Dietary early-life exposure to contaminated eels does not impair spatial cognitive performances in adult offspring mice as assessed in the Y-maze and the Morris water maze

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ABSTRACT

Many environmental contaminants are introduced via the diet and may act as neurotoxins and endocrine disrupters, especially influencing growing organisms in early life. The purpose of this study was to examine whether dietary exposure of dams to fish naturally contaminated with xenobiotics, especially with polychlorinated biphenyls (PCBs) and heavy metals (e.g. mercury and lead), resulted in cognitive function deficits in adult offspring mice. Daily, four groups of dams (n = 10/group) ingested standard diet plus paste with/without eels, during gestation and lactation, from gestational day (GD) six until post natal day (PND) 21 (weaning). Dams orally ingested a standardized amount of eel (0.8 mg kg\(^{-1}\) d\(^{-1}\)) containing the six non-dioxin-like (NDL) PCBs (\(\Sigma6\) NDL-PCBs: 28, 52, 101, 138, 153, and 180) at 0, 85, 216, and 400 ng kg\(^{-1}\) d\(^{-1}\). Results showed that early-life exposure to contaminated eels did not (compared to non-exposed controls) impair immediate working memory in the Y-maze in the offspring assessed at PND 38. Furthermore, it did not significantly impact spatial learning and retention memory as measured in the Morris water maze in adult offspring mice (PND 120-123). Our results suggest that perinatal exposure to contaminated eels does not affect spatial cognitive performances, as assessed by the Y-maze and Morris water maze at adult age. Adverse effects of xenobiotics reported earlier might be camouflaged by beneficial eel constituents, such as n-3 fatty acids. However, additional studies are needed to differentiate between potential positive and negative effects following consumption of food items both rich in nutrients and contaminants.

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Neurotoxins and endocrine disrupting compounds include many industrial related compounds such as certain pesticides, dioxins, furans, and polycyclic chlorinated biphenyls (PCBs), inflicting changes on the nervous and the hormonal system that may result in abnormal physiological functions, including growth disturbance, immunological aspects, and cognitive functions [1–3]. Especially when consumed during early life stages, those compounds may show stronger effects, as the immature organism has less defense mechanism available and the stage for physiological and psychological traits is yet to be set [4–6]. For example, we have previously shown that the consumption of eels by the parental generation can result in hyperactive traits associated with a depressed activity in acetylcholinesterase in the hippocampus in female offspring mice (unpublished results).

For the majority of these compounds, diet is the main contributor of exposure [2,7]. One predominant example is the intake via fish and water-living creatures, especially those ranking high in the food chain and being rich in lipids, and fish consumption has been linked to human PCB contamination via the oral route [8–10]. European eel (Anguilla anguilla L.) is a migratory fish with an extraordinary life cycle that begins and ends in the Atlantic Ocean; however, the main part of the eel’s life stages is spent in the freshwater habitats including rivers, streams and lakes. European eels can live over 90 years but more commonly 10 to 15 years [11], during which they consume a variety of various smaller prey. Eels have a carnivorous regime as they eat a variety of animal species such as insects, worms, caterpillars and spiders, crustaceans such as shrimps as well as frogs, fish eggs, fry and small fish [11]. Eels are fatty fish with fat in their flesh unlike white fish that accumulate fat rather in their livers, and are very low in fat [10]. In general, fatty fish are composed of 5% to 20% fat while white fish contains 1% to 2% fat, with estimated averages of long chain ω-3 polyunsaturated fatty acids (PUFAs) of 2 g and 0.3 g/100 g, respectively (total n-3 PUFAs calculated as the sum of eicosapentaenoic acid [20:5n-3], docosapentaenoic acid [22:5n-3] and docosahexaenoic acid [DHA; 22:6n-3]) [10]. Thus, eels may accumulate more readily xenobiotic contaminants, in particular lipophilic ones such as PCBs, but also heavy metals, e.g. mercury (especially in its xenobiotic contaminants, in particular lipophilic ones such as DHA; 22:6n-3) [10].

In addition, eel is a highly appreciated item and thus might pose a toxicological risk to consumers, especially for pregnant or lactating women and young children. Due to the long-standing human tradition of eel usage as food, this fish represents a popular food item in many populations, e.g. in many European, Asian and North America countries. Thus, several eel dishes have been developed such as kabayaki, smoked eel, eel pie, jellied eels, angulas, eel stew, eel curry and matelote d’anguille [11]. Its high lipid content represents a rich source of essential nutrients including linoleic and linolenic acid, iodine, and vitamin D [15]. The increasing demand for eels as a food source has resulted in the use of eel aquaculture as an alternative source of maintaining a stable supply of eels to the market. Thus, 90% to 95% of current eel production is attributed to aquaculture of wild-caught juveniles, which is practiced in several countries including the UK, France, the Scandinavian countries, Morocco, Australia, China, Taiwan, and Japan [16,17].

To the best of our knowledge, this is the first animal study that aimed to investigate the effect of early-life exposure to naturally PCB and heavy metal polluted fish on cognitive functions of offspring mice. We hypothesized that exposure to chemically contaminated eels would impact the cognitive function of offspring adult mice. To test our hypothesis, the research objective was to use the Y-maze to assess immediate working memory and the Morris water maze spatial navigation task to assess spatial learning and retention memory in mice. The contamination level of eels was also assessed, focusing on PCB and heavy metal contamination.

2. Methods and materials

2.1. Chemicals and standards

Unless otherwise stated, all chemicals were of analytical grade or superior. High purity (≥98%) 13C12 labelled analogues of the six non-dioxin-like NDL-PCB were supplied by Cambridge Isotope Laboratories, Andover, MA, USA. Only solvents of dioxins analysis purity grade (n-hexane: ≥95%; dichloromethane: ≥99.5%; toluene: ≥99.5%) were used. They were provided by Biosolve, The Netherlands. Nitric acid (trace analysis grade, minimum 67%) was from LGC Standards, France, hydrogen peroxide (20–35 w/w) from Fisher Scientific, Belgium. High purity (Milli-Q) water was employed throughout and was produced in the laboratory by a Millipore system, Brussels, Belgium.

2.2. Animals

Sexually mature male (n = 40) and female (n = 50) Swiss albino mice (CD 1, Charles River, France), 9 weeks of age (30–40 g), were employed in this trial. Mice (5 per gender) were housed in a cage that was maintained under a reversed light/dark cycle (light: 8 PM–8 AM) at 22°C ± 2°C, and 55 ± 10% relative humidity. A commercial rodent diet that supplied all nutrients for mice was provided (SDS Dietex, St Gratien, France), and diet and tap water were provided ad libitum.

2.3. Preparation of the diets

Standard diet consisted of food pellets enriched or not with chemically contaminated eels. Contaminated diet (paste with eels) was prepared by mixing sea and river eels at various proportions: NDL-PCBs 85 ng kg⁻¹ d⁻¹ (sea eels only), 216 ng kg⁻¹ d⁻¹ (a mix of sea and river eels), and 400 ng kg⁻¹ d⁻¹ (river eels only). Constituents of the diets were mixed with a kitchen household blender (Robot monofonction Seb Valentin 8553, France), i.e. 10 g of powdered food pellets (SDS Dietex, St Gratien, France) with 10 mL water, 0.5 mL sweep syrup, 1 mL...
2.4. Study design

After an acclimatization period (14 days), mating was done overnight (female mice to males 2:1) and were examined for copulatory plugs the following morning. The mating session was terminated with forty fecundated females (10 dams per group). These females were then housed individually. Two weeks post-fecundation nesting material was provided (cotton nest square). Parturition day was considered as postnatal day (PND) 1, and the gender and individual pup weight were recorded. When possible, litters were reduced to ten pups (composed equally of both sexes as recommended by the Organization for Economic Co-operation and Development (OECD) [20]) as to avoid the effect of litter size on pup development [21, 22]. Nurturing females were allocated to experimental groups by stratified randomization to result in comparable mean body weights in each litter across all groups on PND 1.

An overview of the experimental design of dam exposure is given in Fig. 1. From GD 6 until PND 21 (weaning), 40 dams daily ingested the standard diet plus paste with/without eels at the 3 concentration levels. The appropriate pastes were filed daily into the cage of each female during the exposure period. Paste was completely eaten by each female and no residual was found in the cage. After weaning (PND 21), mice pups were housed with their same-sex littermates in groups of 5/cage (OECD [20]). Male and female mice were confined into two different rooms to exclude the effect of sexual pheromones at adult age on behavior. To avoid introducing variance, all behavioral tests were conducted between 10 AM and 1 PM. All results were recorded and evaluated by an experienced human observer.

All animal experiments were in accordance with the European Union (Directive 2010/63/EU), and were approved by the institutional ethics committee of the University of Lorraine (authorization number CELMEA-2013-0010). Eel fishing was performed in agreement with the Ministry of the Walloon region, Belgium (authorization number DNF/DCP/CD/05.1/Sortie 2007: 31416).

2.5. NDL-PCB analyses

Contamination level of each NDL-PCB was determined for the two pools of eels by a fully validated method in an ISO 17025 accredited laboratory (Center of Analytical Research and Technology, CART). The used methodology is based on the...
EN16215:2011 European standard and consists in: a fat extraction by pressurized solvent extraction with the ASE system (Thermo-Fisher, Palo-Alto, CA, USA), followed by a multi-column liquid-solid chromatography purification achieved on a PowerPrep automat system (FMS, Watertown, CA, USA). The analysis was performed by gas chromatography coupled with a sector high-resolution mass spectrometer (GC-MS Autospec Premier Waters, Millford, MA, USA). The quantitative aspect is raised by the isotope dilution involving a 13C12 analog of each NDL-PCB.

2.6. Heavy metal analysis

2.6.1. Sample mineralization
Prior to inductively coupled plasma-mass spectrometry (ICP-MS) analysis, samples were submitted to total mineralization using microwave digestion (Multiwave Pro microwave oven, Anton Paar). About 250 mg of sample material was weighted into a PFA reactor, then 7 mL of nitric acid and 3 mL of hydrogen peroxide were added. The microwave power was increased to 1500 W in 10 minutes, then kept during 15 minutes. After cooling, the samples were recovered in 25 mL of water, then diluted 5 times with Millipore water before the ICP-MS analysis.

2.6.2. Inductively coupled plasma-mass spectrometry analysis
The metals (namely Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Pb, and Pt) were analysed in the digested samples by ICP-MS on an Elan DRC-e (Perkin Elmer, MA, USA), equipped with a microwave-USC associated with a cyclonic spray chamber. The samples were quantified using internal calibration, based on standard solutions prepared with a Multi-element ICP Standard Solution (30E) (Chemlab, Zedelgem, Belgium), and a Platinum ICP Standard (LGC Standards, Molsheim, France).

2.6.3. Mercury analysis
Mercury was analysed on an ICPMS 100 (Perkin Elmer) direct Mercury analyser, based on thermal desorption, gold amalgamation and atomic absorption. For the eel samples, triplicates of about 20 to 30 mg were analysed. For matrices containing lower amounts of Hg (feed excipients), about 400 mg were analysed to increase the sensitivity. The samples were quantified with calibration solutions prepared with Mercury Standard Solution (Plasma HIQU) (Chemlab).

2.7. Behavioral tests

According to the OECD guidelines [20] for the testing of chemicals, the weighing, and behavioral tests, were conducted in one pup of each gender, selected at random from each litter group i.e., 10/gender/group, resulting in a total of n = 20/group.

2.7.1. Y-maze for assessing working memory
Immediate working memory performance was assessed on PND 38, by recording spontaneous alternation behavior in a Y-maze [22]. The maze was of black painted wood, each arm 25 cm long, 14 cm high, 5 cm wide and positioned at equal angles. Mice were placed at the end of one arm and allowed to move freely through the maze during 3 min. The number of arm entries was recorded. An arm entry was considered to be completed when the four paws of the mouse were completely placed in the arm. Alternation was defined as triplets of explored arms and counted only if the mouse entered into the three arms of maze (without revisiting the first arm at the third visit). The percentage of spontaneous alternation was calculated as the ratio of successful overlapping alternations by the total possible triplets (defined as the total number of arm entries minus 2) multiplied by 100 [22].

2.7.2. The Morris water maze for spatial learning and memory
The Morris water maze was performed on PND 120 and PND 123 to evaluate spatial learning and memory [23,24]. For this purpose, an open circular pool (100 cm in diameter) was chosen, filled ca. half of the height with water (23°C ± 1°C). Four different geometric shapes were glued in the surroundings of the pool, marking each quadrant. The animal objective was to locate a platform that was hidden 1 cm underneath the water surface (placed in a fixed position in the middle of one quadrant of the pool). The platform was camouflaged by placing milk in the water. Mice were given five consecutive trials per day (a single training session [25]), each trial lasting 120 s, with a 30 sec inter-trial interval. In this step, the animal had to learn to navigate a direct path to the hidden platform, resulting in less time for reaching it. On PND 120, the spatial learning task started by gently placing the mouse into the water with its head towards the pool wall in any of the three quadrants without the platform. If an animal found the platform within 120 s, it was left to stay on the platform for 30 s. If not, animals were gently guided to the platform by the experimenter. To assess retention memory, 72 h (i.e. PND 123) post spatial learning task, the mouse had to undergo another trial. The latency (time) to escape to the platform was recorded for each animal.

2.8. Statistical analyses

As for the dependent variables including offspring body weight and the acquisition phase in the Morris water maze i.e., the latency escape, they were analyzed using three-way ANOVA, considering perinatal exposure to chemically contaminated eels and gender as between-subject fixed-factors, and trials as the repeated, within-subject factor. For the retention memory, the latency escape was analyzed using two-way ANOVA with exposure and gender as the main factors. The same procedure was used to analyze the spontaneous alternation in the three arms of the Y-maze. All data were reported as the means (±SEM). Significance was set at P < .05 (2-sided). All statistical analyses were performed using the Statview 4.5 statistical package (Abacus Concepts, Inc). Assumptions of normality and equal variance were assessed by normality plots and box plots, respectively.

3. Results

3.1. Body weight of offspring mice
No significant effect of the dams’ exposure to contaminated eels on offspring growth was observed across all groups over
the 130 days of the study; [exposure: \( F(3.633) = 0.05, P = .98 \); exposure × gender: \( F(3.629) = 0.02, P = .99 \); exposure × age: \( F(21.605) = 1.30, P = .16 \); exposure × age × gender: \( F(21.375) = 0.304, P = .99 \)]. Nevertheless, offspring mice gained weight over time, as shown by a significant effect of age \( [F(7.629) = 1782.326, P < .001] \). In addition, postnatal evolution of the body weight was gender-dependent \([F(7.635) = 2.52, P = .11]\) (Fig. 2).

### 3.2. PCB and metal analysis

As seen in Table 1, the concentration of the \( \sum_6 \) PCB-NDLs is below the maximum permissible level in eel muscle as defined by the EU. River eels caught in la Meuse, Belgium were slightly below the permissible level, and had five times higher PCB levels than reared eels. Mercury, lead and cadmium levels in both river and reared eels were below the maximum permissible levels in fish. Arsenic was more concentrated in river eels than reared eels. However, the risks associated from consuming a portion of eels (70-140 g) should be low, as it was below the tolerable weekly intake for an adult of 60–70 g (Table 2).

### 3.3. Y-maze

No significant effects were found for perinatal exposure to contaminated eel exposure: \( [F(3.76) = 1.305, P = .27] \) nor for gender \([gender: F(1.78) = 0.441, P = .50]\) or their interaction \([exposure \times gender: F(1.78) = 0.441, P = .50]\) on the percentage of spontaneous alternation. This result suggests that early-life exposure to chemically contaminated eels does not impair immediate spatial working memory (Table 3).
3.4. Morris water maze

Three-way ANOVA revealed a significant main effect for perinatal exposure to contaminated eels during the acquisition phase [exposure: F(3.396) = 3.065, P = .02]. Neither a gender effect [F(1.397) = 0.102, P = .75] nor trial effect [F(4.395) = 1.849, P = .12] were found. No interaction between the 3 main factors was found [exposure x trials: F(12.380) = 0.52, P = .89; exposure x gender F(3.91) = 0.54, P = .65; gender x trials: F(4.38) = 0.703, P = .59]; exposure x gender x trials F(12.359) = 1.10, P = .35]. At each trial, one-way analysis of variance revealed no significant differences between groups (P > .05), indicating the absence of exposure effect. As for retention memory, no significant effects were found for perinatal exposure to contaminated eels [exposure: F(3.76) = 0.340, P = .79] or gender [gender: F(1.78) = 0.109, P = .74] or their interaction (on the latency time to find the escape platform in the pool) [exposure x gender: F(3.91) = 0.56, P = .63] (Table 3).

4. Discussion

In this study, selected aspects of learning and memory were examined in offspring mice exposed at early life-stage to a complex of dietary derived contaminants including PCBs and heavy metals accumulated in eels during their life cycle. Exposure to contaminated eels based on their concentration of the \( \Sigma_6 \) NDL-PCB (85-400 ng kg\(^{-1}\) d\(^{-1}\)) indicators were chosen to mimic human diet PCBs i.e., average dietary intakes to high exposure. Cognitive performance of offspring mice whose mothers were exposed to chemically contaminated eels in the Y-maze were not significantly different to those whose

<table>
<thead>
<tr>
<th>PCB indicators</th>
<th>River eels (la Meuse, Belgium)</th>
<th>Reared eels (Netherlands)</th>
<th>Maximum permissible level in eel muscle</th>
<th>Tolerable daily intake (ng/kg b.w. day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 PCB-NDL congeners</td>
<td>PCB 28 8.9</td>
<td>6.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>PCB 52 35.6</td>
<td>14.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB 101 31.8</td>
<td>9.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB 138 59.3</td>
<td>8.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB 153 78.6</td>
<td>10.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB 180 36.4</td>
<td>3.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total PCBs</td>
<td>250.5</td>
<td>52.9</td>
<td>300</td>
<td>10*</td>
<td>[48,49]</td>
</tr>
<tr>
<td>( \Sigma_6 ) PCB-NDLs</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

ND, no data available.

* Based on the estimation that \( \Sigma_6 \) PCB-NDLs reflects almost half of the total PCBs [49].

### Table 2 – Metal concentrations in eel muscle (reared vs. river eels), maximum permitted levels and tolerable weekly intakes

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal concentrations (µg/g) weight</th>
<th>Maximum levels in the muscle meat of fish (kg b.w.)</th>
<th>Tolerable weekly intake (TWI: µg/kg b.w.)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>River eels (la Meuse, Belgium)</td>
<td>Reared eels (Netherlands)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aluminium 7.076</td>
<td>6.899</td>
<td>ND</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Vanadium &lt;0.588</td>
<td>&lt;0.588</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Chromium 0.594</td>
<td>&lt;0.151</td>
<td>ND</td>
<td>637</td>
</tr>
<tr>
<td></td>
<td>Manganese 0.853</td>
<td>0.562</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Iron 10.94</td>
<td>8.351</td>
<td>ND</td>
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<tr>
<td></td>
<td>Cobalt 0.015</td>
<td>0.028</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Nickel 0.140</td>
<td>0.066</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>Copper 0.718</td>
<td>0.725</td>
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<td></td>
<td>Zinc 38.28</td>
<td>49.24</td>
<td>ND</td>
<td>7000</td>
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<tr>
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<td>Arsenic 3.019</td>
<td>&lt;0.475</td>
<td>ND</td>
<td>15</td>
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<tr>
<td></td>
<td>Selenium 1.802</td>
<td>0.399</td>
<td>ND</td>
<td>ND</td>
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<td></td>
<td>Molybdenum 0.052</td>
<td>0.042</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Cadmium 0.024</td>
<td>&lt;0.010</td>
<td>0.1</td>
<td>2.5*</td>
</tr>
<tr>
<td></td>
<td>Lead 0.043</td>
<td>0.033</td>
<td>0.3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Platinum &lt;0.012</td>
<td>&lt;0.012</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Mercury 0.277</td>
<td>0.550</td>
<td>1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

ND, no data available.

* Estimation based on the values expressed as TDI.

* Weight given as µg/g dry weight; average water content 51.08%.
mothers were not exposed (controls), suggesting that immediate spatial memory was not affected at young-adult age (PND 38). Furthermore, performances of perinatally exposed offspring mice during the acquisition phase in the Morris water maze were statistically not different from controls, suggesting that spatial learning ability was not altered at adult age (PND 120). In addition, on PND 123, retention of the previously learned task was not impaired by early-life exposure to contaminated eels.

Working memory involves storage and manipulation of temporary information that implicates both the hippocampus and prefrontal cortex [26,27]. The Y-maze is a spatial memory test based on the natural tendency of rodents to exert spontaneous alternation in the three arms of the maze with about 60–70% of success [28]. Our results showed that successful spontaneous alternation behavior of exposed offspring mice was about 63% to 71%, which corresponds to the usual working memory performance of untreated mice in a Y-maze, a score also reached by control offspring (63%). Spatial working memory task was performed at PND 38, an age in rodent development that is considered as analogues to the periadolescence period (ca. 11-13 years) in human. Thus, our study suggests that immediate working memory was preserved in offspring mice despite an early-life exposure to xenobiotics including PCBs and Hg.

Furthermore, in this study, the Morris water maze, a well-known test to assess spatial learning and memory in rodents [23–25], was employed. Acquisition of spatial information in rodents consists of learning to escape from the pool by finding the hidden platform during a session of trials. The time required to reach the submerged platform (escape latency) is a valid variable to assess spatial learning. Our results indicated that early-life exposure to contaminated eels does not impair the acquisition process in the Morris water-maze training session. A memory test for the location of the escape platform, maintained in the same position in the pool, was performed 3 days later. Retention testing during the spatial water-maze task indicated that offspring mice (exposed vs. controls) had a similar spatial long-term memory performance. This finding indicated that early-life exposure to contaminated eels does not affect memory consolidation process in offspring mice. Therefore, our results from the Morris water maze suggest that consuming contaminated eels by dams during pregnancy and lactation does not result in cognitive deficits in their offspring at adult age.

Although there is contradictory evidence on the relation between maternal fish consumption and cognitive deficits in offspring; several studies have shown negative effects upon dietary consumption of persistent organic pollutants at several stages of life. For example, in adult humans, ingestion of Great Lake fish rich in PCBs was associated with lower scores of memory and learning ability [29] compared to controls (non-fish eaters). In animals, e.g. Rhesus monkey, it has been demonstrated that in utero exposure to PCBs (Aroclor 1016 or Aroclor 1248) caused cognitive deficits in the offspring (assessed at age 4-6) [30]. PCB exposure showed to reduce circulating T4 levels in 60 d old rats, and resulted in modest memory defects [31]. However, the fact that no effects were detected in the present study raises the possibility that healthy components of eels, such as n-3 fatty acids, have counterbalanced the adverse effects of xenobiotics. Indeed, it has been recognized that these essential fatty acids could overlay negative effects of pollutants such as dimethylmercury neurotoxicity, underestimating toxicity [32]. It is important to emphasize that eels during their life cycle may accumulate an array of toxic compounds including PCBs, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), organochlorine pesticides, perfluorinated compounds, brominated flame retardants, heavy metals and many more [11–14,33–35]. For instance, in freshwater and marine fish, NDL-PCBs levels and DL-PCBs + total dioxins (PCDD/Fs) were found to be positively correlated. This further raises the possibility that with a complex mixture of xenobiotics, besides synergistic and additive effects, antagonistic effects may also occur.

To date, balancing the risk of fish consumption constitutes a complex task for public health agencies, particularly due to differences in human sensitivity to toxins. It is assumed that women of childbearing age, pregnant or nursing women, and young children constitute sensitive populations for neurological developmental risks from exposure to contaminants in fish. In their report, the Scientific Advisory Committee on Nutrition (SACN) published advices for fish consumption, with particular reference to fatty fish, based on nutritional and

<table>
<thead>
<tr>
<th>Table 3 – Cognitive spatial performance of offspring mice in the Y-maze and the Morris water maze. Contaminated eel was fed to mothers during pregnancy and lactation, from GD 6 until PND 21 (weaning), at the following concentrations (expressed as the ∑6 NDL-PCBs): 0; 85; 216, and 400 ng kg(^{-1}) d(^{-1}).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive function: related measured performances</td>
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<td><strong>Y-maze</strong></td>
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<td><strong>Morris water maze</strong></td>
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Values represent means ± SEM of 20 individuals per group.
toxicological considerations. For the general and sensitive individuals it has been recommended to eat fish at least twice a week, of which one should be fatty, with a restriction concerning pregnant and lactating women on certain fish e.g., marlin and swordfish due to methylmercury contamination [10]. A methylmercury intake of 1.6 μg/kg b.w./week was used as a guideline to protect against neurodevelopmental effects in the fetus [10].

In our study, it was surprisingly found that reared eels contained more Hg (550 ng/g dry weight [d.w.]), lead (0.05 μg/g d.w.) and chromium (0.59 μg/g d.w.) than river eels (276 ng/g d.w., 0.03 μg/g d.w. and <0.15 μg/g d.w., respectively). In contrast, in river eels, arsenic (3 μg/g d.w.) and cadmium (0.02 μg/g d.w.) were detected at higher concentration (vs. reared eels: <0.47 and <0.01 μg/g d.w., respectively). Permissible levels in the EU for fish for babies and infants are 0.5 μg/g mercury, 0.3 μg/g (lead) and 0.1 μg/g for Cd [36], thus levels of heavy metals in eel cannot be considered high or even extreme. Nevertheless, infant food formula has much more stringent recommendations with 10 fold lower maximum residue concentrations. Differences in the concentration of these inorganic contaminants, which have both natural but mainly anthropic origins, depend on the quality of aquatic mediums, in which the eels have lived. However, selenium, a generally considered beneficial nutrient in fish was found in our metal analysis at higher concentrations in river eels (1.8 μg/g d.w.) than in reared eels (0.4 μg/g d.w.). A more stringent recommendation with respect to fish consumption was proposed by the National Food Agency Finland, advising children and young people at fertile age not to consume high levels of herring and salmon more than once or twice a month, owing to the high concentration of dioxins (PCDD/Fs) detected in these fish caught in the Baltic Sea [12]; and Norway has also recently issued a warning concerning farmed salmon.

From nutritional considerations, consuming fatty fish does have beneficial effects on fetal development. For the human developing brain during growth, n-3 fatty acids including DHA and arachidonic acid (AA, 20:4n-6) play an essential role as they are integral components of neuronal membranes [37,38]. Fatty fish is an important dietary source to deliver DHA and AA (ca. 500 and 125 mg/100 g [15]) for fetuses and newborns that depend on the maternal supply in particular during the last trimester of pregnancy till the end of the first year of life. For instance, during the brain's growth spurt (last trimester of pregnancy), 15–22 mg/week of DHA enter the fetal brain; however, during the first year of life approx. 76 mg/week is needed for brain growth [37]. In adult human brain, lipids represent 50 to 60% of dry weight, mainly (ca. 35%) in form of PUFAs, of which AA and DHA are the principal ones [37].

It has been thought that the optimal efficiency of n-3 fatty acid supplementation on cognitive performances including spatial memory is reached when starting early in life; however, it becomes less effective when starting at later ages [39]. This response difference between the immature and mature brain might be due to the temporal windows of opportunity and vulnerability. Nevertheless, it has been shown that fish ingredients such as PUFAs and selenium were unable to counterbalance the deleterious effects of fish-associated mercury on working memory in animals exposed at young age (3 weeks old) [40]. Although high fish consumption (1.2 portions/wk) by pregnant women was associated with better infant cognition (assessed at 6 months of age) in a prospective US pregnancy and child cohort study; higher mercury levels were associated with lower cognition [41]. As a result, women have been advised to continue eating fish during pregnancy, choosing species with lower mercury contamination such as small fatty fish including sardines, and canned tuna [41]. Furthermore, no adverse effects of perinatal exposure to MeHg from fish consumption on cognitive development were found in cohort studies of Seychellois children (assessed at 66 months [42] and at 9 years of age [43]); mothers reported consuming 12 fish meals per week [43]. In contrast, prospective studies have associated high fish consumption with high mercury exposure in both Spanish preschoolers (4 years old) [44] and children (7 years old) from the Faroe Islands [45] in whom cognitive performance deficits were detected. Besides methylmercury, deficits in cognitive function were also attributed to perinatal exposure to lead and PCBs; which can alone or in mixture act as neurotoxins. For instance lead, even at low doses, impairs cognitive functions in childhood such as attention and intellectual function [46]. In a cohort study, maternal consumption of PCB-contaminated fish was associated with poorer visual recognition memory in babies at 7 months postpartum [47].

In general, chemically contaminated fish and seafood involves exposure to multiple contaminants. In such a scenario, it is hard to attribute adverse effects to one contaminant or even a family of contaminants. It has been postulated that the combined exposure to MeHg and PCBs may have an interactive effect, but in vitro at higher concentrations or longer durations, the two chemicals antagonized each other [3]. In a recent study, it has been shown that prenatal exposure to PCBs, mercury, and lead resulted in impaired cognitive development in Inuit infants from Arctic Québec, assessed at 6.5 and 11 months. It has been postulated that each type of contaminants may have its own “behavioral signature”, with PCBs being associated with visual recognition memory, MeHg with working memory and an early precursor of executive function, and lead with processing speed [46].

In conclusion, we investigated with an accepted mouse model of behavior the effects of consuming a diet rich in contaminated eels on the following generation. This is an important dietary approach relevant also to humans, were adverse effects of dietary constituents or contaminants may especially be observed in the early childhood, following postnatal exposure. Our results could not demonstrate any cognitive deficits at adult age in offspring mice of dams consuming eels contaminated by PCB and heavy metals during pregnancy and lactation. These results do not prove that consuming environmentally contaminated eels does not pose any risk for brain development. The absence of effects might also be due to the fact that contaminant concentrations were not in the range affecting cognitive function, as contaminants could act on different nervous function with different thresholds of sensitivity. In addition, adverse effects on other brain functions which were not assessed in this study may become detectable at later life stages, e.g. induced hyperactivity (not at PND 47 but at PNDs 195, 305 and 329, unpublished results). Extrapolation of these results from mice.
to humans should also be carried out prudently as species differences with regard to sensitivity to contaminants, due to differences in absorption, metabolism, and excretion do exist. Further studies on the relationship between maternal fish consumption and cognitive functions in offspring at various stages of life are warranted to predict cognitive functions of future generations.

Acknowledgment

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