Author's response to reviews

Title: Feeding mice with diets containing mercury-contaminated fish flesh from French Guiana: a model for the mercurial intoxication of the Wayana Amerindians

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Author's response to reviews: see over
Cover letter

Dear editor,
We have now changed the text to accommodate the desired formatting. The English language has been corrected by a professional, Christine Schwimmer, a specialist of scientific editing.

Answer to Luigi Manzo

We agree with the referee that an experimental study on mice should be carried out using an exposure timing encompassing the animal lifespan. To make it clearer the objective of our study we have now incorporated the following paragraphs in the Introduction section: “Dietary MeHg is readily and efficiently absorbed by the human gastrointestinal tract, to a reported level of 95% to 100% [9]. However, nothing is known about the MeHg trophic transfer rate in mice at such low exposure doses. Thus, to establish our model and although our long-term objective is a follow-up of the dietary MeHg impact on mice metabolism all along the animals’ life, we had first to determine the fish regimen which given to mice would as closely as possible mimic a human contamination. More precisely, the main goal of this experiment was to select the MeHg-contaminated diet giving kidney and brain Hg concentrations in the order of what has been recorded in human kidneys and brains of heavy fish consumers in a general population [10,11]”, and in the Mat & Meth section: “However, the main goal of the present study is first to select a Hg diet content resulting in mice brain and kidneys mercury accumulations of the same amplitude than that of human heavy fish consumers. We thus made prepare three diets supplemented with 0.1, 1, and 7.5% of lyophilized fish flesh, along with a control diet devoid of flesh.”
A discussion of our results in line with the current MeHg risk assessment data is outlined in the discussion section.

Answer to Kunihiko Nakai

1/ About the mice fed the highest fish diet that died after 2 months. We have not recorded the changes in body weight because there were only 2 mice remaining, and thus statistical analysis was not possible. We agree that this reported fact could as well be coincidental and therefore we have deleted this observation from the text: “Interestingly, when some mice were kept alive for longer time periods, control, low and mid level diet fed mice could live for months, whereas mice fed with the highest fish diet died one month after day 34.”

2/ About the fact that the higher fish diet did not trigger effects that are nevertheless observed at lower MeHg concentrations. We have now developed this topic and incorporated into the text the following paragraph: “The biggest impact of fish diets on behaviour, mitochondrial respiratory rates and kidney gene expressions were observed with the low and mid level diets, not with the highest. Although surprising at first glance, many experimental results are now showing that above a given dose of contaminant, the effects observed at low dose vanish or differ qualitatively. For instances, the effects of arsenic at 5 or 50 µM on human lung cells exposed for 4 hours were compared. Increasing the dose of arsenic from 5 to 50 µM did not simply increase the magnitude of the change in the same set of genes or add additional genes. Rather, a completely different pattern of gene response between the lower and the higher dose was observed. Over the 1200 genes examined at both doses, only 16 of the 160 affected genes were altered at both doses [33]. In another study authors carried out a serial analysis of gene
expression (SAGE) on kidneys from mice exposed to chronic or acute uranyl nitrate contamination [34,35]. Only 16 genes were common to both SAGE lists and expressed the same way; 147 genes were differentially regulated in either one of the two conditions; 10 genes were common to both SAGE lists but expressed the opposite way, up-regulated under chronic exposure but down-regulated under acute exposure. These comparative patterns of gene expressions mean that when shifting from chronic to acute exposure the intensity of gene response does not increase as might have been expected but rather that the qualitative nature of the gene response is completely changed resulting in a modified tissue metabolism. In keeping with this, it has been shown that: a/ uranium is an endocrine disrupter in mice at low but not at high doses [36]; b/ after 7 days exposure, copper induced in the aquatic plant Hydrilla verticillata an increase of superoxide dismutase, glutathione peroxidase and catalase activities at low but not at high doses [37]; c/ cadmium triggered greater genotoxic damages on Xenopus laevis larvae at 0.5 than at 1 mg/l [38]; d/ carbon nanotubes induced in rainbow trout gills and intestine an increase of the Na\(^+\)K\(^+\)-ATPase activity at low but not at high doses [39]; e/ nanofullerenes induces in largemouth bass gills and liver a change in the pattern of lipid peroxidation at 0.5 ppm but not at 1 ppm [40]. These results suggest a model in which low doses of pollutant cause mild effects compatible with life, allowing animal resistance through adaptive response, whereas higher doses trigger acute effects threatening animal life, resulting in general stress response instead of adaptative. Additionally, the highest regimen is rich in fish flesh and it has been argued that the beneficial influence of nutrients from fish may counter any adverse effects of MeHg on the developing nervous system [41].”

3/ About a possible external contamination of hairs samples. The only source of Hg contamination could have been the food pellet dust polluting mice furs. However, for food pellets containing 5, 62, and 520 ng Hg/g we found hairs mercury concentrations equal to 81, 1579, and 10,364 ng/g, respectively. So, food pellet dust cannot explain the hairs data.

4/ Wayanas incorporate 10.7% of aimara flesh on a fresh weight basis. Since the corresponding sentence was quite unskilful, we deleted it, and rather make our goal clearer adding a new sentence: “However, besides its mercury content, Wayana’s diet incorporates up to 10.7% of aimara flesh, not 0.1%. To establish our model, we then had first to determine whether the aimara fish content of the Wayana’s regimen could exert any influence. However, the main goal of the present study is first to select a Hg diet content resulting in mice brain and kidneys mercury accumulations of the same amplitude than that of human heavy fish consumers. We thus made prepare three diets supplemented with 0.1, 1, and 7.5% of lyophilized fish flesh, along with a control diet devoid of flesh.”

5/ The article contains now two new tables. Table 1 is describing the nutriment content of the control and fish diets, and Table 2 is giving concentrations of various toxic metals including Cd. Details of nutriment and metal composition have been added in the text in the Mat & Meth section as follows: “These special diets have been manufactured by Special Diets Services (Witham, Essex, United Kingdom; French commercial representation: Dietex, Saint-Gratien, France). The control diet was mainly vegetal (Rat and Mouse n°1 maintenance diet, abbreviated to RM1 diet, Special Diets Services). According to Special Diets Services information RM1 diet is made up by blending wheat, barley, wheateed, de-hulled extracted toasted soya, soya protein concentrate, macro and micro minerals, soya oil, whey powder, amino acids, and vitamins. The nutriment compositions of the control RM1 and the three prepared regimen are given in Table 1 (the analysis have been carried out by Special Diets Services). A macro analysis of lyophilized H. aimara flesh nutriments has also been done. This flesh contains: 8.1 % moisture, 1.8 % crude fat, and 89.9 % crude protein. A comparison
of the diet compositions shows that there are no substantial differences between the control and the low and mid level diets. The main difference between the control and the high level diets lies in the crude protein content: 14% against 20% respectively. The mercury species contained in the control RM1 diet is the inorganic form since it is the one accumulated by plants whereas the contribution of the methylated species to the total mercury load was found to be over 95% in aimara flesh. The content in several other metals of the control and the three fish-containing regimen has been addressed, along with that of the lyophilized aimara flesh (Table 2). Metals have been assayed by ICP-MS (Antellis, Toulouse, France). The diets and fish flesh levels were below the detection threshold for Ag (<0.02 mg.kg\(^{-1}\)), As (<0.1 mg.kg\(^{-1}\)), Au (<0.05 mg.kg\(^{-1}\)), Bi (<0.02 mg.kg\(^{-1}\)), Sb (<0.5 mg.kg\(^{-1}\)), Sn (<0.5 mg.kg\(^{-1}\)), Tl (<0.05 mg.kg\(^{-1}\)), and V (<0.5 mg.kg\(^{-1}\)). The RM1 control diet contains greater metal concentrations than aimara flesh probably due to the fact that plants accumulate heavy metals from soil. Nevertheless, aimara flesh in addition to mercury is also richer in selenium than RM1 diet, a known feature of fish flesh. Consequently, besides mercury, the low and mid level diets are not distinguishable from the control diet in term of metal content, and the 7.5% fish diet contains two-time more selenium than the control diet.”

6/ We agree with the referee that mt gene expression could have been stimulated by the fish regimen in the kidneys since one might expect demethylation of MeHg. As outlined in the conclusion, we began a long-term study with mice fed the 0.1% fish diet. And in fact we observed mt genes differential up-regulation in the kidneys after 3 months exposure to the 0.1% diet. The extent of demethylation in this organ reached at this time 30% (data not shown). Most probably, 1 month exposure is not enough to produce the necessary quantity of inorganic Hg able to induce mt genes up-regulation within kidney cells.

7/ The numbers displayed in Table 6 are the differential gene expressions. In the Mat & Meth section the definition is now given: “The differential expression of a gene was calculated as the ratio of its β-actin normalized expression in fish-contaminated condition to that in the control condition.”

8/ About the significance of the fish diets. The conclusion has been enriched with the following paragraph: “The 7.5% fish-containing diet results in Hg brain concentrations equivalent to acute exposure cases and therefore cannot be useful to mimic human environmental cases. The 1% fish-containing diet is giving after just one month exposure blood, kidney, and brain Hg concentrations in the order of what has been recorded in human blood, kidneys, and brains of heavy fish consumers in a general population. The 0.1% fish-containing diet brings to mice the same mercury contamination pressure as that afflicting the Wayana Amerindians assuming a Hg trophic transfer rate of 100%, and it can be expected that after several months, the mercury levels in mice tissues be equivalent to those observed after one month feeding with diet containing 1% fish flesh.”