Polish J. of Environ. Stud. Vol. 17, No. 4 (2008), 587-596

Original Research

Brain Catecholamine Concentrations in Adult Rats Exposed Perinatally to Methylmercury and/or PCB 153

R. Świercz*, Z. Grzelińska, W. Majcherek, D. Wiaderna, P. Lutz, K. Sitarek, W. Wąsowicz, S. Gralewicz

Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Św. Teresy 8, 91-348 Łódź, Poland

Received: 28 September, 2007
Accepted: 25 March, 2008

Abstract

The vulnerability of the nervous system to toxic insult is particularly high during foetal life and early childhood. Exposures during this period, via maternal blood and/or milk, may result in neurobehavioural disorders, some of which may not become apparent before in adulthood. Methylmercury (MeHg) and polychlorinated biphenyls (PCB) are persistent environmental pollutants and may be present in some food products. Both are neurotoxic. It is suspected that MeHg and PCBs may act synergistically in inducing neurotoxic effects. Some data suggest that catecholaminergic systems, especially the dopaminergic one, are particularly vulnerable to the harmful action of MeHg and PCBs. This study aimed to assess the influence of separate or combined perinatal exposure to MeHg and PCB 153 on brain catecholamine contents in maturity.

The subjects were adult (90-94 days of age) rats, progeny of mothers exposed to methylmercury (MeHg, CH_3HgCl) or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), or MeHg and PCB 153, from day 7 of gestation to day 21 post partum. MeHg was administered with drinking water at daily doses of 0.5 mg/kg b.w./day. PCB 153 was administered by gavage at daily doses of 5 mg/kg b.w./day. The concentrations of adrenaline (A), noradrenaline (NA), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by HPLC in homogenates of the following brain regions: olfactory bulb, hippocampus, striatum, occipito-temporal cortex, diencephalon, mesencephalon and cerebellum.

The results suggest some exposure-related alterations in amine contents and point to the significant role of gender in their development. In the female MeHg-exposed progeny, the content of NA, DA and DOPAC in the mesencephalon was significanrtly elevated (by 43.1, 68.9 and 65.1%, respectively) while in the male progeny no differences were noted in any region of the brain. On the other hand, in the progeny exposed to PCB 153, some differences, i.e. increased NA concentration in the olfactory bulb (by 59.9%) and hippocampus (by 124.5%) and increased DA (by 75.0%) in the striatum were found in males, whereas in females significant differences were not found. In the case of combined exposure, the data suggest some effects in the female progeny only: a decreased concentration of A in the hippocampus (by 40.9%) but increased in the stratum (by 53.1%) and an increased DA concentration in the mesencephalon (by 78.9%). Summing up, the results confirm that gender may be an important determinant of the rat's vulnerability to MeHg and PCB153. They provide no evidence, however, of a synergism in the action of these neurotoxicants when given perinatally on the brain catecholaminergic systems in adulthood. A rough analysis of the obtained data allows one to suspect an antagonistic rather than synergistic type of interaction.

Keywords: neurotransmitters, catecholamine, noradrenaline, adrenaline, dopamine, 3,4-dihydroxyphenylacetic acid, MeHg, PCB 153, brain areas, rats

*e-mail: radek@imp.lodz.pl

Introduction

Methylmercury (MeHg) and polychlorinated biphenyls (PCB) are ubiquitous and persistent environmental pollutants. They enter the food chain and may be present and co-present in some food products, especially marine food (fish, shellfish and sea mammals). They are toxic to the nervous system. The vulnerability of the nervous system to toxic insults is the highest at early developmental stages [1]. Owing to this, consumption of marine food during pregnancy and breast feeding may endanger the development of the child's nervous system.

Results of the epidemiologic studies obtained so far are ambiguous. For example, studies of children from the Faroe Islands yielded results suggesting that maternal diet based largely on seafood may detrimentally affect a child's neurodevelopment. Contrasting results have been obtained in studies of a children's cohort from the Seychelles Islands (the Seychelles Child Developmental Study) in spite of the fact that, as suggested by the maternal hair Hg concentration, MeHg exposure was, on average, higher in the Seychelles than in the Faroe Islands (6.8 vs. 4.27 ppm, respectively) [2, 3]. According to some authors these inconsistencies may be due to differences in the kind of consumed seafood in and possible co-exposure to other neurotoxicants. Whereas the Seychellois populations eat mainly fish which contain MeHg, the mothers from the Faroe Island also eat whale meat and blubber, which contain PCBs. Thus, it has been hypothesized that the co-exposure to MeHg and PCBs may contribute to the incidence of adverse effects in children from the Faroe Islands but not in children from the Seychelles [4]. The validity of this assumption finds support in the results of some in vitro studies indicating that PCB and MeHg may act synergistically and that the effect of combined exposure may be stronger than the sum of effects produced by each of these compounds alone [5, 6]. An interaction of MeHg and PCB has also been suggested by some epidemiological studies [7, 8], and in vivo animal experiments [9].

Some reports, however, do not confirm MeHg-PCB synergism. Thus, for example, Castoldi et al. [10] assessed aminergic transmission in a 21-day progeny of rat mothers exposed to MeHg (1.0 mg/kg/day, from GD7 to PND21) and/or PCB 153 (20 mg/kg/day from GD10 to GD 16). Depending on the analyzed endpoint, the effects of the combined exposure did not differ significantly from those noted in rats exposed to MeHg or PCB 153 alone. No differences suggesting synergism were noted. Studies by Vettori et al. [11], on PC12 cells, show that the effects of the combined exposure to PCB153 and MeHg may be significantly weaker than the effects of separate exposures, indicating an antagonistic effect of those two chemicals. Considering the above, discrepancies and a significant contribution of frutti di mare (which may contain both contaminants) to the diet, further research intended to elucidate the character of MeHg-PCB interaction is necessary.

The present study aimed to assess the influence of separate or combined perinatal exposure to MeHg and a selected PCB congener on brain catecholamine contents in maturity. Of the 135 PCB congeners detected in environmental samples and biological specimens, PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl) is the congener which is always present at the highest concentration and constitutes a large portion of the quantified PCBs [12]. Therefore, PCB 153 was the congener selected for the present study.

Data obtained in animal studies suggest that prenatal low-level exposure to MeHg may result in altered content of catecholamines and their turnover in certain brain regions, and that these effects become apparent after weaning [13]. PCB 153 is a di-ortho substituted noncoplanar PCB congener and there is evidence that perinatal exposure to ortho-substituted PCB congeners results in a decreased concentration of dopamine (DA) in some areas of the rat brain [14]. Alterations in catecholaminergic functions are also suggested by some behavioural observations done in animals perinatally exposed to MeHg [15] or PCBs [16]. If MeHg and PCB act synergistically to affect catecholaminergic functions, the alterations in the catecholamine concentrations (if any) produced by a combined exposure should be considerably more evident than the alterations produced by separate exposures to these compounds.

Materials and Methods

Animals

Adult (90- to 96-day) white Wistar rats, both genders, born to females exposed during pregnancy and breast feeding to the selected neurotoxicants were used as the material for the experiment. Female rats were exposed from day 7 of pregnancy up to day 21 after delivery to MeHg (CH_3HgCl, CAS No: 115-09-3, from ALDRICH, Cat. No. 442534-5G-A) in drinking water, or PCB 153 (2,2',4,4',5,5'-Hexachlorobiphenyl, CAS No: 35065-27-1, supplied by Fluka, Cat. No. 35602) by gavage, or to both these neurotoxicants in combination, at predetermined daily doses. In the control groups, the females received the vehicle (water was given to the MeHg controls, while the PCB 153 controls received maize oil. To determine possible developmental effects at the early stages of life, the progeny underwent routine examinations and a series of tests (negative geotaxis, forepaw suspension, free-fall righting, pinna detachment, incisor eruption and eye opening). Detailed descriptions of animal breeding conditions, mating procedures, monitoring of pregnancy, exposure conditions, maternal health conditions and the results of the assessment of developmental effects in the progeny during the preweaning period of life will be available in a separate report. After weaning (on PND 21), the animals, divided into gender/exposure groups, were kept undisturbed (except for the routine procedures, including weekly weighing and change of bedding material) in cages (4 animals per cage) until about the 75th day of life. After that time, the animals were divided into cohorts as required by the experiments to be performed and transferred into single cages, where they were kept undisturbed until the time of the experiments. During the classification into the cohorts, care

was taken to ensure that animals from the same litter were distributed to different groups.

The animals used for assessment of the brain content of catecholamines consisted of three cohorts: the MeHg cohort, the PCB cohort and the MeHg+PCB cohort. The MeHg cohort consisted of 7 females and 8 males born to mothers of the control (unexposed) group and 11 females and 11 males born to mothers of the MeHg-exposed group (0.5 mg/kg/day). The PCB cohort consisted of 12 females and 12 males born to mothers of the control group, and 8 females and 7 males born to mothers of the PCB exposure group (5.0 mg/kg/day). The MeHg+PCB cohort consisted of 10 females and 10 males born to mothers of the control group and 10 females and 10 males born to mothers exposed to PCB 153 (5.0 mg/kg/day) and MeHg (0.5 mg/kg/day). Observations during the period since birth until weaning did not reveal significant effects of exposure in the offspring of groups exposed to MeHg (0.5 mg/kg/day) alone, or to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day). Some effects, such as accelerated growth rate but reduced muscular strength and endurance, were noted only in the male pups born to females exposed to PCB 153 (5.0 mg/kg/day) alone. On the day of the selection, significant differences in body mass were not recorded between groups within each gender, irrespective of exposure type.

Sacrifice, Brain Dissection and Tissue Preprocessing

At the day of sacrifice the animals were at 90–97 days of age. The sacrifice (decapitation) took place between 8 and 11 a. m. The brains were removed from skulls as quickly as possible, chilled in ice-cold physiological saline for approximately 5 min and sectioned on ice into the following parts: olfactory bulb, hippocampus, striatum (n. caudatus and putamen), occipito-temporal cortex (with underlying white matter), diencephalon, mesencephalon and cerebellum. Corresponding tissue samples from right and left hemisphere were pooled, weighed (with 0.001 g accuracy), supplemented with five parts of ice-cold water (HPLC) and homogenized in the Ultra – Turrax T 8 (IKA – WERKE) homogenizer for 30 s. Homogenate samples (450 µl) were supplemented with 50 µl of 4 M $HClO_4$ and centrifuged at 15,000 rpm, 4ºC for 15 min. The supernatants were filtered through 0.22 µm GV (Millipore, Bedford, Mass., USA) and stored at –20ºC until analysis.

The brain concentrations of noradrenaline (NA), adrenaline (A) dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by HPLC.

Apparatus

Tissue amine concentrations were quantified using high performance liquid chromatography (WATERS) with electrochemical detection using a glassy carbon electrode set at + 0.8 V and an Ag/AgCl reference electrode. The chromatography was performed using a reversed-phase column Discovery® C 18, 15 cm x 4.6 mm, 5µm (SUPELCO) eluted in isocratic mode with a mixture of phosphoric buffer (150 mM, pH 3.4), 1-octanesulfonic acid (0.5 mM), EDTA (0.1mM), sodium chloride (5 mM), methanol (12%) at a flow-rate of 0.4 ml/min. The reagents for mobile phase were of HPLC grade and all other reagents were of analytical grade. Dihydroxybenzamine was used as an internal standard.

Drugs and Reagents Used for the HPLC Assays

Ethylenediaminetetraacetic Acid (EDTA), 1-octanesulfonic acid (Approx 98%), Trizma® base (minimum 99.9% titration), Trizma® hydrochloride, (±)-arterenol hydrochloride (NA), (-)-epinephrine (+)bitartrate salt (A), dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and aluminum oxide (type WN-3 neutral) were purchased from SIGMA. Acetic Acid (99-100%), perchloric Acid (70 – 72%), phosphoric acid (85%), potassium phosphate (monobasic ultrapure), methanol and sodium chloride (ultrapure) were purchased from J.T. BAKER. 3,4-Dihydroxybenzylamine hydrobromide (98%) was purchased from ALDRICH.

Procedure

DOPAC was assayed directly in the supernatant. Prior to chromatographic analysis noradrenaline, adrenaline and dopamine were purified by absorption on aluminum oxide [17, 18, 19, 20]. Twenty mg of aluminum oxide, 200 µl of the supernatant, 10 µl of the internal standard (concentration: 2.5ng/ml) and 1.0 ml of 2 M Tris buffer – were shaken on vortex for 2 min., and centrifuged for 10 min. at 12,000 rpm. The supernatant was decanted, shaken with the addition of 1.0 ml of cold deionized water for 2 min., centrifuged for 2 min. and decanted again. This sequence was repeated three times. After the last centrifuge, in order to extract the catecholamines, 200 µl of 2% acetic acid were added to the sediment. The sample was shaken for 5 min. and then centrifuged for 2 min. at 12,000 rpm. For analysis, 20 µl of the supernatant were injected on the chromatographic column.

Preparation of Standards

The stock standard solutions were prepared by solving 10 mg of each standard in 10 ml of 0.4M HClO4. Standards were prepared by thinning the stock solutions to the required concentrations: in the range of 1–100 ng/ml for noradrenaline, adrenaline and dopamine, and 2–200 ng/ml for 3,4-dihydroxyphenylacetic acid.

Statistical Analysis

Tissue concentrations of the determined substances were determined in terms of ng/g tissue. The variability of data obtained from each brain region in each group was high. In order to reduce the variability, in each group and in each brain region two scores (assays), the highest and the

lowest one, were excluded from analysis. Individual data (values of amine concentrations) were normalized by expressing them as percentages of the mean control value.

Statistical comparisons were performed with the use of the Kruskall-Wallis one-way ANOVA. Scheffe's test was used for detailed, multiple comparisons. Differences were regarded as significant when the probability of the null hypothesis was < 0.05.

Results

Masses of body and brain from animals are given in Table 1. Compared with controls, no statistically significant differences were found either in brain weight, body weight and the body weight/brain weight ratios of animals exposed to MeHg and /or PCB 153.

Despite the exclusion of the highest and the lowest score from the raw data, the variability of the determination values – the direct as well as indirect ones – within each brain region and in each group remained very high. Results of determinations of the noradrenaline (NA), adrenaline (A), dopamine (DA) and 3,4-dihydrophenylacetic acid (DOPAC) concentrations in the brain regions studied are presented in Table 2 (females) and Table 3 (males).

Effects of Exposure to MeHg Alone

In females of the exposed group, in diencephalon the concentrations of NA, DA and DOPAC were significantly increased (by 43.1%, 68.9% and 65.1%, respectively) compared to the control group. A marked increase in NA concentration (by 44.7%) was noted also in the cortex but, due to the high individual variability, this difference appeared insignificant statistically. In males, significant differences between groups were not found in any of the brain regions investigated. The only notable, but insignificant difference was a reduced (by 36.8%) DA concentration in the midbrain.

Effects of Exposure to PCB 153 Alone

In females, the concentrations of NA, A, and DA in the striatum appeared markedly increased (by 70.4%, 47.0%, and 69.6%, respectively). The NA concentrations were also markedly increased in the olfactory bulb (by 76.6%), cortex (by 46.0%), and the cerebellum (by 43.3%). Notable was also a decrease in the DA concentration in the diencephalon and mesencephalon (by 37.8% and 39.7%, respectively, and a decrease in the DOPAC concentration in the hippocampus (by 41.4%). None of the above-mentioned differences, however, appeared statistically significant. In males, subjects of the exposed group differed significantly from controls in their NA content in the olfactory bulb (by 59.9% higher in the exposed group) and in the hippocampus (by 124.5% higher in the exposed group), and in their DA concentration in the striatum (by 75.0% higher in the exposed group). Notable, but statistically insignificant were the increases in the NA concentration in the mesencephalon (by 55.0%) and the cerebellum (by 43.6%), and a decrease in the DOPAC concentration in the hippocampus (by 39.8%).

Effects of Exposure to MeHg + PCB 153

Compared to females of the control group, the exposed females differed significantly in the A concentration in the hippocampus (decreased by 40.9%), the A concentration in the striatum (increased by 53.1%), and the DA concentration in the mesencephalon (increased by 78.9%). Other worthy consideration differences between the control and the exposed females were not observed. In case of the male

Table 1. Brain weight (means ± SD) and body mass of rats from which the biological material was collected.

Treatment (n = animals per treatment)		Body weight (g)	Brain weight (g)	$\text{Quotient} = \frac{\text{Body weight (g)}}{\text{Brain weight (g)}}$
female	Control (n=7)	235.7 (13.0)	1.88 (0.03)	125.3 (7.1)
	MeHg (n=11)	242.3 (20.2)	1.78 (0.08)	138.3 (9.7)
	Control (n=12	226.7 (16.7)	1.80 (0.13)	125.1 (9.7)
	PCB 153 (n=8)	240.6 (17.6)	1.86 (0.09)	128.8 (8.7)
	Control (n=10)	237.0 (13.0)	1.93 (0.13)	123.2 (14.0)
	MeHg and PCB 153 (n=10)	229.5 (15.9)	1.90 (0.06)	124.9 (8.7)
male	Control (n=8)	383.1 (18.1)	1.98 (0.04)	193.2 (9.5)
	MeHg (n=11)	374.2 (24.7)	1.88 (0.09)	201.5 (18.6)
	Control (n=12)	381.3 (28.1)	1.99 (0.08)	186.5 (12.9)
	PCB 153 (n=7)	371.4 (16.5)	1.91 (0.11)	195.0 (13.9)
	Control (n=10)	379.5 (13.8)	2.07 (0.19)	181.5 (20.2)
	MeHg and PCB 153 (n=10)	370.0 (21.5)	2.01 (0.09)	186.2 (13.4)

progeny, the only marked, but not significant statistically, difference was an increase, by 82.4%, of the DA concentration in the cortex.

Discussion

Results presented above point to some changes occurring in the catecholaminergic system of brains of the adult rats exposed perinatally to MeHg, PCB 153 separately or in combination. However, considerable inter-individual differences and the resultant doubts about the reliability of the results of our statistical analyses prevent precise conclusions on the profile and location of the changes produced by each studied neurotoxin or on the character of possible interactions.

Several reports are accessible in the literature on the levels of catecholamines in rat brain. However, they differ significantly from our present work both in exposure ranges and in the age of the animals. From the viewpoint of the subject of our current work, most essential are the observations on DA levels. In our present study, like in reports by some other authors [21-23], striatum was the region where the concentration of that amine and its metabolite was highest. However, the absolute values quoted by various authors differ fundamentally. Thus, according to Tsuzuki et al. [21], mean DA concentration in the striatum of the control rats was 12270 ng/g, according to Zahalka et al. [22] it is ca. 5000 ng/g (value read from the diagrams), while according to Rea et al. [23] it is estimated at 1899 ng/g. Those values relate to ca. 2-month rats, both genders [22] or males only [21, 23]. In our present study, mean striatal DA values of the control groups were about four times lower than those reported by Tsuzuki et al. [21] and about two times lower than in Zahalka et al. [22], but nearly twice higher than those specified by Rea et al. [23] (Tables 2 and 3). It is worth noting that, judging from the description, Rea et al. [23] and Tsuzuki et al. [21] used the same (spectrofluorometric) method, but different rat strains (Spraque-Dawleys were used by Rea et al. [23] and Wistars by Tsuzuki et al. [21]). Zahalka et al. [22] used Long-Evans rats. In our experiment we used the same method (HPLC with electrochemical detection) which was used by Zahalka et al. [22] but the subjects were outbred Wistar rats from our own breeding colony. The above comparison shows that a) values of striatal DA determinations obtained in our study are within the range of the corresponding values specified by other authors and, therefore, are reliable, and b) differences in the experimental material (animal strain, origin and breed) are the main source of discrepancies in the results from different laboratories. In each individual experiment of our present study, the control rats came from the same cohort as the exposed animals and, until they were sacrificed, were handled exactly in the same way as the progeny of the exposed mothers. Thus, it is reasonable to use the values determined in the control groups as the reference values for those determined in the exposed groups.

If we consider only the statistically significant differences, then the results (Table 4) can be summarized as follows: Exposure to MeHg results in some changes in the female rats (higher mesencephalic Na, DA and DOPAC levels), while it does not affect catecholamine levels in the males. Exposure to PCB 153 causes some changes (higher NA in the olfactory bulb and hippocamus, and increased DA in the striatum) in the males, while catecholamine levels in the female rats are not affected by exposure. The combined exposure to both chemicals does not produce changes in the male rats, while it does produce changes in the female rats (higher striatal A and mesencephalic DA levels) in regions different than in the case of MeHg exposure. The above may indicate a) a significant role of gender in the determination of the vulnerability to each of the two neurotoxins (MeHg affects mainly female progeny whereas PCB 153 affects mainly male progeny) and b) that copresence of MeHg may make the males less susceptible to PCB 153. When we consider the marked (~>40% or more), albeit statistically insignificant changes, the above picture becomes somewhat more complicated, particularly for PCB 153 results. When we look at the results in Table 4, we feel that the role of gender in determining susceptibility to PCB 153 is not so important; in females, the number of locations with marked changes is nearly the same as in males. Besides, in some areas, the trend of the changes was the same in both genders (increased striatal DA snd lower hippocampal DOPAC). It is also interesting to note the evidently opposite changes of DA and DOPAC levels in the mesencephalon of the females exposed to MeHg (increase) and those exposed to PCB 153 (reduction), and n changes in females exposed to the combination of both chemicals.

It is generally recognized that the functional state of the catecholaminergic systems, and of the dopaminergic system in particular, significantly affects behaviour. [24]. Thus, it would be reasonable to expect the exposure-related changes within those systems to correlate with the behavioural changes. Behavioural effects of separate and combined exposures to MeHg and PCB 153 were the subject of another study. The results of that study revealed a number of effects. However, the association between those effects and the changes described in our present study is not clear.

It was demonstrated, for example, that perinatal MeHg (0.5 mg/kg/day) exposure results in lower spontaneous motor activity in the females and increased susceptibility to behavioural sensitization in the males. PCB 153 (5.0 mg/kg/day) exposure, on the other hand, resulted in a higher motor activity in the females and an impairment of sensor motor coordination (poorer rota-rod performance) in the males. None of these effects occurred after the combined exposure to these two neurotoxins in either male or female rats [Wiaderna D. et al. and Lutz P. et al. – in preparation].

At present no literature data are available which could be directly compared with our data. Reports on exposure to MeHg suggest that high (toxic) doses administered at an early postnatal period result in increased tissue concentrations of 5-HT and 5-HIAA, and increased NE and DA concentrations. However, those effects were studied shortly after weaning, and their persistency was not determined [25].

Table 2. Brain contents of noradrenaline (NA), adrenaline (A), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC), in adult female progeny of rat mothers exposed separately or jointly to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day) during gestation and breast feeding.

Brain region	Exposure group	NA		A		DA		DOPAC	
		Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)
Olfactory bulb	MeHg	67.6 (30.5)	105.8 (43.3)	31.5 (20.2)	78.5 (21.2)	29.4 (16.3)	96.8 (17.5)	21.4 (6.29)	115.9 (12.8)
	PCB 153	53.3 (20.4)	176.6 (111.4)	26.1 (9.8)	127.8 (85.4)	26.6 (18.7)	130.44 (50.3)	32.9 (7.3)	78.0 (25.4)
	MeHg + PCB 153	93.4 (21.3)	95.0 (15.9)	21.3 (2.8)	110.5 (14.8)	37.5 (14.6)	120.4 (28.5)	22.5 (5.9)	91.7 (29.4)
Cortex	MeHg	62.6 (28.1)	144.7 (55.7)	21.2 (5.1)	133.5 (27.5)	30.4 (9.7)	85.5 (31.4)	55.0 (30.9)	67.8 (20.1)
	PCB 153	72.9 (30.3)	146.0 (73.9)	26.9 (12.8)	129.9 (32.9)	22.6 (10.6)	139.3 (44.4)	46.7 (22.4)	101.0 (47.1)
	MeHg + PCB 153	104.4 (13.0)	123.5 (37.0)	30.2 (15.2)	98.7 (42.9)	43.6 (22.8)	139.8 (113.2)	22.8 (9.7)	117.8 (53.6)
Hippocampus	MeHg	81.9 (22.0)	99.7 (40.5)	27.5 (9.4)	82.5 (32.9)	205.4 (53.7)	93.5 (28.7)	68.9 (13.5)	118.2 (46.4)
	PCB 153	84.2 (38.8)	87.0 (44.4)	23.3 (13.2)	119.0 (38.0)	135.9 (73.0)	81.6 (30.1)	96.9 (48.2)	58.6 (27.1)
	MeHg + PCB 153	129.3 (18.2)	93.4 (16.5)	25.6 (6.3)	59.1 (18.7)*	114.5 (29.7)	134.5 (54.6)	46.8 (9.0)	116.7 (42.1)
Striatum	MeHg	116.7 (60.2)	83.2 (31.2)	31.7 (5.7)	82.3 (16.1)	3247.6 (1417.2)	125.8 (60.5)	784.3 (112.4)	120.4 (31.7)
	PCB 153	95.2 (70.0)	170.4 (98.4)	25.8 (13.6)	147.0 (62.7)	2837.1 (1813.5)	169.6 (58.5)	801.0 (184.5)	84.1 (27.8)
	MeHg + PCB 153	149.9 (16.0)	127.2 (39.2)	25.0 (6.7)	153.1 (40.7)*	2235.8 (727.7)	117.3 (65.1)	840.4 (238.4)	81.2 (24.4)
Diencephalon	MeHg	115.2 (17.1)	143.1 (45.0)*	30.3 (10.0)	115.5 (46.2)	117.7 (20.1)	168.9 (77.2)*	35.0 (10.7)	165.1 (69.5)*
	PCB 153	126.2 (86.2)	118.2 (30.0)	31.5 (13.6)	70.7 (22.2)	100.6 (65.5)	62.2 (23.0)	42.8 (17.4)	62.5 (32.6)
	MeHg + PCB 153	125.1 (21.6)	110.4 (17.1)	30.2 (10.4)	96.3 (21.0)	73.0 (34.1)	111.2 (53.3)	32.2 (8.6)	88.7 (23.4)
Mesencephalon	MeHg	85.7 (29.9)	106.3 (38.5)	30.6 (8.4)	102.4 (56.4)	54.8 (14.0)	113.0 (81.3)	26.5 (5.5)	113.9 (32.5)
	PCB 153	91.9 (62.4)	113.3 (26.7)	27.1 (16.2)	94.4 (25.1)	32.0 (25.9)	60.3 (19.3)	47.4 (18.9)	78.5 (28.8)
	MeHg + PCB 153	101.4 (26.9)	101.2 (16.3)	31.1 (11.0)	111.4 (38.2)	34.9 (13.0)	178.9 (88.7)*	19.5 (4.8)	111.3 (23.4)
Cerebellum	MeHg	83.4 (16.4)	106.7 (38.9)	36.4 (13.5)	86.8 (35.1)	23.4 (11.3)	125.2 (33.4)	14.0 (0.2)	106.3 (11.3)
	PCB 153	50.6 (27.7)	143.6 (66.3)	19.9 (8.6)	107.5 (40.8)	17.8 (6.3)	97.7 (23.8)	15.5 (2.5)	104.8 (25.8)
	MeHg + PCB 153	106.6 (34.6)	119.1 (16.7)	31.0 (10.8)	128.0 (47.3)	17.6 (1.3)	b. d.	14.5 (1.5)	b. d.

Values specified in the columns represent mean ± SD; * - $p \leq 0.05$ compared to control; b. d. - close or below the detection limit, data regarded unreliable.

Table 3. Brain contents of noradrenaline (NA), adrenaline (A), dopamine (DA)and 3,4-dihydroxyphenylacetic acid (DOPAC), in adult male progeny of rat mothers exposed separately or jointly to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day) during gestation and breast feeding.

Brain region	Exposure group	NA		A		DA		DOPAC	
		Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)	Control (ng/g)	Treated (% Control)
Olfactory bulb	MeHg	72.5 (11.1)	68.5 (64.4)	36.4 (1.3)	68.6 (39.0)	31.3 (16.0)	131.2 (87.9)	19.9 (4.3)	98.6 (23.7)
	PCB 153	56.8 (16.3)	159.9 (16.7)*	19.0 (5.7)	175.2 (96.7)	18.0 (4.1)	150.0 (72.8)	21.2 (6.2)	124.8 (79.7)
	MeHg + PCB 153	104.0 (13.7)	95.6 (16.3)	21.7 (4.3)	105.8 (20.5)	33.9 (11.7)	109.1 (27.0)	19.3 (2.9)	94.5 (21.2)
Cortex	MeHg	89.8 (30.9)	83.9 (26.1)	24.7 (6.1)	93.0 (19.6)	32.9 (11.5)	84.5 (29.2)	38.1 (17.1)	75.8 (30.0)
	PCB 153	72.7 (44.7)	129.9 (43.5)	23.3 (10.8)	170.2 (90.4)	25.4 (10.3)	92.0 (28.0)	35.5 (14.8)	70.1 (23.8)
	MeHg + PCB 153	125.8 (19.2)	98.1 (17.1)	32.0 (11.2)	110.2 (46.9)	44.7 (23.1)	182.4 (100.7)	27.6 (13.1)	127.4 (35.2)
Hippocampus	MeHg	82.7 (23.0)	101.3 (29.2)	26.9 (5.8)	109.2 (56.0)	162.2 (51.8)	124.4 (36.9)	65.8 (16.9)	106.6 (41.7)
	PCB 153	99.8 (69.0)	224.5 (55.5)*	26.1 (7.4)	142.3 (17.8)	120.7 (63.2)	160.3 (55.0)	119.7 (51.5)	60.2 (5.3)
	MeHg + PCB 153	130.9 (30.4)	102.9 (16.6)	28.1 (9.7)	127.8 (21.1)	166.8 (67.4)	78.7 (30.7)	57.0 (10.9)	78.6 (28.2)
Striatum	MeHg	99.6 (67.1)	99.1 (41.7)	30.9 (14.4)	81.3 (34.3)	2975.7 (1981.2)	111.8 (71.5)	874.4 (255.7)	81.4 (28.2)
	PCB 153	92.5 (53.2)	111.2 (34.5)	30.3 (12.7)	126.8 (60.4)	3013.9 (2072.6)	175.0 (76.2)*	857.9 (294.1)	88.0 (26.3)
	MeHg + PCB 153	93.1 (12.3)	107.4 (25.9)	29.8 (11.3)	87.9 (28.5)	2612.1 (840.7)	96.5 (34.3)	825.7 (304.3)	95.4 (23.3)
Diencephalon	MeHg	128.0 (41.4)	103.6 (34.9)	38.5 (15.1)	80.4 (44.0)	144.4 (54.0)	92.1 (30.2)	48.7 (14.1)	84.2 (19.4)
	PCB 153	172.3 (95.2)	111.6 (31.4)	21.8 (7.4)	128.8 (9.5)	97.5 (59.3)	81.5 (16.3)	48.5 (30.9)	121.3 (47.4)
	MeHg + PCB 153	153.1 (24.8)	108.0 (26.4)	33.1 (17.4)	85.0 (22.3)	78.9 (27.5)	85.8 (40.5)	33.2 (14.1)	85.2 (24.5)
Mesencephalon	MeHg	85.4 (21.1)	104.1 (43.9)	29.2 (10.2)	97.1 (44.8)	56.7 (19.1)	63.2 (27.7)	30.6 (13.1)	105.1 (29.3)
	PCB 153	109.6 (54.1)	155.0 (26.4)	19.9 (6.4)	130.0 (37.7)	53.0 (16.8)	132.5 (21.2)	49.4 (17.9)	70.4 (15.5)
	MeHg + PCB 153	116.8 (23.6)	97.3 (18.3)	23.4 (6.1)	118.6 (49.5)	54.6 (17.9)	83.3 (38.6)	22.1 (4.4)	92.6 (18.2)
Cerebellum	MeHg	61.6 (18.6)	151.2 (63.4)	35.4 (15.5)	71.7 (27.5)	23.2 (7.5)	71.8 (28.0)	14.3 (0.7)	102.5 (8.4)
	PCB 153	69.1 (36.4)	143.6 (23.8)	22.8 (10.1)	147.4 (19.5)	16.3 (3.0)	114.4 (6.3)	14.7 (1.4)	98.7 (9.2)
	MeHg + PCB 153	112.2 (17.6)	112.7 (20.1)	30.8 (11.1)	104.7 (26.7)	18.6 (3.3)	98.3 (11.3)	14.1 (0.6)	b. d.

Values specified in the columns represent mean ± SD; * - $p \leq 0.05$ compared to control; b. d. - close or below the detection limit, data regarded unreliable.

Two reports deserve particular attention due to the period of the exposure and the applied doses [10, 13]. In the first, MeHg was administered subcutaneously to pregnant rats from GD8 to the day of delivery at doses 0.5, 1.0 and 2.5 mg/kg/day [13]. In the other work, MeHg was administered to dams from GD7 to PND7 with drinking water at doses 0.5 or 1.0 mg/kg/day [10]. Also in those studies, the progeny was tested shortly after weaning. The authors of the first report found that the exposure resulted in shortfalls in both the levels and turnover rate of brain dopamine and no changes in the NA content. The authors of the other work report significantly lowered MAO-B activity and significantly elevated 5-HIAA concentration of in cerebellum, and significantly reduced HVA concentration in the hippocampus of both genders. It is also worth noting that the described effects were observed after exposure to the dose of 1.0 mg/kg/day or higher. Exposure at 0.5 mg/kg i e. the dose used in our experiment, was completely ineffective. In the context of those observations, the result obtained in our experiment: significantly elevated NA, DA and DOPAC concentrations in the diencephalon of the female rats is puzzling. Such a result may indicate that the perinatal exposure to MeHg, even at very low doses, may cause some alterations in neurotransmission systems which become evident as late as after reaching maturity.

PCB effects on the aminergic systems have been extensively studied. The published reports show that, among the aminergic systems, the dopaminergic one is the most sensitive to PCBs. Structure Activity Relationship (SAR) studies indicate that the ortho-substituted noncoplanar PCB congeners (like PCB 153) are the most potent in affecting the DA system [14]. Studies on various animals, including primates, show that exposure to those congeners in adulthood results in prolonged drop of dopamine and noradrenaline levels in various parts of the brain [21, 22, 26-29]. The effects of exposure at the early stages of development are ambiguous. For example Morse et al. [30] found no dose-dependent effects on the concentration of DA, DOPAC, NA and HVA in any brain region of the 21 or 90 days old progeny of rat mothers exposed to Aroclor 1254 (0.5 or 25 mg/kg/day) from GD10 to GD 16 [30]. Meerts et al. [31] studying Aroclor 1254 obtained similar results. Further studies showed that longer (since GD 6 till PND 21) exposure to the single congeners may significantly and permanently affect DA levels in the brain of the progeny (the changes were detectable at the age of 35, 60 and 90 days). Exposure to dioxin-like coplanar PCB congener (3,4,3',4'-TCB) resulted in elevated, and to non-coplanar di-ortho-substituted PCB congener (2,4,2',4'-TCB) in reduced DA level in the frontal cortex and caudate nucleus [28].

Table 4. Changes in noradrenaline (NA), adrenaline (A), dopamine (DA)and 3,4-dihydroxyphenylacetic acid (DOPAC) of brain regions in adult female and male progeny of rat mothers exposed separately or jointly to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day) during gestation and breast feeding.

Brain region	MeHg				PCB 153				MeHg + PCB 153			
	NA	A	DA	DOPAC	NA	A	DA	DOPAC	NA	A	DA	DOPAC
	Females											
Olfactory bulb					+							
Cortex	+				+							
Hippocampus								-		--		
Striatum					+	+	+			++		
Diencephalon	++		++	++			-	-				
Mesencephalon							-				++	
Cerebellum					+							
	Males											
Olfactory bulb					++	+	+					
Cortex						+					+	
Hippocampus					++	+	+	-				
Striatum							++					
Diencephalon												
Mesencephalon			-		+							
Cerebellum	+				+	+						

+ : Insignificant increase; ++: significant increase; - : insignificant decrease; --: significant decrease.

Considering the information quoted above it would be reasonable to expect that perinatal exposure to the di-ortho-substituted PCB 153 would result in lower levels of DA (and of other amines) in certain regions of the brain. Results of the present work did not confirm this expectation. On the other hand, Castoldi et al. [10] have found that prenatal exposure to PCB 153 resulted, in both genders, in a significant reduction of DA, HVA and 5-HIAA levels in the striatum, cortex and hippocampus. In the Castoldi et al. [10] experiments, however, the PCB 153 daily doses were higher (20 mg/kg/body weight) and the exposure duration was shorter (from GD10 to GD16) than in our experiments. Moreover, the offspring was sacrificed at PND 21-22, whereas in ours at PND 90-96. In the existing literature no data is accessible on the effects of perinatal exposure to PCB 153 on the level of bioamines in the brain during maturity. Thus, the question whether, and if so, to what extent, the differences in the methods employed in the Castoldi et al. [10] study could be responsible for the differences in the results (at least in the part on DA) must for the time being remain unsolved.

Little is known at present about the type of MeHg/PCB interaction in their effect on the aminergic systems. Results of some *in vitro* studies suggest that MeHg co-occurrence in the medium may, depending on the concentration, augment or weaken the effects of PCBs [5, 6]. Results of Vettori et al. [11] *in vitro* study on PC12 cells are in line with that conjecture. They have demonstrated that PCBs (PCB 153) reduce intracellular DA concentration and that co-occurrence of MeHg at certain concentrations may block the effect of PCBs [11]. On the other hand, however, in the already mentioned *in vivo* Castoldi et al. [10] study the pattern of changes detected both in male and female progeny after the combined exposure to MeHg and PCB 153 did not differ significantly from that observed after exposure to PCBs alone.

As we have already stressed, the considerable variability of the values obtained in our current experiment prevents us from being very positive in our conclusions. However, the fairly evident relationship between the incidence of the differences and gender in the separate exposures and the changed character of that relationship in the combined exposure seems noteworthy.

When we compare the results of exposure to MeHg, we can see that the occurrence of changes in the catecholaminergic systems during adulthood is more likely in the female progeny. Sex-related differences in the effects of MeHg exposure during the early phases of development has been reported in behavioural studies on mice [32] and rats [15], which may indicate that our results are not random. The results of PCB 153 exposure suggest, like the behavioral observations by Holene et al. [33, 34], somewhat higher sensitivity of males. From the viewpoint of the aim of our current work, the effects of the combined exposure are most interesting. For males, the results point obviously to an antagonism, which is in line with the observations by Vettori et al. [11]. In females, the situation seems to be somewhat more complicated although also in that case the antagonistic type of interaction seems to dominate.

It is worth mentioning that an antagonistic type of interaction is also suggested by the results of parallel neurobehavioral studies. They have revealed evidently weaker effects of the combined MeHg/PCB 153 exposure compared to single MeHg and PCB 153 exposures [Wiaderna D. et al. and Lutz P. et al. – in preparation].

To sum up, the results of the work presented in this report suggest that perinatal exposures to MeHg, PCB 153 and the combined exposure to those two substances may cause some changes in the catecholamine levels in the mature brain, but the pattern of those changes does not confirm the conjecture about the synergistic activity of those two substances. An antagonistic type of interaction is more likely.

Acknowledgements

This study was performed under the “DEVNERTOX” project supported by the European Commission: Contract no. 6PR/03/506143).

References

1. GRANDJEAN P., LANDRIGAN P. J. Developmental neurotoxicity of industrial chemicals. Lancet **368**, 2167, **2006**.
2. GRANDJEAN P., WEIHE P., WHITE R. F., DEBES F., ARAKI S., YOKOYAMA K., MURATA K., SORENSEN N., DAHL R. Cognitive deficit in 7-year old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. **19**, 417, **1997**.
3. DAVIDSON P. W., MYERS G. J., COX C., AXTELL C., SHAMLAYE C., SLOANE-REEVES J., CERNICHIARI E., NEEDHAM L., CHOI A., WANG Y., BERLIN M., CLARKSON T. W. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment. J. Am. Med. Assoc. **280**, 701, **1998**.
4. NAKAI K., SATOH H. Developmental neurotoxicity following prenatal exposures to methylmercury and PCBs in humans from epidemiological studies. Tohoku J. Exp. Med. **196**, 89, **2002**.
5. BEMIS J. C., SEEGAL R. F. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro. Environ. Health Perspect. **107**, 879, **1999**.
6. BEMIS J. C., SEEGAL R. F. Polychlorinated biphenyls and methylmercury alter intracellular calcium concentrations in rat cerebellar granule cells. Neurotoxicology **21**, 1123, **2000**.
7. GRANDJEAN P., WEIHE P., BURSE V. W., NEEDHAM L. L., STORR-HANSEN E., HEINZOW B., DEBES F., MURATA K., SIMONSEN H., ELLEFSEN P., BUDTZ-JORGENSEN E., KEIDING N., WHITE R. F. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol. Teratol. **23**, 305, **2001**.
8. STEWART P. W., REIHMAN J., LONKY E. I., DARVILL T. J., PAGANO J. cognitive development in preschool children prenatally exposed to PCBs and MeHg. Neurotoxicol. Teratol. **25**, 11, **2003**.
9. ROEGGE C. S., SCHANTZ S. Motor function following developmental exposure to PCBs and/or MeHg. Neurotoxicol. Teratol. **28**, 260, **2006**.

10. CASTOLDI A. F., BLANDINI F., RANDINE G., SAMUELE A., MANZO L., COCCINI T. Brain monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153). Brain Res. **1112**, 91, **2006**.
11. VETTORI M. V., GOLDONI M., CAGLIERI A., POLI D., FOLESANI G., CECCATELLI S., MUTTI A. Antagonistic effects of methyl-mercury and PCB153 on PC12 cells after a combined and simultaneous exposure. Food Chem. Toxicol. **44**, 1505, **2006**.
12. LONGNECKER M. P., WOLFF M. S., GLADEN B. C., BROCK J. W., GRANDJEAN P., JACOBSON J. L., KORRICK S. A., ROGAN W. J., WEISGLAS-KUPERUS N., HERTZ-PICCIOTO I., AYOTTE P., STEWART P., WINNEKE G., CHARLES M. J., JACOBSON S. W., DEWAILLY E., BOERSMA E. R., ALTSHUL L. M., HEINZOW B., PAGANO J. J., JENSEN A. A. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. Environ. Health Perspect. **111**, 65, **2003**.
13. BARTOLOME J., WHITMORE W. L., SLOTKIN T. A. Effects of neonatal mercuric chloride administration on growth and biochemical development of neuronal and no neuronal tissues in the rat: comparison with methylmercury. Toxicol. Lett. **22**, 101, **1884**.
14. SHAIN W., BUSH B., SEEGAL R. F. Neurotoxicology of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicol. Appl. Pharmacol. **111**, 33, **1991**.
15. BEYROUTY P., STAMLER Ch. J., LIU J. N., LOUA K. M., KUBOW S., CHAN H. M. Effects of prenatal methylmercury exposure on brain monoamine oxidase activity and neurobehaviour of rats. Neurotoxicol. Teratol. **28**, 251, **2006**.
16. NGUON K., BAXTER M. G., SAJDEL-SUŁKOWSKA E. M. Perinatal exposure to polychlorinated biphenyls differentially affects cerebellar development and motor functions in male and female rat neonates. Cerebellum **4**, 112, **2005**.
17. MEFFORD I. N. Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: measurement of catecholamines, serotonin and metabolites in rats brain. J. Neurosci. Methods **3**, 207, **1981**.
18. HOLMES C., EISENHOFER G., GOLDSTEIN D. S. Improved assay for plasma dihydroxyphenylacetic acid and other catechols using high – performance liquid chromatography with electrochemical detection. J. Chromatogr. B **653**, 131, **1994**.
19. RAGGI M. A., SABBIONI C., CASAMENTI G., GERRA G., CALONGHI N., MASOTTI L. Determination of catecholamines in human plasma by high-performance liquid chromatography with electrochemical detection. J. Chromatogr. B **730**, 201, **1999**.
20. FUERTES G., LAORDEN M. L., MILANES M. V. Noradrenergic and dopaminergic activity in the hypothalamic paraventricular nucleus after naloxone-induced morphine withdrawal. Neuroendocrinology **71**, 60, **2000**.
21. TSUZUKI Y. Effect of methylmercury exposure on different neurotransmitter system in rat brain. Toxicology Letters **13**, 159, **1982**.
22. ZAHALKA E. A., ELLIS D. H., GOLDEY E. S., STANTON M. E., LAU CH. Perinatal exposure to polychlorinated biphenyls Aroclor 1016 or 1254 did not alter brain catecholamines nor delayed alternation performance in Long-Evans rats. Brain Research Bulletin **55** (4), 487, **2001**.
23. REA T. M., NASH J.F., ZABIK J. E., BORN G. S., KESSLER W.V. Effects of toluene inhalation on brain biogenic amines in the rat. Toxicology **31**, 143, **1984**.
24. ALCARO A., HUBER R., PANKSEPP J. Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective. Brain Res. Rev. **56** (2), 283, **2007**.
25. O'KUSKY J. R., BOYES B. E., MCGEER E. G. Methylmercury-induced movement and postural disorders in developing rat: regional analysis of brain catecholamines and indoleamines. Brain Research **439**, 138, **1988**.
26. LINDSTRÖM H., LUTHMAN J., OSKARSSON A., SUNDBERG J., OLSON L. Effects of long-term treatment with methyl mercury on the developing rat brain. Environ. Res. **56**, 158, **1991**.
27. CHOKSI N. Y., KODAVANTI P. R. S., TILSON H. A., BOOTH R. G. Effects of polychlorinated biphenyls (PCBs) on brain tyrosine hydroxylase activity and dopamine synthesis in rats. Fundam. Appl. Toxicol. **39**, 76, **1997**.
28. SEEGAL R. F., BROSCH K. O., OKONIEWSKI R. J. Effects of in utero and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'- tertrachlorobiphenyl on dopamine function. Toxicol. Appl. Pharmacol. **146**, 95, **1997**.
29. RICHARDSON J. R., MILLER W. Acute exposure to aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. Toxicology Letters **148**, 29, **2004**.
30. MORSE D. C., SEEGAL R. F., BORSCH K. O., BROUWER A. Long-term alterations in regional brain serotonin metabolism following maternal polychlorinated biphenyl exposure in the rat. Neurotoxicology **17**, 631, **1996**.
31. MEERTS I. A. T. M., LILIENTHAL H., HOVING S., VAN DEN BERG J. H. J., WEIJERS B. M., BERGMAN A., KOEMAN J. H., BROUWER A. Developmental exposure to 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (40OH-CB107): long-term effects on brain development, behavior, and brain stem auditory evoked potentials in rats. Toxicol. Sci. **82**, 207, **2004**.
32. GOULET S., DORE F. Y., MIRAULT M. E. Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. Neurotoxicol. Teratol. **25**, 335, **2003**.
33. HOLENE E., NAFSTAD I., SKAARE J. U., SAGVOLDEN T. Behavioural hyperactivity in rats following postnatal exposure to sub-toxic of polychlorinated biphenyl congeners 153 and 126. Behav. Brain Res. **94** (1), 213, **1998**.
34. HOLENE E., NAFSTAD I., SKAARE J. U., KROGH H., SAGVOLDEN T. Behavioural effects in female rats of postnatal exposure to sub-toxic doses of polychlorinated biphenyl congener 153. Acta Paediatr. Suppl. **88** (429), 55, **1999**.