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Research Article

Dietary Mercury Exposure Resulted in Behavioral Differences in Mice Contaminated with Fish-Associated Methylmercury Compared to Methylmercury Chloride Added to Diet

Jean-Paul Bourdineaud,[1] Masumi Marumoto,[2] Akira Yasutake,[2] and Masatake Fujimura[2]

[1] *Bordeaux University-CNRS, UMR EPOC 5805, Arcachon Marine Station, place du Docteur Peyneau, 33120 Arcachon, France*
[2] *Pathology Section, Department of Basic Medical Sciences, National Institute for Minamata Disease, 4058-18 Hama, Minamata, Kumamoto 867-0008, Japan*

Correspondence should be addressed to Masatake Fujimura, fujimura@nimd.go.jp

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Methylmercury (MeHg) is a potent neurotoxin, and humans are mainly exposed to this pollutant through fish consumption. However, in classical toxicological studies, pure methylmercury chloride (MeHgCl) is injected, given to drink or incorporated within feed assuming that its effects are identical to those of MeHg naturally associated to fish. In the present study, we wanted to address the question whether a diet containing MeHg associated to fish could result in observable adverse effects in mice as compared to a diet containing the same concentration of MeHg added pure to the diet and whether beneficial nutriments from fish were able to counterbalance the deleterious effects of fish-associated mercury, if any. After two months of feeding, the fish-containing diet resulted in significant observable effects as compared to the control and MeHg-containing diets, encompassing altered behavioral performances as monitored in a Y-shaped maze and an open field, and an increased dopamine metabolic turnover in hippocampus, despite the fact that the fish-containing diet was enriched in polyunsaturated fatty acids and selenium compared to the fish-devoid diets.

1. Introduction

Methylmercury chloride (MeHgCl), the most toxic form of mercury, is a potent neurotoxin, to which human beings are mainly exposed through fish consumption. In classical toxicological studies, MeHg is most often injected intraperitoneally, instilled in the trachea, or dissolved in drinking water, which are not the natural routes for MeHg entrance into the body. In other cases MeHgCl is added to the diet. On the other hand, we have in a recent past exposed mice to diets containing fish and mimicking the Wayana Amerindians mercurial contamination or the Western populations' average fish consumption [1, 2]. A more classical approach consisting in dispersing a given quantity of methylmercury (MeHg) within diet preparations had been precluded because our working postulate is that the supramolecular form under which MeHg enters the body is of crucial importance, contrarily to the common assumption according to which the toxicological outcome of MeHg incorporated into fish tissue is identical to that from MeHgCl added to the same matrix. In this context, a higher faecal excretion and lower tissue accumulation, as well as metallothionein induction in rats following exposure to methylmercury naturally incorporated in fish compared to methylmercury chloride added to the same matrix, have been reported [3]. Moreover, the chemical form of methylmercury in fish has been identified as methylmercury-cysteine (MeHg-cysteine), probably as part of larger peptides [4]. Therefore, one can suspect a different trophic transfer rate through the intestinal barrier and a different early toxicity for ingested free and

protein-bound methylmercury. It has well been established that MeHg-cysteine was not hydrolyzed by gastric acidic juice and remained intact [4]. The acute toxicity of solubilized MeHg-cysteine has been reported to be considerably lower than MeHgCl both *in vivo* [5] and *in vitro* [6]. In addition, many compounds within fish flesh or brought by diet are likely to influence the trophic transfer rate and the toxicity outcomes of MeHgCl. For instance, selenium and vitamin E are decreasing the overall toxicity of MeHgCl [7, 8], whereas fruit or tea consumption decreases mercury accumulation within body for the same number of eaten fish meals [9, 10]. The precise quality of dietary fats also modulates the MeHg toxicity and the steroidogenesis in rats [11, 12].

Therefore, the aim of the present work was to address the questions of whether a diet containing "naturally" accumulated MeHg in fish flesh could result in differential biological outcomes as compared to MeHgCl added pure to diet. After two months of feeding with such diets, we analyzed the mercury accumulation in tissues, the mice body growth kinetics, the possible behavioral impairments, and the concentration of neurotransmitters in various brain structures.

2. Materials and Methods

2.1. Preparation of the Mice Diets. The *H. aimara* fish whose flesh was used was caught in French Guiana in the Sinnamary River, known to be contaminated by methylmercury mostly originating from the Petit-Saut hydroelectric reservoir [13]. The dry flesh of this animal contained 5 μg Hg/g. The *H. aimara* fish were thawed, minced, and lyophilized. Then the fish powder was ground in a kitchen blender. The nutrient composition and the metal content of the *H. aimara* fish powder have already been described [1]. The special diets were manufactured by Special Diets Services (Witham, Essex, United Kingdom; French commercial representation: Dietex, Saint-Gratien, France). The control diet was vegetarian (Rat and Mouse no. 1 maintenance diet, abbreviated to RM1 diet, Special Diets Services). The *H. aimara* fish-containing diet was prepared by adding 4.88% of lyophilized fish powder to the diet ingredients. The methylmercury chloride-containing diet was prepared by adding 1 L of a 1.5 mg/L solution of MeHg in a blend of ingredients allowing the preparation of 6 kg of diet. The nutrient compositions of the control RM1 and the two prepared regimens are given in Table 1 (the analyses were carried out by Special Diets Services). A comparison of the diets' compositions showed some minor differences between the control and the MeHg diets on one side, and the fish diet on the other side. Apart from the content in mercury, the control and MeHg diets are identical. The fish diet contained 3.7% more protein than the control and MeHg diets (18.1% compared to 14.4%). It also contained more of some polyunsaturated fatty acids (PUFA): 290, 70, 40, and 150 ppm of docosahexaenoic, docosapentaenoic, erucic, and eicosapentaenoic acids against <20, <20, 20, and <20 ppm for control and MeHg diets. We quantified the total mercury content of the two prepared regimens and found 253 ± 38 and 237 ± 23 ng Hg/g of food pellets for the MeHg and fish diets, respectively.

Table 1: The composition of the diets used[a].

	Control and MeHg diets	Fish diet[b]
Moisture	10	10
Fat	2.71	2.66
Protein	14.38	18.08
Fibre	4.65	4.45
Ash	6.00	5.94
Carbohydrates		
Starch	45.0	43.3
Sugar	4.05	3.85
Pectin	1.52	1.45
Hemicellulose	10.17	9.67
Cellulose	4.32	4.11
Lignin	1.68	1.60
Fatty acids		
Saturated fatty acids		
C12:0 lauric	0.02	0.02
C14:0 myristic	0.14	0.14
C16:0 palmitic	0.31	0.31
C18:0 stearic	0.04	0.04
Monounsaturated fatty acids		
C14:1 (ω5) myristoleic	0.02	0.02
C16:1 (ω7) palmitoleic	0.09	0.09
C18:1 (ω9) oleic	0.77	0.74
Polyunsaturated fatty acids		
C18:2 (ω6) linoleic	0.69	0.66
C18:3 (ω3) linolenic	0.06	0.05
C20:4 (ω6) Arachidonic	0.13	0.13
C20:5 (ω3) (EPA) Eicosapentaenoic	**<0.002**	**0.015**
C22:1 (ω9) erucic	0.002	0.004
C22:5 (ω3) (DPA) Docosapentaenoic	**<0.002**	**0.007**
C22:6 (ω3) (DHA) Docosahexaenoic	**<0.002**	**0.029**

[a] Nutrients and compounds are given as their percentages in the diets.
[b] Fish diet : mice were fed a 4.9% aimara flesh-containing diet.

The control RM1 diet contained 7.8 ± 1.9 ng Hg/g of food pellets. The content of several other metals in the control, MeHg and fish-containing regimens was assessed (Table 2). Metals were assayed by ICP-MS (Antellis, Toulouse, France). The diets metal levels were below the detection threshold for Ag (<0.02 mg/kg), As (<0.1 mg/kg), Au (<0.05 mg/kg), Bi (<0.02 mg/kg), Sb (<0.5 mg/kg), Sn (<0.5 mg/kg), Tl (<0.05 mg/kg), and V (<0.5 mg/kg). The MeHg and fish diets contained equal amounts of mercury whereas the control diet was 30-times poorer in this metal. The fish diet was richer in selenium than the control and MeHg diets (0.48 ppm against 0.30), and apart from mercury and selenium, the 3 diets were comparable.

Table 2: The metal and selenium composition of the diets used[a].

Element	Diets		
	Control diet	MeHg[b]	Fish[c]
Al	41.1	41.1	39.1
Cd	64.10^{-3}	64.10^{-3}	62.10^{-3}
Co	0.80	0.80	0.76
Cr	0.72	0.72	0.69
Cu	7.99	7.99	7.63
Hg (total)	**8.10^{-3}**	**253.10^{-3}**	**237.10^{-3}**
Ni	0.39	0.39	0.38
Pb	0.165	0.165	0.161
Se	**0.30**	**0.30**	**0.48**
Zn	41.1	41.1	40.0

[a] Metals and selenium are given in mg/kg.
[b] MeHg: mice were fed a MeHg-containing diet.
[c] Fish: mice were fed a 4.9% aimara flesh-containing diet.

2.2. Mice Treatment and Tissue Sampling. Subjects were naïve male mice of the C57Bl/6 Jico inbred strain obtained from Japan Clear (Tokyo, Japan) at the age of 3 weeks weighing 10.4 ± 0.24 g. Young animals were used because they grow quickly and because they are more sensitive to MeHg than older animals. They were first ink-labeled on the ear skin. They were socially housed in standard conditions: room temperature (23°C), 12/12 light cycles, and *ad libitum* food and water. Experiments were performed in compliance with the European Community Council directive of 24 November 1986 (8616091 EEC). Three groups of 16 mice each (8 of which dedicated for each kinetic time points at 29 and 57 days) were fed for 29 and 57 days as follows: one with the control RM1 diet and the two other groups with the MeHg and fish diets. Mice from the three groups were weighed twice a week throughout the experiment. At the end of the exposure period, mice were subjected to an open-field maze test, in order to quantify anxiety levels, and to a Y-shaped maze test, to assess cognitive ability. Thereafter, mice were anesthetized with Pentothal (50 mg/kg, i.p.), blood was immediately collected by puncturing the vena cava, and the vascular system was washed for traces of blood by perfusion with saline (0.9% NaCl). Then, tissues were dissected for mercury quantification (blood, several brain structures, liver, kidney) and neurotransmitters quantification (6 different brain structures).

2.3. Activity Test Using an Open-Field Maze. The anxiety test was assessed using the open-field apparatus after Tanaka et al. [14] with slight modifications. This behavioural test was performed using randomly chosen animals (8 mice per group) on days 28 and 56, one day before their sacrifice for tissue sampling. The apparatus consisted of a floor (50 × 50 cm) surrounded by a 50 cm high opaque wall. A CCD camera fixed above the apparatus was connected to a Macintosh computer, and the movement of the mouse was analyzed using Image OF (O'Hara & Co., Ltd., Tokyo, Japan), a modified NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). In this software, the position of the animal was defined as the position of the gravity center of the animal's image, which was calculated every 0.5 s. The total distance traversed by the gravity center was calculated and converted into centimeters. Also, floor area was divided into 25 squares (10 × 10 cm), and the location of the animal was classified either as central (nine areas that did not have direct contact with the walls) or peripheral (remaining sixteen areas). Behavior was monitored for 5 min after placing one mouse at the center of the floor. Between each trial, floor and walls were cleaned with 70% alcohol followed by wiping with wet cotton.

2.4. Cognitive Test Using a Y-Shaped Maze. To assess spatial learning and memory, mice were tested in the Y-maze. This behavioural test was performed using randomly chosen animals (8 mice per group) on days 27 and 55, two days before their sacrifice for tissue sampling. Each mouse was placed at the end of one arm facing the centre of the maze and allowed to move freely within the maze for a period of 5 min. The arm length was 40 cm, the arm width, 10 cm, and the arm height, 35 cm. The total number of arms entered by the mouse and the order of arm entries were recorded. The total number of arms entered provides an indication of locomotor activity, and the order of arm entries provides a measure of spontaneous alternation behaviour [15]. Success in this test is indicated by a high rate of alternation in the control group, indicating that the animals can remember which arm was entered last.

2.5. Mercury Quantification. Animals were chosen randomly for mercury analysis (at day 57, 8 for blood, 6 for liver and kidney, and 3 for each brain structure). Samples were analyzed for total mercury at the National Institute for Minamata Disease (NIMD), Japan, using reliable and sensitive techniques following described procedures [16]. Tissue samples were homogenized (10%, w/v) in distilled water using a Polytron homogenizer (Kinematica GmbH, Littau, Switzerland). Blood was collected from the femoral vein into heparinised tubes. A portion of blood was haemolysed by distilled water (1 : 50). Total Hg levels in the homogenates (100 μL) and haemolysed blood (100 μL) were determined by an oxygen-combustion gold amalgamation method using an atomic absorption mercury analyzer MD-A (Nippon Instruments Co., Ltd., Osaka, Japan). To control the quality of measurements, a standard reference material from dogfish, DORM2 (National Research Council, Canada) was included in analyses. The certified value of total mercury is 4.64 ± 0.26 μg/g (mean ± SEM). Our qualification data were 4.51 ± 0.14 μg/g for total mercury (mean ± SEM).

2.6. Neurotransmitters and Associated Metabolites Quantification. The brain homogenates were prepared as follows. Brain regions including the frontal cortex, cortex, hypothalamus, striatum, cerebellum, and midbrain were dissected out from frozen brains. Dissected tissues were frozen immediately in liquid nitrogen and stored at −80°C until use. These tissues were homogenized in 0.2 M perchloric acid (Nacalai Tesque, Kyoto, Japan) containing 100 mM

EDTA and isoproterenol (ISO) (100 ng/μL) as an internal standard. Homogenates were centrifuged for 15 min at 0°C and 20,000 g. The pH of each supernatant was adjusted to 3.0 with 1 M sodium acetate and supernatants were then used to determine monoamine levels. Each sample (10 mL) was injected into an HPLC system (HTEC-500MAD) with electrochemical detection (ECD-300 EICOM, Kyoto, Japan) with an ODS column (EICOMPAC SC-5, 3.0 mm × 150 mm, EICOM). The mobile phase was sodium acetate-citric acetate buffer (pH 3.5)/methanol/sodium octane-1-sulfonate (85%/15%/0.21 g), the flow rate was maintained at 0.5 mL/min, and the temperature of the column was 25°C. Tissue levels of dopamine (DA), 5-hydroxytryptamine or serotonin (5-HT), and noradrenalin (NE) as well as their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytryptamine (3-MT) and homovanillic acid (HVA) as DA metabolites, and 5-hydroxyindole-acetic acid (5-HIAA) as a 5-HT metabolite were measured. Each of the standard solutions for the 6 catecholamines (DA, NE, DOPAC, HVA, 3-MT, and ISO) and 2 indoleamines (5-HT and 5-HIAA) was prepared at a concentration of 0.1 ng/mL. Each standard (10 μL) was analyzed by HPLC, and the standard chromatographic peaks per 1 ng for each sample were obtained. The amount of each monoamine was determined with peak-area ratios using an HPLC chromatogram analysis software, eDAQ Power Chrom (eDAQ, NSW, Australia). Values were normalized for the amount of protein in each sample.

2.7. Statistical Analysis. Statistical significance was determined by a one-way ANOVA followed by a Dunnett's multiple comparison test. Data were expressed as the mean ± SEM. A difference was considered statistically significant when $P < 0.05$.

3. Results and Discussion

During the timecourse of the present experiment we observed no mortality, and no clinical or histopathological signs of lesions in mice brains whatever the diet given. The mice growth was recorded for two months. It appeared that the weight gain was faster for mice fed the MeHg and fish diets than the control diet during the first 10 days (Figure 1). At days 3, 7 and 10, the mercury-contaminated mice got body weights 7, 4, and 4% higher than the control mice, respectively. This effect is easily attributable to the beneficial effects of fish nutriments in the case of the fish-containing diet but remains hard to explain in the case of the MeHg diet.

3.1. Mercury Quantification. As expected, after 57 days of exposure the mercury concentrations in tissues of both groups of mice dietary contaminated with mercury were much higher than those from mice fed the control diet (Table 3). The mercury concentration increases ranged between 11 and 25 times those recorded in tissues from mice fed the control diet. We could notice a 20-fold increase in blood mercury for both MeHg and fish mice compared to control mice. Kidneys were the organs accumulating the highest concentrations of mercury in the organism, reaching

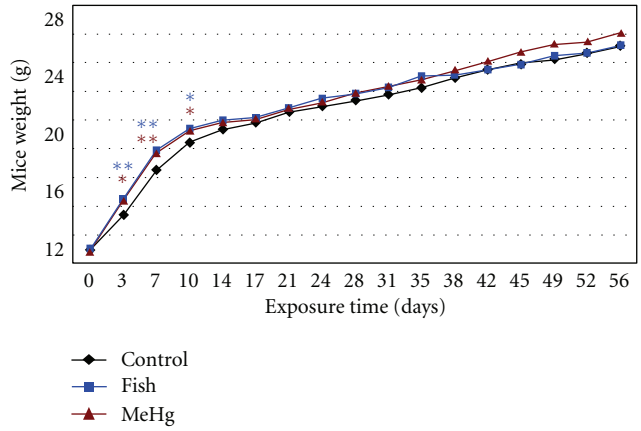

Figure 1: Influence of diet on the body weight growth. Mice were exposed to a control diet ("Control" plot with black diamonds), or a MeHg-containing diet ("MeHg" plot with red triangles), or a fish-containing diet ("Fish" plot with blue squares). Weights (in grams) were collected at the indicated kinetic time points and the mean weights plotted. Means and standard deviations were calculated from 16 control-, MeHg- and fish-fed mice from the beginning to the 35th day. From days 38 to 56, they were calculated from 8 animals per diet. The asterisks indicate a significant difference in body weight for mice fed the MeHg- and fish-containing diet compared to those fed the control diet, as determined with a one-way ANOVA followed by a Dunnett's multiple comparison method, $^{*}P < 0.05$, and $^{**}P < 0.01$.

7.3 and 6.8 mg Hg/g in mice fed the MeHg and fish diets, respectively, a multiplication factor of 17 and 16 compared to the control diet. Worth to note is the heterogeneity of brain structures in terms of mercury accumulation since, for instance frontal cortex accumulated 2.7 and 2 times more mercury than brain stem in mice fed the MeHg and fish diets, respectively. Except in the striatum, which accumulated 33% less mercury in animals fed the fish diet compared to those fed the MeHg diet, the mercury concentrations in tissues of mice fed the MeHg and the fish diets displayed no statistical significant differences, although a trend of smaller concentrations was observed in tissues of mice fed the fish diet. This lower mercury concentration in the striatum of mice fed the fish diet compared to those fed the MeHg diet cannot be explained by a lower blood mercury concentration since we measured the same blood mercury concentrations for both groups of mercury-contaminated mice. This is probably due to the fact that different mercurial chemical species are not transported into tissue cells with the same efficiency by transporters. For instance, the ubiquitous transporters LAT1 and LAT2 efficiently catalyze the uptake of MeHg-L-cysteine but not that of MeHg-D-cysteine or MeHg-glutathione [17]. Furthermore, solubilised MeHg-cysteine is thought to be, at least partly, actively transported via neutral amino acids carrier systems [18–20], while MeHgCl would be transported via other facilitated processes [20].

In order to mimic the mercury exposure in the Amazon riverside population, rats have been fed with a diet containing 20% of carnivorous fish caught in the Tapajós River and which flesh contained 1.95 μg MeHg/g, making a mercury concentration in feed of 0.39 μg MeHg/g [21].

Table 3: Total mercury concentrations in mice tissues[a].

Tissues	Control	MeHg[b]	Fish[c]
Blood ($n = 8$)	0.010 ± 0.002	0.21 ± 0.02	0.20 ± 0.01
Brain stem ($n = 3$)	0.016 ± 0.006	0.27 ± 0.07	0.20 ± 0.07
Cerebellum ($n = 3$)	0.016 ± 0.002	0.33 ± 0.06	0.26 ± 0.04
Frontal cortex ($n = 3$)	0.031 ± 0.013	0.73 ± 0.16	0.41 ± 0.13
Hippocampus ($n = 3$)	0.024 ± 0.002	0.62 ± 0.26	0.34 ± 0.08
Midbrain ($n = 3$)	0.020 ± 0.003	0.41 ± 0.20	0.23 ± 0.04
Striatum ($n = 3$)	0.018 ± 0.002	0.49 ± 0.05	***0.33 ± 0.01**
Liver ($n = 6$)	0.038 ± 0.008	0.94 ± 0.11	0.79 ± 0.12
Kidney ($n = 6$)	0.416 ± 0.091	7.3 ± 1.4	6.8 ± 0.5

[a] In μg/g for solid tissues or μg/mL for blood (mean ± SD).
[b] MeHg: mice were fed a MeHg-containing diet.
[c] Fish: mice were fed a 4.9% aimara flesh-containing diet.
All total mercury concentrations in MeHg and fish diet samples significantly higher than those in control diet samples, as determined with a one-way ANOVA followed by a Dunnett's multiple comparison method ($P < 0.01$).
The symbol * indicates a significant difference in mercury concentrations for mice fed the fish-containing diet compared to those fed the MeHg-containing diet, as determined with a one-way ANOVA followed by a Dunnett's multiple comparison method. $^*P < 0.05$. The corresponding value appears in bold characters.

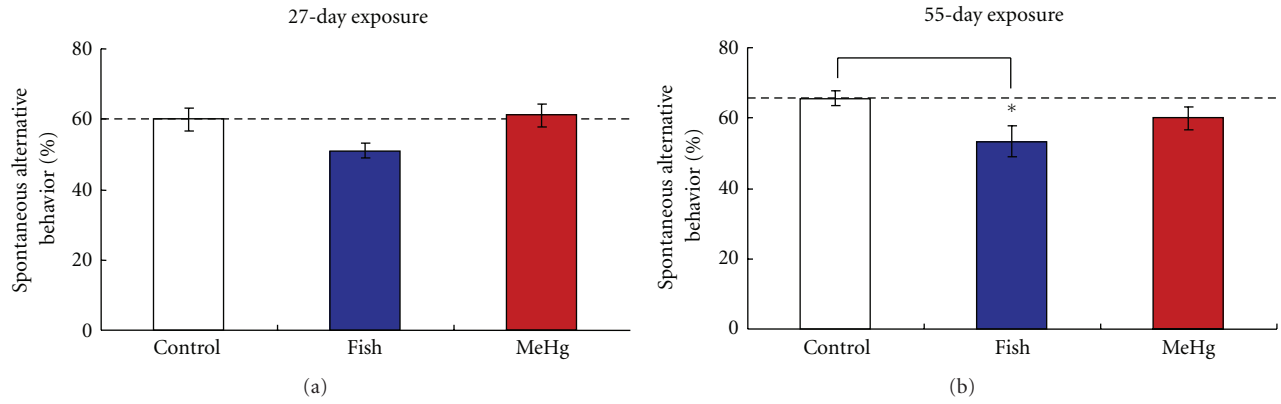

Figure 2: Mice behavior in a Y-shaped maze. Spontaneous alternations were recorded for 5 min (mean ± SEM, $n = 8$). The asterisk indicates a significant difference between the spontaneous alternations presented in the Y maze by mice fed the fish-containing diet compared to those fed the control diet, as determined with a one-way ANOVA followed by a Dunnett's multiple comparison method, $^*P < 0.05$, and $^{**}P < 0.01$.

After 84 days of contamination rats presented in their blood cells an increased lipid peroxidation and genotoxicity and an increased systolic blood pressure. Mercury concentrations were 1.31 μg/mL in blood, 0.87, 0.89, and 0.1 μg/g in liver, kidney, and brain, respectively. In the present study, we gave to mice a diet containing 0.24 μg MeHg/g, resulting after 57 days of exposure in mercury concentrations 6.5 and 1.1 times weaker in blood and liver but 8.6 and 4.1 times larger in kidney and brain, meaning that the distribution of methylmercury from blood to tissues is differing between rats and mice.

3.2. Behavioral Modification. The activity of mice was assessed in an open-field maze (Table 4). The quantity of emitted feces, along with the number of rearing events and the number of squares crossed, could not distinguish the three different groups of mice tested after 28 or 56 days of exposure. However, after 56 days of exposure the number of grooming events was significantly decreased for the fish diet mice as compared to the MeHg-fed and control mice, with this decrease reaching 45 and 40% of the values of the MeHg-fed and control mice, respectively. Additionally, after 56 days of exposure the time spent in center was significantly higher for the fish diet mice as compared to the MeHg-fed and control mice, with this increase representing 2 and 3-fold the values of the MeHg-fed and control mice, respectively. Anxious mice tend to avoid the center of the open field and to groom more frequently. Therefore, a decrease of grooming events associated to an increase of time spent in the center of the open field indicates an antianxious behaviour.

The Y-maze allowed for the testing of memory function in mice (Figure 2). Spontaneous alternation is a behavioural test dealing with spatial learning and memory. The purpose of the test for the mouse consists in remembering which

Table 4: Mice behavior in an open field maze[a].

	Control		MeHg[b]		Fish[c]	
	28th day	56th day	28th day	56th day	28th day	56th day
Squares crossed	93 ± 7	86 ± 4	86 ± 6	83 ± 5	78 ± 6	84 ± 5
Rearing	23 ± 2	26 ± 2	21 ± 2	28 ± 3	22 ± 3	24 ± 2
Grooming	1.7 ± 0.3	1.25 ± 0.16	1.5 ± 0.2	1.38 ± 0.18	1.9 ± 0.3	$^{\circ,\S}$**0.75 ± 0.16**
Fecal pellets	1.4 ± 0.8	3.8 ± 1.2	3.7 ± 1.8	1.7 ± 0.6	3.9 ± 1.1	2.3 ± 0.9
Time spent in center (s)	5.3 ± 1.0	3.6 ± 0.4	3.8 ± 0.5	4.7 ± 1.2	3.7 ± 0.6	$^{\circ\circ,\S}$**10.0 ± 1.5**

[a] Mean ± SEM, $n = 8$.
[b] MeHg: mice were fed a MeHg-containing diet.
[c] Fish: mice were fed a 4.9% aimara flesh-containing diet.
Values appearing in bold characters highlight those significantly different from the control values.
Circles indicate significant differences in behavioral parameters for mice fed the fish-containing diet compared to those fed the control diet, as determined with the Students test, $^{\circ}P < 0.05$, and $^{\circ\circ}P < 0.01$.
The symbol § indicates significant differences in behavioral parameters for mice fed the fish-containing diet compared to those fed the MeHg-containing diet, as determined with the Students test, $^{\S}P < 0.05$.

maze arm was last visited and trying to enter as many different arms as possible. The task is testing hippocampal memory and can be weakened by lesions to the hippocampus. The sequence of arm entries and total amount of arm entries are scored and a percentage is calculated. The cognition of the animal can be assessed based on the score where a lower score is considered cognitively impaired. After 55 days of exposure, mice fed the fish diet displayed a significant lower rate of spontaneous alternations in the maze compared to MeHg-fed and control mice, strongly suggesting that these animals had difficulties remembering which arm was entered last. After 27 days of exposure, mice fed the fish-containing diet already presented a downward trend concerning the rate of spontaneous alternations but this was statistically nonsignificant.

4. Neurotransmitters and Associated Metabolites Quantification in Various Brain Structures

The levels of noradrenalin (NE), dopamine (DA), and 5-hydroxytryptamine or serotonin (5-HT) were quantified in various brain structures after two months of feeding with mercury-containing diets. In addition to these three neurotransmitters, the metabolite resulting from the degradation of serotonin, 5-hydroxyindole-acetic acid (5-HIAA) and those from the degradation pathway of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were also quantified (Table 5).

No differences in levels of neurotransmitters or their metabolites could be observed after one month of exposure in the various brain structures collected. Only after 2 months of exposure could we see differences. The levels of serotonin appeared to be 2 times decreased in brainstem of mice fed the MeHg diet as compared to those fed the control diet. However, the level of serotonin in the same brain structure from mice fed the fish diet was not significantly different from that of the MeHg-containing diet, and therefore this decrease cannot account for the differences observed in mice behavior. And the same holds true with the 5-HIAA level which was 67% higher in the striatum of mice fed the fish diet compared to control mice, since the value from the MeHg-fed mice was not significantly different. In cerebellum of mice fed the fish diet, the DOPAC level was 2-times higher than that of the control mice. For mice fed the MeHg diet, the DOPAC level in this brain structure was as high as for mice fed the fish diet, but due to a great variability of values the mean could not be considered significantly different from that of control mice, and therefore the values from both the MeHg and fish diets cannot be taken as different. The dopamine metabolite DOPAC levels were significantly increased in hippocampus and striatum of mice fed the MeHg diet as compared to those fed the control diet, with increase factors reaching 15- and 2.6-fold the values of the control mice, respectively. Accordingly, the dopamine metabolite HVA level was 2.6 times increased in striatum of mice fed the MeHg diet as compared to those fed the control diet. It has already been described the striatal increase in dopamine and its acidic metabolite DOPAC after exposure of rats to MeHgCl [22]. However in this latter study the dose of injected MeHgCl was very high (0.1–2 mg/kg/day) compared to the dose used through diet in the present study (50 μg/kg/day), suggesting that striatum is more sensitive to methylmercury associated to fish than added pure in the diet. In hippocampus of mice fed the fish diet, the DOPAC level was significantly intermediate between those of mice fed the control and MeHg diets, since it reached 5 and 0.33 times those of control and MeHg-fed mice, respectively. Additionally, the metabolic turnover was calculated as the ratio of the sum of DOPAC and HVA levels to dopamine to estimate the activity of this metabolic pathway. We calculated a dopamine metabolic turnover of 0.36, 0.26, and 0.52 for mice fed the control, MeHg-containing, and fish-containing diets, respectively. This indicates that the rate of consumption of dopamine is greater in hippocampus of mice fed the fish-containing diet compared to the other two diets. Previous reports demonstrated that hippocampal dopamine receptors might be necessary for executive function including working memory [23]. The greater dopamine consumption in hippocampus might show the abnormality of hippocampal dopamine receptors. This may explain the decreased

Table 5: Neurotransmitter levels and associated decay products in brain structures of mice fed MeHg- and fish-containing diets.

Brain structures and diets	Days	Neurotransmitters and decay products (pg/mg, mean ± SEM, $n = 3$)					
		NE	DA	DOPAC	HVA	5-HT	5-HIAA
Frontal cortex							
Control	28	10.8 ± 3.7	2.1 ± 0.8	1.4 ± 0.6	3.5 ± 1.4	4.3 ± 1.8	8.2 ± 3.2
	56	11.1 ± 3.2	1.8 ± 0.4	0.25 ± 0.02	1.4 ± 0.2	4.4 ± 2.6	3.6 ± 0.8
MeHg[a]	28	9.4 ± 1.5	2.0 ± 0.6	1.1 ± 0.5	1.7 ± 1.0	2.2 ± 0.6	4.4 ± 0.6
	56	5.8 ± 0.7	1.7 ± 0.3	0.36 ± 0.08	0.9 ± 0.6	0.3 ± 0.3	2.6± 0.5
Fish[b]	28	16.9 ± 4.9	2.2 ± 0.7	0.9 ± 0.2	3.8 ± 1.5	5.8 ± 2.2	6.9 ± 0.7
	56	6.8 ± 3.9	2.0 ± 0.2	0.7 ± 0.2	1.1 ± 0.6	2.1 ± 1.2	5.7 ± 2.9
Brainstem							
Control	28	15.7 ± 2.8	7.2 ± 1.8	1.1 ± 0.5	2.0 ± 1.0	4.4 ± 0.8	21 ± 7
	56	12.3 ± 1.4	3.4 ± 0.4	0.56 ± 0.11	0.8 ± 0.1	4.7 ± 0.1	10 ± 1
MeHg	28	13.2 ± 0.2	3.6 ± 0.3	0.73 ± 0.04	1.04 ± 0.08	4.1 ± 0.3	11 ± 1
	56	8.4 ± 4.0	2.3 ± 0.7	0.68 ± 0.19	0.73 ± 0.05	*__2.3 ± 0.6__	8.9 ± 0.7
Fish	28	14.3 ± 1.7	6.3 ± 1.5	1.2 ± 0.6	1.8 ± 0.8	4.8 ± 0.6	15 ± 3
	56	10.2 ± 2.2	3.2 ± 0.8	0.63 ± 0.15	0.8 ± 0.1	3.1 ± 1.0	10 ± 2
Hippocampus							
Control	28	18.5 ± 4.6	9.4 ± 3.6	6.6 ± 5.8	5.4 ± 3.6	7.1 ± 2.1	26 ± 7
	56	7.9 ± 1.8	2.4 ± 0.7	0.17 ± 0.03	0.7 ± 0.1	5.0 ± 1.2	7.6 ± 1.9
MeHg	28	13.8 ± 2.4	4.6 ± 0.2	2.0 ± 0.8	3.2 ± 1.6	5.4 ± 1.3	12.3 ± 0.4
	56	14.4 ± 10.6	12.8 ± 0.8	*__2.55 ± 0.06__	0.8 ± 0.2	12.4 ± 9.7	49 ± 3
Fish	28	10.5 ± 2.4	4.2 ± 0.4	1.0 ± 0.7	1.8 ± 0.7	6.1 ± 1.9	10.1 ± 1.3
	56	12.5 ± 3.3	4.3 ± 1.2	°,§__0.85 ± 0.18__	1.4 ± 0.2	7.5 ± 2.8	13 ± 3
Striatum							
Control	28	6.0 ± 1.3	120 ± 29	68 ± 15	56 ± 18	11.6 ± 4.8	19 ± 7
	56	1.3 ± 0.7	79 ± 9	31 ± 5	22.0 ± 1.4	5.7 ± 0.1	5.2 ± 0.3
MeHg	28	4.6 ± 1.5	53 ± 17	60 ± 21	32 ± 10	6.5 ± 1.9	10 ± 2
	56	2.8 ± 0.6	65 ± 10	*__82 ± 12__	*__43 ± 5__	7.5 ± 3.3	13.4 ± 5.1
Fish	28	5.2 ± 1.6	49 ± 11	23 ± 8	15 ± 4	5.0 ± 1.1	6 ± 1
	56	4.9 ± 1.6	61 ± 10	54 ± 14	32 ± 7	5.0 ± 1.0	°__8.7 ± 1.0__
Cerebellum							
Control	28	4.7 ± 1.4	1.2 ± 0.4	0.33 ± 0.11	1.0 ± 0.5	0.4 ± 0.1	5.1 ± 2.4
	56	9.0 ± 2.8	1.0 ± 0.3	0.36 ± 0.03	0.81 ± 0.09	2.5 ± 1.0	3.4 ± 0.7
MeHg	28	6.2 ± 0.9	0.96 ± 0.08	0.29 ± 0.16	0.47 ± 0.05	0.38 ± 0.07	2.6 ± 0.1
	56	6.3 ± 1.4	0.5 ± 0.2	0.59 ± 0.21	0.5 ± 0.1	1.6 ± 1.0	3.3 ± 1.1
Fish	28	5.1 ± 0.7	0.5 ± 0.2	0.18 ± 0.05	0.44 ± 0.05	0.23 ± 0.06	2.5 ± 0.2
	56	3.4 ± 1.7	0.6 ± 0.3	°°__0.61 ± 0.03__	0.3 ± 0.1	0.16 ± 0.09	1.7 ± 0.9
Midbrain							
Control	28	13.8 ± 2.8	6.8 ± 1.8	2.0 ± 0.8	3.3 ± 1.3	3.7 ± 0.8	22 ± 5
	56	17.0 ± 2.9	9.0 ± 1.4	2.1 ± 0.2	3.5 ± 0.5	9.5 ± 2.2	23 ± 4
MeHg	28	14.1 ± 2.5	8.6 ± 1.0	2.2 ± 0.4	3.7 ± 0.8	5.6 ± 1.2	19 ± 3
	56	11.8 ± 7.3	4.0 ± 1.1	2.5 ± 0.4	3.1 ± 0.4	7.8± 5.6	14.5 ± 0.9

Table 5: Continued.

Brain structures and diets	Days	Neurotransmitters and decay products (pg/mg, mean ± SEM, $n = 3$)					
		NE	DA	DOPAC	HVA	5-HT	5-HIAA
Fish	28	13.1 ± 1.4	7.1 ± 1.2	1.7 ± 0.6	3.2 ± 1.2	5.7 ± 1.0	17± 4
	56	10.0 ± 1.3	5.8 ± 1.1	1.8 ± 0.1	3.29 ± 0.05	2.8 ± 1.3	16 ± 1

[a] MeHg: mice were fed a MeHg-containing diet.
[b] Fish: mice were fed a 4.9% aimara flesh-containing diet.
NE: noradrenalin, DA: dopamine, DOPAC: 3,4-dihydroxyphenylacetic acid, HVA: homovanillic acid, 5-HT: 5-hydroxytryptamine or serotonin, 5-HIAA: 5-hydroxyindole-acetic acid.
Values appearing in bold characters highlight those significantly different from the control values.
Asterisks indicate significant differences in the levels of neurotransmitters or decay products in the brain structures of mice fed the MeHg-containing diet compared to those fed the control diet, as determined with the Students test, $^{*}P < 0.05$.
Circles indicate significant differences in the levels of neurotransmitters or decay products in the brain structures of mice fed the fish-containing diet compared to those fed the control diet, as determined with the Students test, $^{\circ}P < 0.05$, and $^{\circ\circ}P < 0.01$.
The symbol § indicates significant differences in the levels of neurotransmitters or decay products in the brain structures of mice fed the fish-containing diet compared to those fed the MeHg-containing diet, as determined with the Students test, $^{\S}P < 0.05$.

cognitive performance of the mice fed the fish diet after 2 months of exposure.

5. Conclusion

The two mercury-containing diets are differing by the fact that mercury was brought by the addition of either pure methylmercury chloride or by mercurial species associated to fish. Therefore, any differential effects observed between MeHg-containing and fish-containing diets should be attributed to different chemical species of mercury present in one diet and absent from the other and vice-versa along with the possible intervening role of fish PUFA and selenium. If the beneficial role of fish nutriments such as PUFA and selenium was to counteract MeHg effects, the pattern of effects displayed after exposure to the fish-containing diet should appear less severe than that observed with the MeHg-containing diet. But in the present study, the mice fed the fish-containing diet displayed worse behavioral performances than those fed the control and the MeHg-containing diets, although the brain structures of both mercury-contaminated groups of mice contained comparable levels of mercury and even less in the striatum of those fed the fish diet. Therefore, the different chemical species of mercury within fish flesh are likely to explain the deficit in cognitive performance in the Y maze and the decreased locomotory activity in the open-field maze.

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