National Institute for Public Health and the Environment
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RIKILT WAGENINGEN UR

FRONT OFFICE FOOD AND CONSUMER PRODUCT SAFETY

Intake of methylmercury in children aged 2 to 15 in the Netherlands

Risk assessment requested by:	NVWA-BuRO and Netherlands Nutrition Centre
Risk assessment performed by:	RIVM and RIKILT
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Subject
Intake of methylmercury by children aged to 2 to 15 years in the Netherlands

Question
The Front Office received a question of the Office for Risk Assessment and Research of the Netherlands Food and Consumer Product Safety Authority (NVWA-BuRO) and the Netherlands Nutrition Centre (Voedinsgcentrum) in relation to the report "Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood" of the European Food Safety Authority (EFSA, 2015). In this report, EFSA indicates that (young) children may exceed the tolerable weekly intake of methylmercury (MeHg) via fish consumption. However, due to differences in fish consumption (both amount and type of fish) between countries within Europe, EFSA states that a general advice about fish consumption at the European level is not feasible.

The Netherlands Nutrition Centre advises women before and during pregnancy not to consume predatory fish, such as pike, pike perch, tuna or mackerel, due to a possible high intake of MeHg. For other population groups, including children, no specific advices are given. Based on the opinion of EFSA, NVWA-BuRO and the Netherlands Nutrition Centre have asked RIVM to perform a risk assessment of the exposure to methylmercury via food in (young) children in the Netherlands. The outcome of this assessment will form the basis for a decision on the need for such an advice for (young) children.

Specific questions to be answered in this assessment are:
1. What is the exposure to MeHg of (young) children in the Netherlands?
2. Does the current exposure to MeHg of (young) children result in a possible health risk related to MeHg?
3. Which fish types contribute most to the exposure?

Conclusion
1) The mean exposure to methylmercury (MeHg) equalled 0.2 µg/kg bw per week for children aged 2 to 15 years. The high exposure (99th percentile) of children was 0.9 µg/kg bw/week in the age class 2 to 6 years and 1.8 µg/kg bw/week in that of 7 to 15 years.
2) The health risk related to the exposure to MeHg via food in children aged 2 to 15 years in the Netherlands is negligible.
3) Cod and tuna were the main contributors to the exposure of MeHg at both the mean and high level of exposure. The reason for this is that these two fish types are consumed at high levels and that their MeHg concentrations are relatively high.

Additional remarks:

- It is noted that on the website of the Netherlands Nutrition Centre regarding a healthy diet before and during pregnancy, it is stated that the consumption of canned tuna during pregnancy is no problem[1]. There is however no difference between canned tuna and fresh tuna with respect to the levels of MeHg (EFSA,2012).
- It is also noted that on the same website page, it is stated that consumption of predatory fish during pregnancy should be avoided[1]. However, in order to reach a low-level exposure of the unborn child, and given the bioaccumulating properties of MeHg, it would be prudent for women with a wish to conceive to reduce their MeHg exposure by avoiding the consumption of fish with a high level of MeHg well before the intended pregnancy. This advice is given on the website of the Netherlands Nutrition Centre regarding heavy metals[2], but not repeated on the one related to a healthy diet before and during pregnancy. This information may therefore not be easily found by the relevant target population.

[1] http://www.voedingscentrum.nl/nl/mijn-kind-en-ik/zwanger/wat-kan-ik-wel-en-niet-eten-tijdens-mijn-zwangerschap-.aspx
[2] http://www.voedingscentrum.nl/encyclopedie/zware-metalen.aspx

Executive summary
In this risk assessment, the exposure to methylmercury (MeHg) via food in children aged 2 to 15 in the Netherlands was estimated and compared to the health-based guidance to assess whether the estimated exposure poses a possible health risk for this age group. The exposure was calculated by combining food consumption data, obtained from two national food consumption surveys conducted among 2 to 6-year-olds and 7- to-69 year-olds, with MeHg concentration values in fish and other seafood types. The exposure was calculated taking all possible consumption levels recorded in the food consumption databases into account resulting in a distribution of the exposure. From this distribution, it was estimated that the mean exposure to MeHg equalled 0.2 µg/kg bw per week for children aged 2 to 15. For reasons of comparison, also the exposure in 16- to 69-year-olds was estimated. The high exposure (99th percentile) of children was 0.9 µg/kg bw/week in the age class of 2 to 6 and 1.8 µg/kg bw/week in that of 7 to 15. Cod and tuna were the main contributors to the exposure at both the mean and high level of exposure. To assess whether these exposure levels pose a possible health risk, they were compared to the tolerable weekly intake (TWI) of 1.3 µg/kg bw. This comparision showed that the mean exposure of children aged 2 to 15 years to MeHg did not pose a health risk in the Netherlands. However, such a risk could not be excluded in the case of a high consumption of fish with a relative high MeHg level. The percentage of children with an intake exceeding the TWI was however low (< 4%). The TWI of MeHg is furthermore defined in such a way that if over a lifetime the intake does not exceed this limit value, the levels of MeHg in the body will remain below the level which might induce adverse effects. A high intake during childhood does therefore not result in an adverse effect, if this intake is offset by lower intakes at higher ages, as was observed in 16- to 69-year-olds. We therefore conclude that the health risk related to the exposure to MeHg via food in children aged 2 to 15 years in the Netherlands is negligible.

Introduction
Mercury (Hg) is a metal that is released into the environment from both natural and anthropogenic sources. Hg appears in three chemical forms: (i) elemental or metallic Hg (Hg^0), (ii) inorganic Hg (mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}) cations) and (iii) organic Hg.

Organic Hg compounds have at least one carbon atom covalently bound to the Hg atom. Methylmercury (MeHg) is by far the most common form in the food chain and exposure to MeHg occurs mostly via food. MeHg collects and concentrates especially in the aquatic food chain. In fish, MeHg accumulates in the muscle mass. This accumulation is related to the age of the fish and its position within the food chain, with predatory fish (shark, swordfish, marlin) having relative high levels. Of the total Hg content in fish muscle, 80 - 100% has been shown to be present as MeHg.

In 2015, EFSA released a report regarding the risks and benefits of consuming fish in relation to MeHg (EFSA, 2015). In this report, EFSA indicates that (young) children may exceed the tolerable weekly intake (TWI) of MeHg via fish consumption. However, due to differences in fish consumption (both amount and type of fish) between countries within Europe, EFSA considered a general advice about fish consumption at the European level as not feasible.

In the present assessment, the exposure to MeHg was calculated in children aged 2 to 15 years in the Netherlands to establish if there is a health risk related to the current consumption levels of fish in the Netherlands regarding MeHg. Furthermore, also the fish types contributing most to the exposure was estimated.

Toxicokinetics (EFSA, 2012)
MeHg is much more extensively and rapidly absorbed after oral intake than inorganic mercuric and mercurous salts. Absorption rates are higher than 80% and do not greatly vary between humans and experimental animals. In humans, MeHg is recycled through the enterohepatic system.

MeHg crosses cell membranes, the placenta and the blood-brain barrier, allowing accumulation in the fetus and the brain. Tissue concentrations tend to be constant relative to blood levels. In umbilical cord blood, total Hg and MeHg levels are generally higher (factor 1.7 – 2.2) than in maternal blood at parturition. Total Hg in cord tissue correlates with that of MeHg in umbilical cord tissue, and total Hg and MeHg in cord tissue correlate with total Hg in cord blood. Total Hg in cord blood has been shown to correlate with maternal total Hg in hair, a biomarker of the maternal body burden of MeHg and, hence, maternal blood. A significant relationship was reported between fish consumption during pregnancy and total Hg in cord blood.

In the human body, MeHg has a half-life of approximately 70 - 80 days. MeHg elimination in humans mainly occurs via the biliary route after conjugation with glutathione in the liver. In the gastrointestinal tract, MeHg is partly converted by the intestinal microflora to mercuric Hg, which is less effectively reabsorbed in the gut and is therefore excreted via faeces. Approximately 90% of MeHg is excreted by the faecal route as mercuric Hg.

Toxicology (EFSA, 2012)
Neurodevelopmental toxicity of MeHg (cerebral palsy and severe developmental retardation) in a population highly exposed from environmental sources was observed in the 1950s in Minamata, Japan, in association with consumption of highly contaminated fish during pregnancy. These findings were confirmed in 1972 in Iraq, in association with the consumption of contaminated seed.

Several prospective studies longitudinally follow the postnatal cognitive development of cohorts of children which have been prenatally exposed to MeHg. The results of cohort studies from the Faroe Islands have consistently indicated that prenatal MeHg exposure has an detrimental effect on cognitive development (follow-up period: 14 years). From the Faroese study, a 95% lower

confidence limit of the benchmark dose corresponding with a 5% decrease in cognitive functioning of 12 mg/kg in maternal hair was selected a health-based guidance value.

A similar study from the Seychelles revealed an apparent no-observed effect level (NOEL) at a Hg level of approximately 11 mg/kg maternal hair.

Dervation of the Tolerable Weekly Intake (EFSA, 2012)
In deriving a Health Based Guidance Value, EFSA applied a "body burden" approach (for details, see Appendix A). Here, based on the results of the Faroese and Seychelles studies, the critical amount of MeHg in the maternal body ("body burden"), i.e. maternal blood, which, when exceeded during pregnancy, may induce neurodevelopmental toxicity in the fetus, was taken as the starting point for the calculation of the long-term daily dietary intake leading to this "body burden". The calculated exposure was corrected by means of an assessment factor, resulting in a daily intake of 0.19 µg/kg bw, equivalent to a TWI of 1.3 µg/kg bw.

Extrapolation to young children
In deriving the TWI, the concentration of MeHg in the blood of pregnant women, which, when exceeded, may induce neurodevelopmental toxicity in the fetus, was estimated. Furthermore, the concentration of MeHg in cord blood may be twice as high as the concentration in maternal blood. Assuming concentrations of MeHg in cord blood to be representative for the exposure of the fetal brain to MeHg and the brain of young children to be as sensitive for MeHg as the fetal brain, the cord blood MeHg concentration may be used as follows as a benchmark for the evaluation of the MeHg exposure of young children: MeHg exposure of young children → human kinetic model → blood concentration in young children ↔ critical cord blood concentration) (for details, see Appendix A).

Exposure

Food consumption data
In the current assessment, the dietary exposure to MeHg in children aged 2 to 15 years was assessed. For this, we used food consumption data of the Dutch National Food Consumption Survey (DNFCS)-Young Children performed in 2005/2006 (Ocké et al., 2008) and the DNFCS 2007-2010 (van Rossum et al., 2011). DNFCS-Young children 2005/2006 covers the dietary habits of children aged 2 to 6 years, and DNFCS 2007-2010 those of people aged 7 to 69 years. For the assessment, the daily consumption patterns were used as input. See Appendix B for a description of the food consumption data used in the assessment. For reasons of comparison, we also estimated the exposure to MeHg in the population aged 16 tot 69 years.

Concentration data
To obtain MeHg concentration data, total Hg concentration data present in the Quality Agricultural Products (KAP) Database) as analysed in fish and other seafood were used. These data were derived from monitoring programmes performed in the Netherlands by RIKILT Wageningen UR, and were targeted at locations where fish and other seafood are generally caught. It is expected that fish and other seafood caught on these sites have similar MeHg concentrations to those present in most other marine waters. All other foods (such as cereals, vegetables, fruit, etc.) were assumed to contain no MeHg, and were therefore not considered in the exposure assessment.
Concentrations below the limit quantification (LOQ) were assigned a concentration equal to ½LOQ[3]. This accounted for < 5% of the samples.

[3] Since the majority of the samples contained total mercury at concentrations above the LOQ (Appendix C), assigning either 0 µg/kg product, ½LOQ, or the limit values themselves to the samples with concentrations below LOQ resulted in negligle differences in exposure estimates. We therefore only present in this assessment the exposure estimates based on assigning ½LOQ to these samples as being the most realistic approach.

In order to assess dietary exposure to MeHg based on total Hg concentrations, it was assumed in accordance with EFSA (2015) that total Hg consisted for 100% of MeHg in fish meat and fish products, and for 80% of MeHg in other seafood. For several consumed fish types and other seafood, no concentration data were available. For these fish types, mean MeHg concentrations were obtained from the report by EFSA (2012).

For more details about the concentration used in the assessment and an overview of the concentration data per fish and other seafood species, see Appendix C.

Matching food consumption and concentration data
Because MeHg is analysed in raw fish and other seafood, the Conversion model Primary Agricultural Products (Boon et al., 2009; van Dooren et al., 1995) was used to translate the MeHg concentrations analysed in fish and other seafood to those in consumed foods. In this way the concentrations analysed could be linked directly to consumption in the assessment. See Appendix D for more details.

Exposure modelling
The exposure to MeHg via food was calculated using Monte Carlo Risk Assessment programme (MCRA), Release 8 (de Boer et al., 2015) with the use of the Model-Then-Add approach (van der Voet et al., 2014). In this approach, the exposure is modelled for subsets of the diet (single foods or food groups), and then added to obtain the overall intake distribution. For more details, see Appendix E. All daily estimated exposures (based on two recorded days per person) were divided by their corresponding individual body weight and expressed in µg/kg bw per week. For this, the daily exposure estimates were multiplied by 7. The reported percentiles of the exposure to MeHg are P50 (median), P95 and P99, as well as the mean. The uncertainty associated with the input variables food consumption and concentration data was evaluated by an uncertainty analysis using the bootstrap approach. This uncertainty analysis covers the sampling uncertainty in the analysed foods and food consumption data. For more details, see Boon et al. (2009). The uncertainty is summarised by a 95% uncertainty interval around the resulting exposure percentiles.

Results
Table 1 lists the exposure percentiles to MeHg for children aged 2 to 6 years and 7 to 15 years. The mean exposure equalled 0.2 µg/kg bw per week in both age groups. The P99 of exposure ranged from 0.9 µg/kg bw per week in 2- to 6-year-olds to up to 1.8 µg/kg bw per week in 7- to 15-year-olds. Given the uncertainty around the exposure estimates due to the sampling size of the concentration and consumption database, the P99 of exposure could be as high as 3.8 µg/kg bw per week in 7- to 15-year-olds.

Of the 2- to 6-year-olds, 0.4% had an estimated intake exceeding the TWI of 1.3 µg/kg bw per week. In the age group of 7 to 15 years, this percentage increased to up to 1.6%. Given the uncertainty around the percentage of children exceeding the TWI, this percentage could be as high as 3.9% in 7- to 15-year-olds. For persons aged 16 to 69 years, only 0.2% exceeded the TWI.

Table 1. Exposure to methylmercury (µg/kg bw per week) of the Dutch population (2-69 years) due to consumption of fish

Age (years)	Exposure to MeHg (µg/kg bw per week)[a]				% exceeding
	mean	P50	P95	P99	TWI
2-6	0.2 (0.1-0.2)	0.1 (0.1-0.1)	0.5 (0.3-0.7)	0.9 (0.5-1.5)	0.4 (0.0-1.4)
7-15	0.2 (0.1-0.3)	0.1 (0.0-0.1)	0.6 (0.3-1.0)	1.8 (0.9-3.8)	1.6 (0.4-3.9)
16-69	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0.5 (0.4-0.8)	0.7 (0.6 -0.9)	0.2 (0.1-0.4)

[a] Concentrations below LOQ were assigned a value of ½LOQ (medium bound scenario).

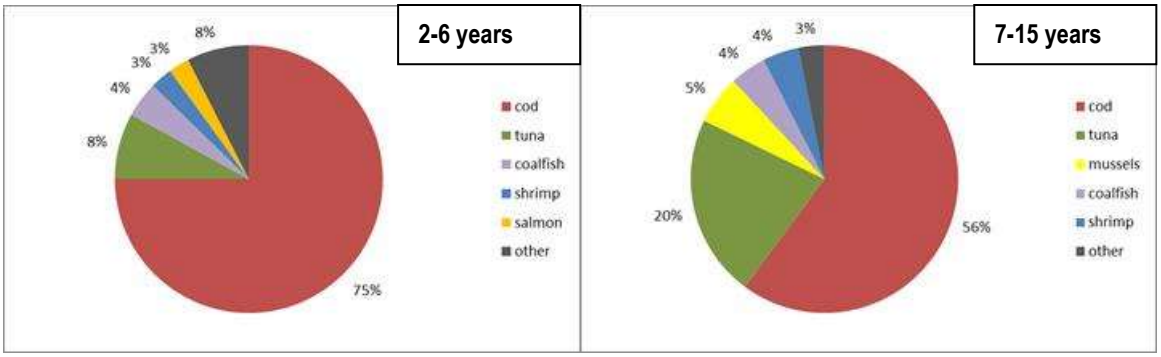

Figure 1. Contribution (%) of top five of fish and seafood types and remaining fish and sea food types to the dietary exposure to MeHg in children aged 2 to 6 years and persons aged 7 to 15 years in the Netherlands in which samples with MeHg concentrations below limit of quantification (LOQ) were assigned a value of ½LOQ (medium bound scenario).

Figure 1 shows the relative contribution of different fish and other seafood types to the total dietary MeHg exposure in both age groups. It is clear that cod and tuna were the main contributors to the exposure of MeHg. The reason for this is that these two fish types are consumed at high levels and that their MeHg concentrations are relatively high (Appendix C).

Risk assessment

In Table 1, the MgHg exposure via food of 2- to 6-, 7- to 15- and 16- to 69-year-olds is compared with the TWI for MeHg. As shown in Appendix A, an exposure at the level of the TWI guarantees that in children the blood concentration of MeHg is a factor 2 lower in comparison with the MeHg level in cord blood, which, when exceeded, is associated with the induction of neurodevelopmental toxicity in the unborn. Assuming that the developing central nervous system in children is as sensitive for MeHg as that in the fetus, the exposures listed in Table 1 do not pose a toxic risk to children in the Netherlands.

However, though the current MeHg exposure of children may not pose a toxic risk for themselves, any structural increase of the long-term dietary intake above the TWI will lead to unwanted accumulation of MeHg in the body. In the case of girls, this could lead to a body burden which, at the beginning of pregnancy, poses an unwanted toxic risk for the fetus.

Discussion

- The mean MeHg intake estimated in Dutch children was higher than that estimated by EFSA (2012), whereas the estimated P95 intake was lower. EFSA estimated the mean intake at 0.09 and 0.14 µg/kg bw per week in 2-year-olds and 3- to 6-year-olds, respectively. Corresponding numbers for the P95 exposure were 0.68 and 0.75 µg/kg bw per week. The results presented here for the Netherlands are expected to be more specific, because detailed information about consumption and concentrations in the Netherlands were used, as well as a more refined exposure assessment model.
- The number of analyses per fish and seafood type included in the assessment were small (Appendix C). To address this, the concentration data were included in the assessment as a distribution instead of using onlyt the observed values. Because of this, also concentrations above and below the observed values were included in the assessment. For tuna, a major contributor to the exposure, we only had a concentration of MeHg obtained from EFSA (2012). This level was however based on 125 samples. For cod, the average level (102 µg/kg) was close the the level reported by EFSA (2015) for cod / whiting (94 µg/kg). Although the number of samples was small, we considered these values as the best available for the exposure assessment to MeHg in the Netherlands.
- In this study, we calculated the exposure distribution of MeHg across three populations of individuals. The time-basis of such a distribution can be chosen freely. MCRA calculates exposures on a per-day basis (µg/kg bw per day). For an easier comparison to the traditional

TWI, these values were multiplied by 7 to express exactly the same exposures on a per-week basis (µg/kg bw per week).
- The current risk assessment of MeHg makes use of one-compartmental modeling for human MeHg kinetics. Although this way of modeling may suffice for the accumulation of MeHg in the body as a whole, it does not allow for the modeling of placental transfer and fetal kinetics, since a placental and a foetal compartment are not included in this model. Therefore, the modeling approach as performed in the present evaluation can only serve as a first tier in assessing human MeHg kinetics during pregnancy and MeHg kinetics in young children.

Conclusion

In the Netherlands, the mean exposure to MeHg was 0.2 µg/kg bw per week in 2-to 15-year-olds (with uncertainty ranging from 0 to 0.3 µg/kg bw per week). The P99 of exposure ranged from 0.9 µg/kg bw per week in 2- to 6-year-olds (with uncertainty ranging from 0.5 to 1.5 µg/kg bw per week) and up to 1.8 µg/kg bw per week in 7- to 15-year-olds (with uncertainty ranging from 0.9 – 3.8 µg/kg bw per week). Of the 2- to 6-year-olds, 0.4% had an intake exceeding the TWI. In the age group of 7 to 15 years, this percentage increased to 1.6%. Given the uncertainty, this percentage could be as high as 3.9% in 7- to 15-year-olds. Cod was the predominant fish species consumed by 2- to -6-year-olds. Next to cod, tuna also substantially contributed to the MeHg exposure of 7- to 15-year-olds.

Compared to the TWI of 1.3 µg/kg bw, the mean exposure of children aged 2 to 15 years to MeHg does not pose a health risk in the Netherlands. However, such a risk cannot be excluded in the case of a high consumption of fish with a relative high MeHg level. The percentage of children with an intake exceeding the TWI was however low (< 4%) (Table 1). The TWI of MeHg is furthermore defined in such a way that if over a lifetime the intake does not exceed this limit value, the levels of MeHg in the body will remain below the level which might induce adverse effects. A high intake during childhood does therefore not result in an adverse effect, if this intake is offset by lower intakes at higher ages, as observed in 16- to 69-year-olds (Table 1). We therefore conclude, based on the current assessment, that the health risk related to the exposure to MeHg via food in children aged 2 to 15 years in the Netherlands is negligible.

Additional remarks:

The authors of this evaluation note the following:
- It is noted that on the website of the Netherlands Nutrition Centre regarding a healthy diet before and during pregnancy[1], it is stated that the consumption of canned tuna during pregnancy is no problem. There is however no difference between canned tuna and fresh tuna with respect to the levels of MeHg (EFSA,2012). There are maximum limits of MeHg in fish, which also apply to canned tuna.
- It is noted that on the same website page, it is stated that consumption of predatory fish during pregnancy should be avoided[1]. However, in order to reach a low-level exposure of the unborn child, and given the bioaccumulating properties of MeHg, it would be prudent for women with a wish to conceive to reduce their MeHg exposure by avoiding the consumption of fish with a high level of MeHg well before the intended pregnancy (see also Appendix A). This advice is present on the website of the Netherlands Nutrition Centre regarding heavy metals[2], but not repeated on the site regarding a healthy diet before and during pregnancy. This information may therefore not be easily found by the relevant target population. EFSA (2015) recommends that women of childbearing age should avoid exposure to MeHg above the TWI.

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Appendix A Tolerable Weeky Intake (EFSA, 2012)

MeHg in maternal hair of pregnant women was chosen by EFSA as a biomonitor for the occurrence of neurodevelopmental toxicity in offspring. In deriving the health-based guidance value the relationship between Hg in maternal hair and neurodevelopmental toxicity in offspring, i.e. disturbed cognitive development after birth, as observed in two long-term prospective epidemiological studies (Seychelles and Faroese studies) was selected. EFSA decided to use 11.5 Hg/kg maternal hair as a health-based guidance value. This is the mean of the apperent no-observed effect level (NOEL) from the Seychelles study of 11 mg/kg maternal hair and the $BMDL_{05}$ from the Faroese study of 12 mg/kg in maternal hair

By application of a maternal hair to maternal blood ratio of 250, the maternal hair concentration associated with no appreciable adverse effect of 11.5 mg/kg was converted into a "steady state" maternal blood concentration of 11.5 mg/kg÷250 = 46 µg/L. Using a one-compartment toxicokinetic model (see below), the "steady state" value of 46 µg/L in maternal blood was converted to a daily dietary Hg intake of 1.2 µg/kg bw per day.

In concordance with WHO (1990) and JECFA (FAO/WHO, 2004), EFSA used the following one-compartment modeling in describing human MeHg kinetics. More specifically the following "steady state" model was used:

$$C = (d \times A \times f \times bw) \div (b * V)$$

with:

C	=	concentration of Hg in blood (µg/L)
d	=	average daily dietary Hg intake (µg/kg bw per day)
A	=	absorption factor (0.95)
f	=	the absorbed fraction distributed to the blood (0.05)
bw	=	body weight (65 kg for a pregnant woman)
b	=	elimination rate constant (ln 2 ÷ half-life in blood = ln2÷50 = 0.014 per day)
V	=	blood volume (9% of the body weight in a pregnant female)

In order to convert the mentioned daily Hg intake of 1.2 µg/kg bw per day via food to a health-based guidance value, EFSA applied an assessment factor of 6.4 to this intake. In concordance with JECFA (FAO/WHO, 2004), this factor consisted of a factor 2 for variation in hair to blood ratio and a standard factor of 3.2 to account for interindividual variation in toxicokinetics. This resulted in a tolerable daily intake of 1.2÷6.4 = 0.19 µg/kg bw, which corresponds with a tolerable weekly intake (TWI) for MeHg of 7 x 0.19 = 1.3 µg/kg bw, expressed as mercury.

Note that the TWI corresponds with a "steady state" maternal MeHg blood level of (0.19 x 0.95 x 0.05 x 65)÷(0.014 x 0.09 x 65) = 7.2 µg/L (expressed as Hg).

As shown in Figure E1, this blood level will be reached after an exposure time of around 600 days, after which a similar wash-out period is needed to clear the body from MeHg. This means that in order to protect the unborn for MeHg toxicity, an effective advice with respect to a restriction of the consumption of MeHg containing (predatory) fish should start well before an intended pregnancy.

Strictly spoken, EFSA's MeHg risk assessment approach is restricted to the protection of the unborn against MeHg exposure via placental transfer during pregnancy. This protection is achieved by setting a limit value for MeHg in maternal blood. As such EFSA's approach is not suited to assess MeHg induced neurodevelopmental toxicity in young children *after* birth.

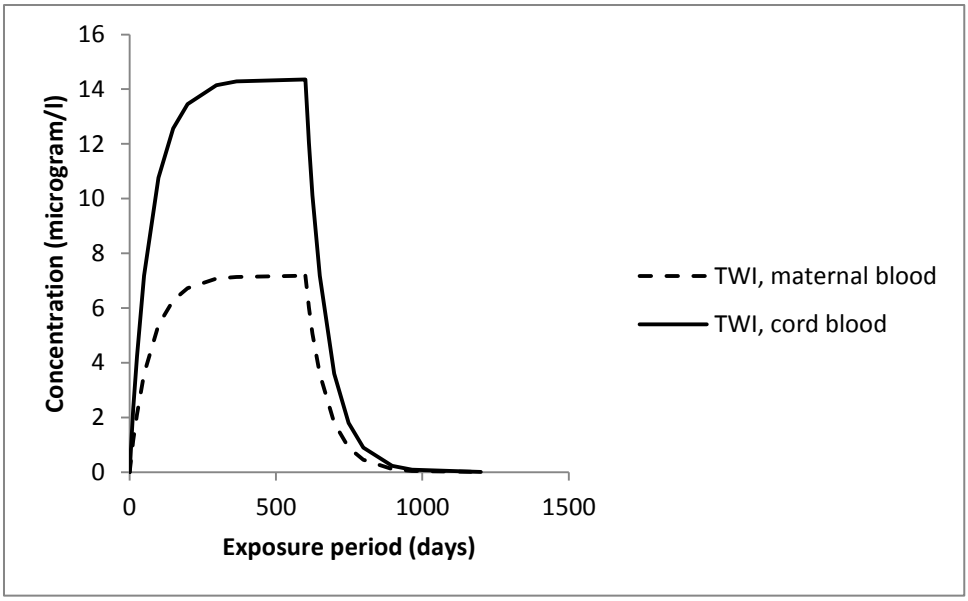

Figure E1. Simulation of the time-course of the blood concentration of MeHg in humans, i.e.pregnant women, exposed to MeHg at the level of the TWI (dashed line, EFSA one-compartment model) and corresponding Hg concentration in cord blood (solid line). Exposure period: 600 days, followed by a similar depurination period.

However, as MeHg concentrations in umbilical cord blood may be twice as high as their corresponding maternal blood concentrations (EFSA, 2012) this implies that at a maternal exposure level equivalent to the TWI, the corresponding MeHg levels in cord blood of 14.4 µg/L is a level with no appreciable adverse effect in the fetus.

Extrapolating of this blood level as a benchmark for the disturbance of neurodevelopment in young children after birth and assuming EFSA's kinetic model parameters to apply across age implies that these negative effects may be expected when the exposure of children transgresses the TWI by a factor of 2 or more.

In EFSA's risk asssement of MeHg one-compartmental modeling is used to describe the accumulation of MeHg in the body and the blood of pregnant females. Indirectly this approach allows for the estimation of MeHg in cord blood. Though the latter may be used as a first tier for the exposure of the fetal brain this approach does not allow for the modeling of fetal MeHg kinetics, including a more refined exposure at the level of the fetal blood and the fetal brain. Clearly, to reach this goal Physiologically Based PharmacoKinetic is needed (see for example Gearhart et al., 1995; Clewell et al., 1999; Zeilmaker et al., 2013).

<u>Developmental immunotoxicity</u>

EFSA identified a rat study on developmental immunotoxicity describinga $BMDL_{05}$ for reduction in antibody response of 0.01 mg/kg bw per day expressed as methylmercuric chloride, which isequivalent to 0.008 mg/kg bw per day expressed as Hg) (Tonk et al., 2010). In this study, rats were given methylmercuric chloride by oral gavage at doses of 0, 0.1, 0.4, 0.7, 1.0, 1.5, or 2.0 mg/kg bw per day, expressed as methylmercuric chloride (equivalent to 0, 0.08, 0.32, 0.56, 0.8, 1.2, or 1.6 mg/kg bw per day expressed as Hg) from gestation day 6 to the tenth day of lactation. Immune parameters were assessed in the male offspring on postnatal day (PND) 21, 42 and 70. The lowest $BMDL_{05}$ was 0.01 mg/kg bw per day expressed as methylmercuric chloride (equivalent to 0.008 mg/kg bw per day expressed as Hg).

EFSA's CONTAM Panel noted that the BMD is below the lowest dose tested (0.008 mg/kg bw per day to be compared with 0.08 mg/kg bw per day). EFSA decided that the data need to be confirmed and therefore did not indicate any new experimental animal studies that could provide a

Table E1. MOE of the exposure to methylmercury (µg/kg bw per day) of the Dutch population (2-69 jaar) due to consumption of fish when compared with the BMDL051 for developmental immunotoxicity in the rat.

Age (years)	Margin of exposure of the MeHg intake Table 1 of the main text) and the $BMDL_{05}$ for immunotoxicity in the rats (Tonk *et al.,*2010)[1]			
	mean	P50	P95	P99
2-6	280 (280-560)	560 (560-560)	112 (86-197)	62 (37-112)
7-15	280 (187-560)	560 (560-inf)	93 (56-187)	31 (15-62)
16-69	560 (280-560)	560 (560-560)	112 (70-140)	80 (62-93)

[1] $BMDL_{05}$ = 8 µg/kg bw per day = 56 µg/kg bw per week

better primary basis than the human epidemiological data for a health-based guidance value.

Although EFSA noted that the BMD from the study of Tonk et al. (2010) is below the lowest dose tested, she mentioned that the TWI provides a margin of 8 µg/kg bw/day x 7 days/week ÷1.3 µg/kg bw/week, i.e. about 40, compared to the BMDL05 for immunotoxicity. For reasons of completeness, Table E1 gives corresponding MOEs for this endpoint with respect to the MeHg intake in the Netherlands as depicted in Table 1 of the main text.

Appendix B Description of food consumption data used in the exposure assessment to methylmercury

DNFCS-Young Children 2005/2006

The target population of the DNFCS-Young Children 2005/2006 consisted of boys and girls aged 2 to 6 years living in the Netherlands (Ocké et al., 2008). Respondents were selected from representative consumer panels of the market research agency GfK. Panel characteristics, such as socio-demographic characteristics, are known to GfK. Persons in these panels participate in all types of surveys and were not specially selected on nutritional characteristics. Institutionalised persons were excluded, as well as children whose parents/carers did not have sufficient knowledge of the Dutch language. Per family, only one child was included to avoid correlations in dietary consumption patterns between children of the same family. In total, 1,634 children were invited to participate in the study, and a consent was obtained for 1,279 (net response of 78%). The study population was representative regarding socio-demographic characteristics (including region and education of the head of the household), but densely populated areas were slightly underrepresented.

The food consumption data were collected in the period October 2005 to November 2006 via a food diary on two non-consecutive days, which were separated by about 8 to 13 days. Parents or carers were visited at home by a trained employee of GfK. During the home visit, survey materials were presented and overall instructions were given.

Portion size of the foods and meals were estimated by using photographs, domestic measures (a small and a large spoon were supplied to standardise estimates), standard units, weight and/or volume. The usual volume of cups and glasses used was measured by the carer. All days of the week were equally represented, but the winter and autumn period were slightly overrepresented compared to the spring and summer period. National and/or religious holidays or holidays of the participants were not included in the survey.

DNFCS 2007-2010

The target population of the DNFCS 2007-2010 consisted of people aged 7 to 69 years living in the Netherlands (van Rossum et al., 2011). Pregnant and lactating women, as well as institutionalised people were not included. Respondents were selected from representative consumer panels of GfK. A maximum of one person per household was included in the survey to avoid correlations in dietary consumption patterns between members of the same family. In addition, the panels only included people with sufficient knowledge of the Dutch language. In total, 5,502 individuals were invited to participate in the study, of which 3,819 consented (net response of 69%). Children were overrepresented in the study population and adults underrepresented. This did not affect the current risk assessment, since only children were addressed in this study. The distribution of levels of education, region and urbanisation in the study population was close to that of the general Dutch population.

The food consumption data were collected over a 3-year period from March 2007 to April 2010 via two non-consecutive 24-hour dietary recalls (separated by 2 to 6 weeks). Children aged 7 to 15 years were interviewed face to face during home visits in the presence of at least one of the child's parents or carers. Participants aged 16 and over were interviewed by telephone, at dates and times unannounced to the participants. Portion sizes of the foods consumed were quantified in several ways: by means of quantities as shown on photos in a provided picture booklet, or in household measures, standard units, by weight and/or volume. The survey covered all days of the weeks and all four seasons. National and/or religious holidays or holidays of the participants were not included in the survey.

Appendix C Total number of samples analysed, the mean methylmercury concentrations (µg/kg product) following three scenarios of assigning concentrations to concentrations analysed below limit of quantification (LOQ) and the mean consumption of fish types and other sea food (g/day) in 2 to 6-year and 7 to 15-year olds.

Product[b]	Mean concentration[a] (µg/kg product)				Mean consumption (g/day)	
	Nr of samples	LB scenario	MB scenario	UB scenario	2-6 years[e]	7-15 years[e]
Fish type						
Anchovy[c]	5	22	62	102	0.001	0.007
Catfish[c]	1	121	121	121	-	-
Coalfish/pollack[d]	10	94	94	94	0.161	0.244
Cod	5	102	102	102	3	3.03
Cuttlefish/squid[c]	1	34.4	37.6	37.6	-	0.02
Dab	3	180	180	180	-	0.14
Eel, farmed	2	48.5	48.5	48.5	0.01	0.09
Eel, wild	17	128	128	128	0.0006	0.005
Flounder	1	110	110	110	0.014	0.4
Gurnard[c]	4	103	109	116	-	-
Herring	3	27.7	27.7	27.7	0.09	0.25
Mackerel	1	61	61	61	0.14	0.13
Pangasius	2	-	2.5	5	-	0.43
Plaice	9	61	61	61	0.11	0.07
Pollack	10	94	94	94	0.16	0.24
Salmon	4	24.5	24.5	24.5	0.42	0.52
Sardine[c]	16	14	58	102	0.007	0.004
Seabass	3	486	486	486	-	-
Shrimp	5	64.3	64.3	64.3	0.19	0.46
Sole	4	49.8	49.8	49.8	0.19	0.02
Tilapia	2	-	2.5	5	-	0.17
Trout	2	22.5	22.5	22.5	0.007	0.04
Tuna[c]	125	220	221	221	0.17	0.52
Other seafood[c]						
Crab (leg)	3	130	130	130	0.02	0.04
Lobsters	3	130	130	130	-	-
Oysters	3	130	130	130	-	-
Mussels	3	130	130	130	0.015	0.18

[a] LB = lower bound, congener concentrations below LOQ were assigned a concentration of 0 µg/kg product; MB = medium bound, concentrations below LOQ equalled ½ LOQ, respectively; UB = upper bound, concentrations below LOQ equalled LOQ, respectively.
[b] Concentration methylmercury in fish equalled 100% of measured concentration total mercury, and concentration methylmercury in other seafood equalled 80% of measured concentration of total mercury.
[c] Mean LB, MB and UB concentration obtained from EFSA (2012)
[d] Based on the concentrations of cod, hake and whiting
[e] Note that a mean consumption of fish or seafood equal to "–" means that the product was not consumed. Where both age groups have "–", the fish or seafood is consumed by the population aged 16 to 69.

The total Hg concentration data on wild and farmed fish and shellfish were obtained from the Dutch monitoring programme on contaminants in Dutch fish and fishery products. Samples analysed in 2009-2014 were included in the exposure assessment. All these concentrations were stored in the

Quality Programme of Agricultural Products (KAP) database[4]. For total Hg concentrations in oysters, lobster or mussels, the Dutch monitoring concentration in crab (leg) was used. In order to assess dietary exposure to MeHg based on total Hg concentrations, it was assumed (in accordance with EFSA, 2015)[5] that total Hg consisted for 100% of MeHg in fish meat and fish products, and 80% in other seafood, and that all other foods (such as cereals, vegetables, fruit, etc.) do not contain MeHg. For anchovy, sardine, tuna, catfish, gurnard and cuttlefish/squid (crustaceans), no concentration data were available. For these fish types, MeHg concentrations (means; lower bound, medium bound and upper bound) were acquired from EFSA (2012)[6].

[4] chemkap.rivm.nl/
[5] EFSA (2015). Scientific Opinion Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. EFSA Scientific Committee EFSA Journal 2015;13(1):3982.
[6] EFSA (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA Journal 2012;10(12):2985.

Appendix D Linkage between food consumption and concentration data

MeHg was analysed in raw fish and other seafood, as raw commodities (RC). For an overview of all concentration data used, see Appendix C. To model the dietary exposure, subsequently a link between the concentrations in fish and other sea food, and consumed amounts of foods was made as follows:

The MeHg concentrations in farmed eel were lower comparedto those in wild eel. Since ~95% of the consumed eel is farmed eel, the farmed and wild eel were treated as separate fish species. The wild (5%) and farmed (95%) eel concentrations were included in the exposure assessment in such a way, that wild or farmed eel concentrations were linked to consumptions of eel with a probability of 5 or 95%, respectively. Although, the number of wild eel samples was limited, we expect that given the low probability of 5%, the impact of these high concentrations on the exposure assessment will be limited.

It is important to realise that foods recorded in food consumption surveys include foods consisting of one fish ingredient (e.g. cooked cod) and composite foods consisting of more than one ingredient (e.g. tuna pizza, fish salads). To include exposure via the consumption of composite foods in the assessment, a food conversion model was used. In this model, chemical concentrations per RC are converted to equivalent concentrations in composite foods (Boon et al., 2014; van Dooren, et al., 1995). This model first converts foods to their corresponding RC ingredients (including their weight fractions). For example, fish salad is split into equivalent amounts of its RC ingredients salmon, tuna, eel, etc. Then, the chemical concentrations analysed in these RC ingredients are assigned to these fractions and summed to result in the chemical concentration in fish salad. This approach was applicable to all analysed fish and other seafood types.

Appendix E Modelling of exposure via food

The exposure to MeHg via food was calculated using Monte Carlo Risk Assessment programme (MCRA), Release 8 (de Boer et al., 2015). To assess the exposure, the Logisticnormal-normal (LNN) model is the first model of choice. LNN models exposure frequencies and exposure amounts separately, followed by an integration step (Goedhart et al., 2012). An important prerequisite for the use of LNN is that the positive daily exposure distribution is transformed into a normal distribution using, for example, a logarithmic function. If this criterion is not met, LNN may result in erroneous exposure results.

Figure D1 shows the observed vs. theoretical residuals of the positive daily exposure distributions to MeHg in children aged 2 to 6 years (A) and children aged 7 to 15 years (B) via the consumption of all fish and other seafood types. When the red line follows the black line, the positive daily exposure distribution is approximately normally distributed, and LNN can be used to assess the long-term intake. For both age groups, it was concluded that the model was however not fit for purpose, since the red line did not follow the black line (Figure D1).

MeHg is only present in fish and the number of actual fish consumption-days is very low (about 11% in both age groups). Due to this, the exposure distribution was skewed to the right with a high percentage (≥ 89%) of zero exposures. Furthermore, fish is not only consumed as product, but as an ingredient in products. The combination of a low percentage of consumption-days and consumption of different fish types and other seafood as product or as ingredient with their unique and diverging concentration ranges made the use of LNN to determine long-term exposure not possible in these age groups.

In this assessment, the Model-Then-Add (MTA) approach was therefore used (van der Voet et al., 2014). This approach does not support the calculation of the exposure as a function of age. The exposure was therefore estimated for the whole group: 2-6-years and 7-15-years. For reasons of comparison, the MTA approach was also used to assess the exposure to MeHg in the age group of 16 to 69 years.

With the MTA approach, foods or groups of foods are identified for which the exposure can be modelled separately using either LNN or the observed individual means (OIM) model, and subsequently the resulting exposure distributions per food (group) are added to obtain an overall exposure distribution. OIM calculates the average exposure over the recording days, and is known to overestimate the exposure in the right tail of the exposure distribution. OIM. The advantage of such an approach is that the exposure per food (group) may show a better fit to the normal distribution model than the exposure via all foods or food groups together. Examination of the positive daily MeHg exposure distribution showed that the MeHg exposure via cod contributed for 75% (2-6 year) and 56% (7-15 year) to the total intake (Figure 1). The analysis showed further that cod eaten as an ingredient (defined as amount within product < 15%) only contributed to the left tail of the intake distribution, whereas cod eaten as a product (defined as amount of cod within product at least 15%) mainly contributed to the right tail of the intake distribution (data not shown). This analysis suggested to split up the exposure via cod consumed as a product and as an ingredient.

As a result of this, the consumption of cod eaten as a product and the remaining fish (excl. cod eaten as ingredient and shrimp) was modelled separately with LNN in children aged 2 to 6, while

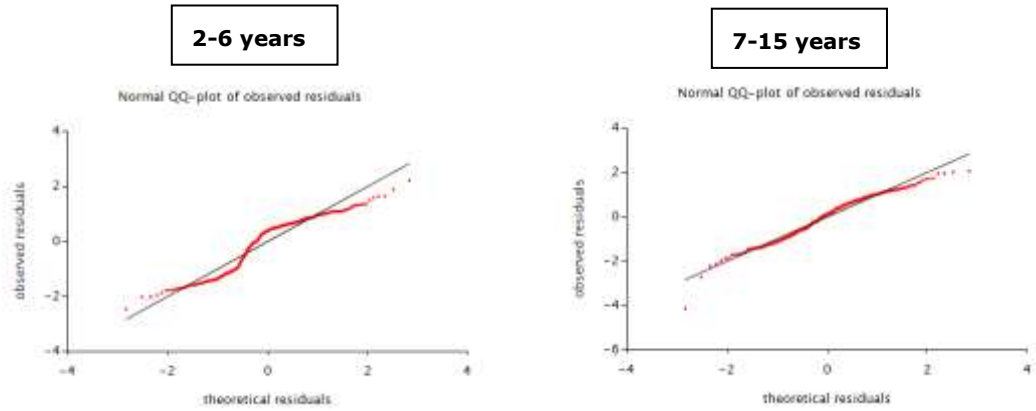

Figure D1. Figure D1. The observed vs. theoretical residuals of the positive daily exposure distributions to methylmercury in children aged 2 to 6 years and those aged 7 to 15 years in the Netherlands in which concentrations below limit quantification (LOQ) equalled ½LOQ (medium bound scenario; LNN modelling).

the exposure via cod eaten as ingredient and shrimp was modelled using OIM (Table D1). Figure D2 shows the model fits of the observed residuals for the two sources modelled with LNN. The three resulting exposure distributions were subsequently added to assess the overall exposure to Hg. In children aged 7 to 15, the exposure via the consumption of shrimp, cod eaten as product and remaining fish (excl. mackerel and salmon) were modelled separately with LNN, whereas the exposure via the consumption of mackerel and salmon was modelled with OIM (Table D1). Figure D2 shows that the fit for MeHg exposure via shrimp, cod eaten as product and remaining fish (excl. mackerel and salmon) was acceptable. In the group aged 16 to 69, the MeHg exposure via the consumption of shrimp and remaining fish (excl. mackerel) was modelled with LNN and via the consumption of mackerel with OIM. Also here the fit was acceptable for the exposures modelled with LNN (not shown).

Table D1. Modelling of the exposure to methylmercury via separate foods or food groups using Model-Then-Add per age group.

Age group (years)	Foods[1]	Model[2]
2-6	Cod eaten as product	LNN
	Cod eaten as ingredient / shrimp	OIM
	All other fish	LNN
7-15	Cod eaten as product	LNN
	Mackerel / salmon	OIM
	All other fish	LNN
16-69	Shrimp	LNN
	Mackerel	OIM
	All other fish	LNN

[1] Selected food groups differ per age group due to differences in food consumption patterns
[2] LNN = Logisticnormal-normal; OIM = Observed Individual Means

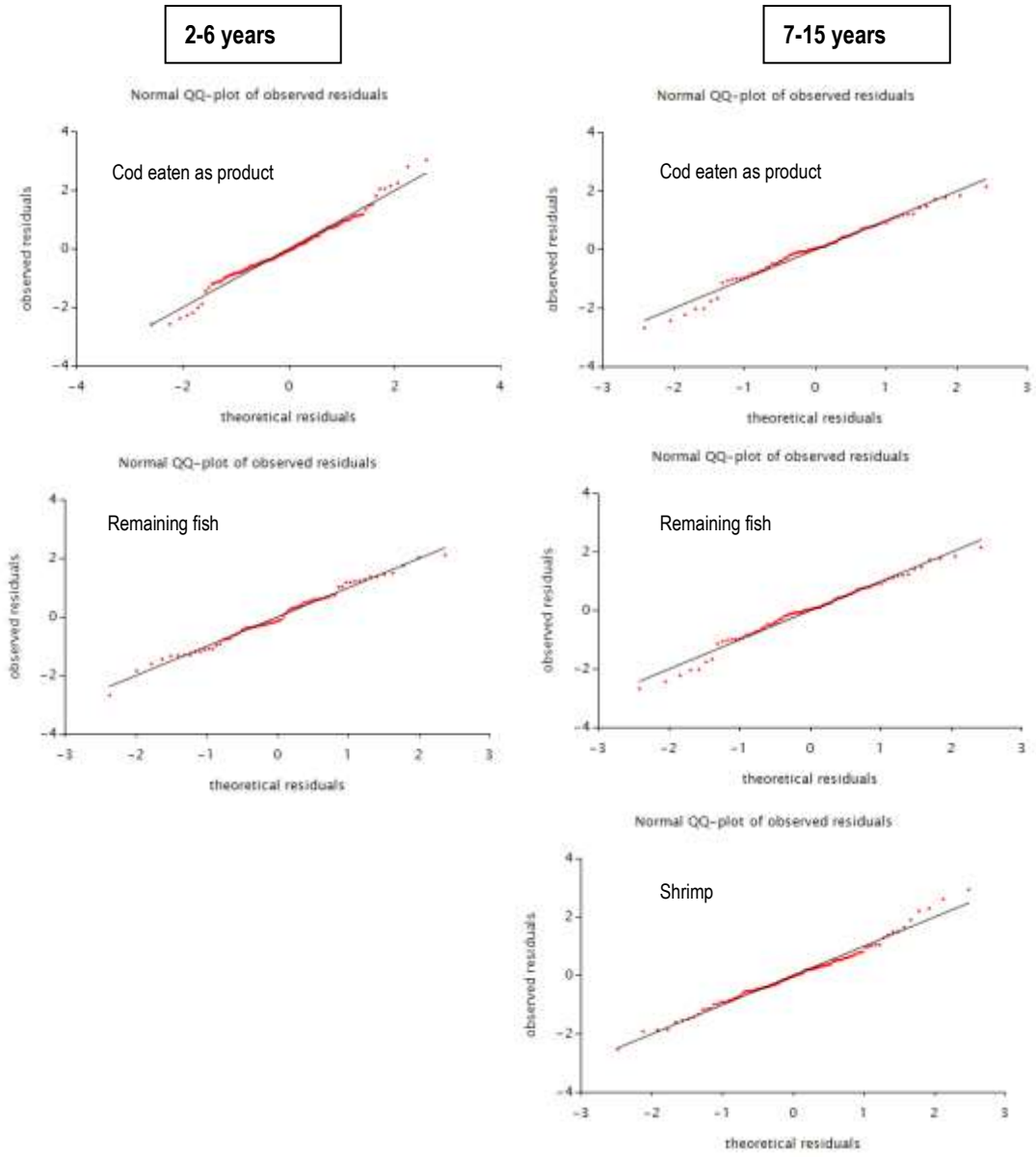

Figure D2. The observed vs. theoretical residuals of the positive daily exposure distributions to methylmercury (MeHg) in children aged 2 to 6 years (left panel) and in children aged 7 to 15 years (right panel) in the Netherlands using the Model-Then-Add approach. Concentrations below limit of quantification (LOQ) equalled ½LOQ (medium bound scenario).

Left panel: exposure via cod eaten as product (amount of cod at least 15%) and remaining fish (excl. cod eaten as ingredient (amount of cod < 15%) and shrimp) modelled with LNN separately, while the exposure via cod eaten as ingredient (amount of cod < 15%) and shrimp was modelled with Observed Individual Means (OIM). Right panel: exposure via mackerel and salmon modelled with OIM, while the exposure via shrimp, cod eaten as product (amount of cod at least 15%) and remaining fish (excl. mackerel and salmon) was modelled separately with LNN.