MATERNAL FISH CONSUMPTION REDUCES LIVER CONCENTRATIONS BUT DOES NOT AMELIORATE EFFECTS OF PBDE47 ON EARLY REFLEX DEVELOPMENT IN MURINE OFFSPRING.

Haave Marte[1], Folven Kristin Ingvaldsen [1], Glover Chris Neill [2], Lundebye Anne -Katrine[1].

1)National Institute of Nutrition and Seafood Research (NIFES), P.O.B 2029 Nordnes, N-5817 Bergen, Norway.
2) School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.

Introduction

Polybrominated Diphenyl Ethers (PBDEs) are a class of compounds widely used as flame-retardants[1]. PBDEs are lipophilic and environmentally persistent. As a result they bioaccumulate in food chains to the extent that they can now be detected in biological samples throughout the chain, including in humans. Furthermore, PBDE accumulation is increasingly linked to adverse effects on biological systems[2]. Harmful effects of PBDEs on the brain, for example, have been documented. These impacts include impaired memory and learning, altered behaviour, affected neurobehavioural development and reduced receptor density in animal models[3,4,5]. Although many studies have been performed to assess the effects of high levels of PBDEs, very few have attempted to mimic the low level, chronic exposure realistic to human consumption. As such there is limited scientific basis for dietary advice regarding the safety of PBDEs in seafood to at-risk-consumers (e.g. pregnant women). Furthermore, little is known regarding the potential for nutrients contained within the seafood matrix to offset, or compensate for, any PBDE-induced impairment. Previous studies, however, have shown the effect of nutrients on both the uptake and toxicity of a diverse range of seafood contaminants[6,7,8,9,10]. The aim of this study was to determine possible neurobehavioural responses to low-level, chronic PBDE exposure in mice, and also the possible interaction of nutrients from seafood on the uptake or effects of PBDE.

Materials and methods

The experiment was approved by the Norwegian Animal Research Authority (NARA/FDU). Female and male Balb-c/A mice were supplied by Taconic Inc., Denmark, and housed under standardized conditions. The females were exposed to experimental feeds during mating, gestation and lactation. Food and water was provided *ad libitum*, and daily feed intake was monitored. Two parallel sets of diets were produced. The standard diet used casein as a protein source and soy-bean oil as a lipid source, while the experimental diet used 15% (per weight) lyophilized, uncontaminated salmon as the main sources of protein and fat. All diets contained 17% protein and 10% lipid. PBDE47 was added to produce three experimental groups within the fish and casein diets: Un-spiked control, Low dose and High dose (0.5, 6 and 2000 ug PBDE47/kg feed, respectively). This resulted in six diets: Fish Control = FC, Fish Low dose = FL, Fish High dose = FH, Casein Control = CC, Casein Low dose = CL and Casein High dose = CH. The detectable, yet low, content of PBDE in the control feed, demonstrates the ubiquitous nature of these compounds in the environment, even in fish raised on diets specifically designed to limit contaminant burden. The low dose resulted in a PBDE concentration which is realistic in fish from many areas, but approximately two to three times higher than the mean concentration presently found in farmed Atlantic salmon (*Salmo salar*) from Norway (www.nifes.no/seafood data). The high dose aimed at the No Observable Adverse Effect Level (NOAEL) to avoid clinical toxicity in both dams and pups.

Pups born to exposed and unexposed mothers were subjected to a suite of assays to assess their neurobehavioural and physical development. Pups were tested every third day from day five after birth (PND5) with a modified "Fox-battery"[11] by an experimenter blinded to the exposure-groups. These tests primarily probed the development of early reflexes. Grip strength was tested on PND15 and PND18. On PND18 a test of motor coordination, and exploratory behaviour in the elevated plus maze was performed. Randomly selected pups from each litter were euthanized on PND5, 11 and 18. Litter sizes were reduced to a maximum of five pups on PND5. PBDE concentrations in livers were analysed by GC/MS (SIM mode) with Negative Chemical Ionization.

The software SPSS15.0 for Windows was used for statistical analysis. The litter was treated as the statistical unit. Litter-means were compared using One-Way ANOVA, or Kruskal-Wallis where appropriate. The statistical level of significance was set at $\alpha=0.05$.

Results

There were no differences in feed intake, and correspondingly no significant group-differences in bodyweight among groups of dams or pups were consistently observed. No clinical symptoms of toxicity or difference in tissue indices (e.g. liver somatic index) were found. The breeding performance and reproductive success was statistically indistinguishable among groups. The reproductive success was low for CL and FL with three and four litters respectively, whereas the other groups had between five and seven litters. The mean litter-sizes of the casein-fed and the fish-fed groups collectively was seven and five pups per litter, respectively, but these were not significantly different.

Significantly lower PBDE concentrations were found in the pup livers from FH compared to CH on PND11 (Figure 1). This was also true when PBDE was normalized to pup body-weight, which could otherwise be a bias due to slightly different litter-sizes and thus pup-sizes. The trend was similar at both PND11 and PND18 for the low PBDE dose, however not significant. Feed- and PBDE intake in dams of the CH and FH groups, as well as liver PBDE47 concentrations, were not significantly different.

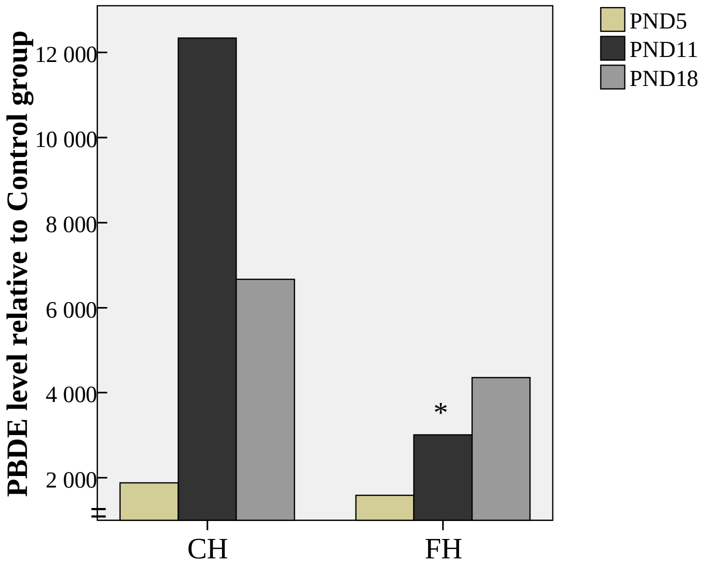

Figure 1: The mean PBDE47 concentrations in livers of high-dose pups at different sampling times relative to control. The fish group generally had lower PBDE levels in their liver than the casein-group, and the difference was significant on PND11 (asterix denotes significant difference). Experimental diets: CH=Casein High-dose, FH=Fish High-dose .

Comparing PBDE in pup livers relative to their mothers on PND18 may give information on transfer rates or uptake in the pups. A high ratio indicates hat much of the PBDE in the dam is transferred to the pup, this pup-to-mother ratio is higher for all casein-fed groups compared to fish-fed groups (Figure 2). Interestingly, this difference is prominent even for the control groups, which have low liver PBDE47 concentrations.

Despite significant differences in accumulated PBDE47, behavioural analyses revealed few significant differences among groups. However, significant differences were found in some of the early reflex-tests.

The “hindlimb grasp” reflex test was significantly less developed in the FH group than FC and CC at PND5 (Figure 3). There was also a trend (not significant) towards a lower grasping reflex with increasing PBDE exposure for the casein groups. In the righting-reflex test on PND8, FH scored significantly lower than CC and CL, and there was a trend towards lower righting reflex with increasing PBDE exposure for both diets (data not shown).

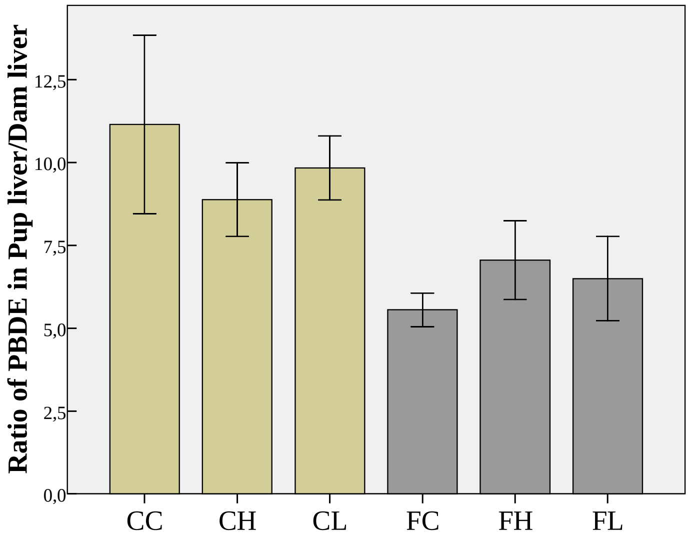

Figure 2. The ratio of PBDE47 (ng/g ww) in the livers of pups relative to their mothers on PND18. Since the PBDE concentrations in dam livers were not significantly different among groups, the PBDE-ratio seems to be related to the source of lipid and protein in the diet, rather than determined by the PBDE concentration in the dam. Experimental diets: CC=Casein Control, CL=Casein Low dose, CH=Casein High dose, FH=Fish Control, FL= Fish Low dose, FH= Fish High dose. Error bars represent +/- 1 Standard Error of the Mean (S.E.M.)

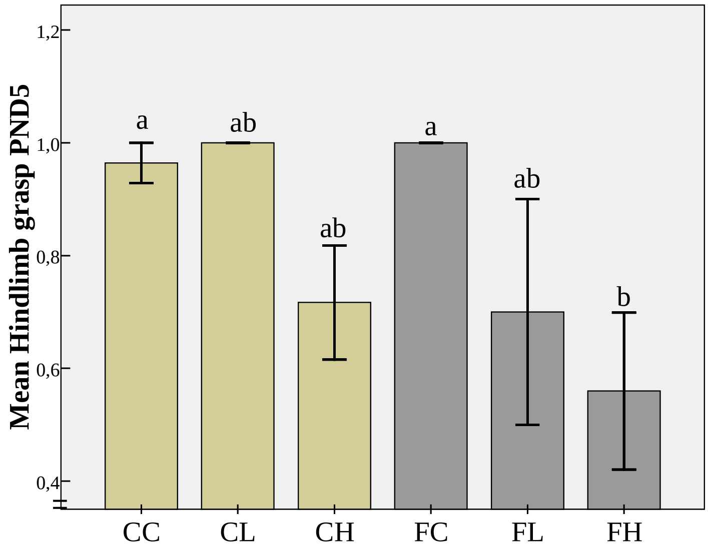

Figure 3. Mean litterscores in the Hindlimb Grasp test on PND5. Different letters above the error bars denote significant differences (p<0.05). . Experimental diets: CC=Casein Control, CL=Casein Low dose, CH=Casein High dose, FH=Fish Control, FL= Fish Low dose, FH= Fish High dose. Error bars represent +/- 1 Standard Error of the Mean (S.E.M.)

Discussion

The present study demonstrated that the PBDE47 levels in pup livers after maternal exposure were dependent on the concentration of PBDE47 in the mother's diet. There was also an effect of the lipid- and protein source in the feed, where inclusion of fish reduced the pup liver concentration of PBDE47 compared to a fish-free diet. This reduction was evident despite normalised pup bodyweight, maternal PBDE47 consumption and dam liver PBDE-concentrations, indicating a difference in PBDE47 uptake, excretion or metabolism in the pups. There are apparently no previous studies suggesting altered metabolism of PBDE's related to fish consumption, however there are studies suggesting that fish oil diets can enhance the metabolism of other persistent organic pollutants, e.g. penta- and hexachlorobenzene (PECB and HCB)[12,13]. Umegaki and Ikegami[12] showed overall lower PECB levels after fish-oil consumption but no differential PECB distribution depending on the diet in mice. However, previous studies have shown that PBDE is found primarily in body fat, with lesser levels in kidney, liver and brain in mice[14]. Further analysis of PBDE levels in other body tissues including dorsal fat and brain, will support the hypothesis that the observed difference in liver concentrations may be due to differential partitioning of PBDE among body fat resources.

The examination of offspring behaviour in the present study showed some impact of PBDE47 on reflex development at an early age, both for the fish and casein diets. This study supports the findings of Branchi *et al*.[4] who found few effects on reflex-development in mice after gestational exposure to PBDE99. Unfortunately, poor reproductive success in the CL and FL groups reduced the n-value, and limited the power of the statistical analyses. A reduction in hind-limb grasp reflex on PND5 was seen for both fish and casein diets with high PBDE exposure. However, the results were only significant for FH. For the righting-reflex, there was also a trend towards reduced reflex development with increasing PBDE-exposure. There seemed to be no protective effect of the fish, as again, only the FH diet significantly impaired reflex development. However, this test may be influenced by pup size, i.e. heavy pups may be less mobile than leaner pups. The fish-groups had more body-fat than the casein-groups, thus these pups may have been less likely to right themselves. This is in contrast to the argument of Branchi *et al*.[4], where larger pups were believed more capable of righting themselves, however this was with a different mouse strain.

Whatever the reason for the reduced liver-concentrations of PBDE47 in the present study, it is assumed that a reduction of liver-PBDE concentration would reduce the toxic effect in the animals. This does not appear to be the case here, and the reason for the discordance between liver accumulation and effect requires further investigation.

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