b.w. administered to adult rats was required to cause impaired body weight gain and skull growth (25). Exposure from PND 1 onwards was selected in the present study to test the hypothesis that PCB lactational exposure would be harmful on offspring growth.

We examined ratios between craniofacial dimensions and body weight to determine which studied parameter i.e., body weight gain or craniofacial growth, is more susceptible to PCB exposure. On PNDs 9 and 22, due to their smaller body weights, all measured craniofacial dimensions relative to body weight were larger in the PCB-169 exposed group and the group exposed to the combination of both PCBs (Table 2). The results suggest that craniofacial growth was less affected by PCB-169, alone or in combination with PCB-155, than body weight gain. This finding is in contrast to results obtained by Hoffman and coworkers (11) on nestling American kestrels, where exposure to doses of PCB-126 that did not significantly decrease body weight, exhibited significantly shorter long bone length.

When the offspring were exposed to PCB-169 alone, skull and mandibular length were reduced more on PND 22 than on PND 9 (Table 2). The differences in adverse effects between PNDs 9 and 22 could also be attributed to PCB accumulation after long lactational PCB exposure. The blood levels of both PCBs increased from PND 9 to PND 22 in all three experimental groups (Table 1), which implies that accumulation of PCBs exceeded the growth of the offspring. The blood levels of the metabolically more stable and super lipophilic planar PCB-169 increased more than the levels of PCB-155. On PND 22, the offspring exposed to the combination of both PCBs had blood PCB-155 levels higher than the levels with exposure to individual PCBs and PCB-169 levels lower than those with exposure to individual PCBs. These results support the finding that when administered together, non-planar PCBs decrease the serum levels of dioxin-like organochlorines (39).

In conclusion, lactational exposure to PCB-169 negatively affected body weight gain and craniofacial growth in rat offspring until PND 22, alone and in combination with PCB-155. Until PND 9, PCB-155 also decreased neurocranium width and body weight gain, but did so less effectively than PCB-169. Evidence is also presented that PCB-155 in combination with PCB-169 may have an additive negative effect on growth rate in early postnatal life based on body weight gain and craniofacial measurements.

Acknowledgements

References

1. Larsen JC. Risk assessments of polychlorinated dibenzo- p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in food. Mol Nutr Food Res 2006; 50: 885–96.

2. Giesy JP, Kannan K. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit Rev Toxicol 1998; 28: 511–69.

3. Safe S, Bandiera S, Sawyer T, et al. PCBs: structure-function relationships and mechanism of action. Environ Health Perspect 1985; 60: 47–56.

4. Patandin S, Dagnelie PC, Mulder PG, et al. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: A comparison between breast-feeding, toddler, and long-term exposure. Environ Health Perspect 1999; 107: 45–51.

5. Ošt'ádalová I, Babický A. Periodization of the early postnatal development in the rat with particular attention to the weaning period. Physiol Res 2012; 61 (Suppl 1): S1–7.

6. Chen YC, Yu ML, Rogan WJ, et al. A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-cheng children. Am J Public Health 1994; 84: 415–21.

7. Guo YL, Lambert GH, Hsu CC. Growth abnormalities in the population exposed in utero and early postnatally to polychlorinated biphenyls and dibenzofurans. Environ Health Perspect 1995; 103 (Suppl 6): 117–22.

8. Patandin S, Koopman-Esseboom C, de Ridder MA, et al. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 1998; 44: 538–45.

9. Miller RW. Congenital PCB poisoning: a re-evaluation. Environ Health Perspect 1985; 60: 211–4.

10. Valvi D, Mendez MA, Martinez D, et al. Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. Environ Health Perspect 2012; 120: 451–7.

11. Hoffman DJ, Melancon MJ, Klein PN, et al. Developmental toxicity of PCB 126 (3,3',4,4',5-pentachlorobiphenyl) in nestling American kestrels (Falco sparverius). Fundam Appl Toxicol 1996; 34: 188–200.

12. Summer CL, Giesy JP, Bursian SJ, et al. Effects induced by feeding organochlorine-contaminated carp from Saginaw Bay, Lake Huron, to laying White Leghorn hens. II. Embryotoxic and teratogenic effects. J Toxicol Environ Health 1996; 49: 409–38.

13. Lind PM, Larsson S, Oxlund H, et al. Change of bone tissue composition and impaired bone strength in rats exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB126). Toxicology 2000; 150: 41–51.

14. Render JA, Aulerich RJ, Bursian SJ, et al. Proliferation of maxillary and mandibular periodontal squamous cells in mink fed 3,3',4,4',5-pentachlorobiphenyl (PCB 126). J Vet Diagn Invest 2000; 12: 477–9.

15. Lundberg R, Lyche JL, Ropstad E, et al. Perinatal exposure to PCB 153, but not PCB 126, alters bone tissue composition in female goat offspring. Toxicology 2006; 228: 33–40.

16. Gutleb AC, Arvidsson D, Orberg J, et al. Effects on bone tissue in ewes (Ovies aries) and their foetuses exposed to PCB 118 and PCB 153. Toxicol Lett 2010; 192: 126–33.

17. Holliday DK, Holliday CM. The effects of the organopollutant PCB 126 on bone density in juvenile diamondback terrapins (Malaclemys terrapin). Aquat Toxicol 2012; 109: 228–33.

18. Gilbertson M, Kubiak T, Ludwig J, Fox G. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J Toxicol Environ Health 1991; 33: 455–520.

19. Olsson M, Karlsson B, Ahnland E. Diseases and environmental contaminants in seals from the Baltic and the Swedish west coast. Sci Total Environ 1994; 154: 217–27.

20. Lind PM, Bergman A, Olsson M, Örberg J. Bone mineral density in male Baltic grey seals (Halichoerus grypus). Ambio 2003; 32: 385–8.

21. Sonne C, Dietz R, Born EW, et al. Is bone mineral composition disrupted by organochlorines in east Greenland polar bears (Ursus maritimus)? Environ Health Perspect 2004; 112: 1711–6.

22. Fox GA, Lundberg R, Wejheden C, et al. Health of herring gulls (Larus argentatus) in relation to breeding location in the early 1990s. III. Effects on the bone tissue. J Toxicol Environ Health A 2008; 71: 1448–56.

23. Johnson KE, Knopper LD, Schneider DC, Ollson CA, Reimer KJ. Effects of local point source polychlorinated biphenyl (PCB) contamination on bone mineral density in deer mice (Peromyscus maniculatus). Sci Total Environ 2009; 407: 5050–5.

24. Chu I, Lecavalier P, Håkansson H, et al. Mixture effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and polychlorinated biphenyl congeners in rats. Chemosphere 2001; 43: 807–14.

25. Alaluusua S, Lukinmaa PL, Pohjanvirta R, et al. Exposure to 2,3,7,8-tetrachlorodibenzo-para-dioxin leads to defective dentin formation and pulpal perforation in rat incisor tooth. Toxicology 1993; 81: 1–13.

26. Render JA, Hochstein JR, Aulerich RJ, et al. Proliferation of periodontal squamous epithelium in mink fed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Vet Hum Toxicol 2000; 42: 85–6.

27. King-Heiden TC, Mehta V, Xiong KM, et al.

Reproductive and developmental toxicity of dioxin in fish. Mol Cell Endocrinol 2012; 354: 121–38.

28. Bowers WJ, Nakai JS, Chu I, et al. Early developmental neurotoxicity of a PCB/organochlorine mixture in rodents after gestational and lactational exposure. Toxicol Sci 2004; 77: 51–62.

29. Chu I, Bowers WJ, Caldwell D, et al. Toxicological effects of in utero and lactational exposure of rats to a mixture of environmental contaminants detected in Canadian Arctic human populations. J Toxicol Environ Health A 2008; 71: 93–108.

30. Elabbas LE, Herlin M, Finnilä MA, et al. In utero and lactational exposure to Aroclor 1254 affects bone geometry, mineral density and biomechanical properties of rat offspring. Toxicol Lett 2011; 207: 82–8.

31. Kattainen H, Tuukkanen J, Simanainen U, et al. In utero/lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure impairs molar tooth development in rats. Toxicol Appl Pharmacol 2001; 174: 216–24.

32. Lukinmaa PL, Sahlberg C, Leppäniemi A, et al. Arrest of rat molar tooth development by lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 2001; 173: 38–47.

33. Allen DE, Leamy LJ. 2,3,7,8-tetrachlorodibenzo-p-dioxin affects size and shape, but not asymmetry, of mandibles in mice. Ecotoxicology 2001; 10: 167–76.

34. Vrecl M, Uršič M, Pogačnik A, et al. Excretion pattern of co-planar and non-planar tetra- and hexa-chlorobiphenyls in ovine milk and faeces. Toxicol Appl Pharmacol 2005; 204: 170–4.

35. Jan J, Uršič M, Vrecl M. Levels and distribution of organochlorine pollutants in primary dental tissues and bone of lamb. Environ Toxicol Pharmacol 2013; 36: 1040–5.

36. Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 2006; 93: 223–41.

37. Ballschmiter K, Zell M. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fresenius Z Anal Chem 1980; 302: 20–31.

38. Hodgson S, Thomas L, Fattore E, et al. Bone mineral density changes in relation to environmental PCB exposure. Environ Health Perspect 2008; 116: 1162–6.

39. Van der Plas SA, de Jongh J, Faassen-Peters M, et al. Toxicokinetics of an environmentally relevant mixture of dioxin-like PHAHs with or without a non-dioxin-like PCB in a semi-chronic exposure study in female Sprague Dawley rats. Chemosphere 1998; 37: 1941–55.

VPLIV NEPLANARNEGA PCB-155 IN PLANARNEGA PCB-169 V MATERINEM MLEKU NA RAST TELESNE MASE IN KRANIOFACIALNEGA PODROČJA PRI SESNIH MLADIČIH PODGANE

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Povzetek: V raziskavi smo ugotavljali škodljive vplive dveh polikloriranih bifenilov (PCB) v materinem mleku, neplanarnega PCB-155 in planarnega PCB-169, posamič ali v kombinaciji, na rast telesne mase in kraniofacialnega področja pri sesnih mladičih podgane, ki so bili izpostavljeni vplivom polikloriranih bifenilov v zgodnjem postnatalnem obdobju. Odraslim podganjim samicam seva wistar (n=15) smo po kotitvi intraperitonealno vbrizgali skupno 12 mg/kg t.t. PCB-155 (skupina 1, n=4), 3 mg/kg t.t. PCB-169 (kar ustreza 90 μg toksičnega ekvivalenta (TEQ)/kg t.t.) (skupina 2, n=4), ali kombinacijo PCB-169 in PCB-155 (skupina 3, n=3). Četrta skupina (n=4) je bila kontrolna. Mladiče smo žrtvovali 9. in 22. dan po skotitvi. Izmerili smo telesne mase in dolžine kraniofacialnega področja. Vsi izpostavljeni mladiči so bili 9. dan po skotitvi lažji (p≤0.001) od mladičev v kontrolni skupini, skupini 2 (p≤0.001) in 3 (p≤0.01) sta bili lažji kot skupina 1. V starosti 22 dni sta bili lažji kot kontrolna skupina le skupini 2 in 3 (p≤0.001). V starosti 9 dni smo izmerili ožji nevrokranij pri skupinah 1 (p≤0.01), 2 (p≤0.05) in 3 (p≤0.001). Lobanje so bile krajše pri skupinah 2 (p≤0.001) in 3 (p≤0.01), škodljivi vpliv na rast lobanje je bil viden tudi v starosti 22 dni (p≤0.001). Pri 22 dneh so imeli mladiči bolj okrogle lobanje v skupinah 2 (p≤0.001) in 3 (p≤0.05) in tudi krajše mandibule (p≤0.001). Na podlagi predstavljenih rezultatov je mogoče sklepati, da se škodljivi vplivi PCB-155 in PCB-169 na rast seštevajo. Dobljeni rezultati kažejo, da je izpostavljenost PCB-155 in PCB-169 preko materinega mleka negativno vplivala na rast telesne mase in kraniofacialnega področja pri sesnih mladičih podgane do 9. dneva po skotitvi. Škodljivi vpliv PCB-169 je bil močnejši in je trajal vse do 22. dneva.

Ključne besede: poliklorirani bifenil; materino mleko; telesna masa; rast kraniofacialnega področja; podgana