Balancing the nutritional benefits of fish consumption against methylmercury toxicity, and evaluating the impact of genetic polymorphisms in glutathione S-transferases on susceptibility to methylmercury in a Swedish population

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Abstract

Methylmercury toxicity in humans was analyzed as a system of three components: a strong negative feedback loop mediated by toxic effects of mercury exposure, a weak positive feedback loop mediated by nutritional benefits from omega-3 polyunsaturated fatty acids (ω3 PUFA), and a genetic component mediated by a putative role of glutathione S-transferases in methylmercury detoxification. The system was implemented as a biological model of mercury pharmacokinetics, with a distribution of hair:blood partition coefficient generated by Monte Carlo simulation. The model predicted the expected distribution of hair mercury concentrations at any given level of methylmercury intake, which was compared to several values for the NOAEL, viz. 11, 12 and 15 ppm in hair, in order to evaluate the risk of toxicity.

The sample population from the northern part of Sweden faced negligible risk of mercury toxicity, based on their daily intake predicted from RBC mercury concentration. Sufficient space existed to recommend increasing fish intake, in order to increase ω3 PUFA intake and maximize health benefits. However, some segments of the population may possess genetic polymorphisms that might slow down their metabolism of methylmercury, and consequently increase their risk of toxicity. While this link has not been empirically established, its absence has to be demonstrated, and this provides rationale for the experimental work that is presently being conducted.

Future revision of the Swedish critical limit to a more stringent value may result in lessening the intake of ω3 PUFA from fish, because the perception of risk from toxicity discourages fish consumption much more than the desire for proper nutrition encourages it. The proposed guideline value could be relaxed, once the absence of toxic risk had been demonstrated for genetically more sensitive population subgroups.

Introduction

Background of the study

Mercury contamination in fish

Mercury (Hg) is a heavy metal used extensively in commerce and industry which, in vapor form (Hg^0), ultimately finds its way into the natural environment. Once there, its long atmospheric residence time allows Hg^0 to be transported globally. Hg^0 is thus deposited over land and water surfaces, from whence it is either re-emitted into the air or converted to methylmercury (MeHg) through biological processes (Schroeder & Munthe 1998). MeHg enters the biosphere, and is bioaccumulated by fish and biomagnified up the food chain (Downs et al 1998). Mercury then ultimately re-enters the anthroposphere as a contaminant in food fish.

The acknowledgment that mercury caused toxic effects sparked the development of a policy for mercury waste management in Sweden. The early phases focused on increased mercury content in food fish due to industrial discharges, which resulted in the blacklisting of lakes and watercourses, as well as the regulation of industrial discharge and the setting of a provisional limit of mercury in fish in 1966. These actions brought results, such that some of the blacklisted lakes were declared clean by the early 1970s, and the mercury problem was thought to have been solved for the time. The later phases focused on the diffusion of mercury from atmospheric transport and old pollution, as well as the uncontrolled spread of mercury-containing products in society. This resulted in several decisions by the Swedish parliament to (1) phase out the use of mercury in society, (2) gather mercury waste, (3) not recycle mercury, and (4) prepare a scheme for the final disposal of mercury (Lidskog & Uggla 2000; Von Rein & Hylander 2000).

The problem of methylmercury contamination of food fish persisted, however, despite the waste management policy outlined above. Out of 83 000 lakes all over Sweden, 10 000 contained fish (pike, *Esox lucius*, was analyzed) with mercury concentrations above the blacklisting limit of 1.0 mg/kg; likewise, 40 000 of these lakes contained pike with mercury concentrations above the guideline value of 0.5 mg/kg. Using a dynamic model of environmental factors regulating lake recovery from pollution, it was estimated that it might take more than three centuries for mercury levels in pike to fall from 1.0 mg/kg to 0.5 mg/kg. The modeling also showed that the duration of the mercury problem depended not on lake processes, but on land and atmospheric transport processes, and ultimately on anthropogenic emission. This implied that the only way to solve the problem would be to reduce emissions (Håkanson 1996). Corollary, this implied that the contamination of food fish would have to be managed in other ways i.e., controlling the exposure of the population by setting a limit on fish consumption (US-EPA 2001).

Human mercury exposure from food fish

Given the high levels of methylmercury in several species of edible fish, the Swedish Food Administration defined a critical limit of 0.5 mg/kg fish (Sverdrup 2000). The interpretation of this critical level meant that fish containing more mercury than 0.5 mgHg/kg was deemed to pose health risks, and was therefore unacceptable for human consumption. Thus, fish advisories were issued against consuming certain types of fish which were known to contain mercury above the critical limit (Von Rein & Hylander 2000). The critical limit in fish represented a proxy guideline for humans, and corresponded to a mercury intake limit of 40 μg/day from food fish (Sverdrup 2000). This is equivalent to 0.57 μg/kgBW/day for a 70-kg person.

Several other guidelines for mercury intake existed, most of which defined the critical limit specifically for human beings, and which prescribed intake levels less than 0.57 μg/kgBW/day (Table 1). The Reference Dose

(RfD), in particular, has been used in the United States to calculate consumption limits based on measured mercury levels in fish tissue. This was derived from a No Observed Adverse Effect Level (NOAEL) obtained from the Iraqi mercury poisoning incident (Table 2), divided by an uncertainty factor of 10 (US-EPA 1997). It represents the most stringent guideline in the literature today, and had been criticized as being too restrictive (Egeland & Middaugh 1997). Given that the Swedish guideline level for mercury corresponds to a higher daily methylmercury intake than the RfD, the present paper sought to answer whether or not this entails a higher risk of toxicity for the Swedish population.

Table 1. Published guidelines for mercury intake from various organizations.

Guideline	Value (μg/kgBW/day)	Agency	NOAEL basis (ppm in hair)	Reference
TDI [a]	0.48	WHO-IPCS [d]	11	WHO (1990)
RfD [b]	0.1	US-EPA [e]	11	US-EPA (1997)
MRL [c]	0.3	ATSDR [f]	15	ATSDR (1999)

[a] Tolerable Daily Intake
[b] Reference Dose
[c] Minimal Risk Level
[d] World Health Organization—International Programme on Chemical Safety
[e] United States—Environmental Protection Agency
[f] Agency for Toxic Substances and Disease Registry

Table 2. Published values for the No Observable Adverse Effect Level (NOAEL) for methylmercury in humans, as measured in maternal hair. The three studies cited below considered the toxic effects of dietary methylmercury intake during pregnancy on the neurological development of the child.

Study	NOAEL (ppm)	Source of Mercury	Manner of Exposure	Reference
Iraq	11	poisoned grain	acute poisoning	Cicmanec (1996), US-EPA (1997)
Faroes	12	oceanic fish	long term dietary intake	Dourson et al (2001)
Seychelles	15	oceanic fish	long term dietary intake	Cicmanec (1996), Dourson et al (2001)

Health benefits from eating fish

The fact that fish consumption is the dominant source of methylmercury exposure does not negate its role as an important source of proteins and the very long chain omega-3 polyunsaturated fatty acids (ω3 PUFA). Dietary intake of ω3 PUFA is necessary for growth and development of fetal and infant brains, and normal functioning of adult brains (Broadhurst et al 2002). The benefits from fish consumption are highlighted by the observation that children in the Seychelles showed enhanced visual motor coordination with increasing methylmercury exposure (Davidson et al 2000), and children in the Faroes being studied for methylmercury toxicity showed excellent visual contrast sensitivity (Grandjean et al 1997). In both instances, the results were interpreted to mean that while fish consumption did increase exposure to methylmercury, the consequently high intake of ω3 PUFA gave superior nutrition.

These nutritional benefits prompted a call to re-examine the basis for setting the US-EPA RfD at 0.1 µg/kgBW/day, which was much lower than the intake guidelines set by the World Health Organization (WHO 1990; Table 1). It was feared that such a stringent guideline might produce fish advisories that would severely restrict nutritional benefits from a fish diet. (Egeland & Middaugh 1997). Given the expressed need to balance mercury toxicity against nutritional benefits of fish consumption, the present study sought to answer whether or not fish consumption could be increased to maximize nutritional benefits from ω3 PUFA, without incurring an unacceptably high risk of methylmercury toxicity.

Genetic differences in susceptibility to mercury toxicity

The relationship between exposure to methylmercury and the appearance of toxic effects is described by a dose-response curve, which assumes that all members of the population are equally sensitive to methylmercury, and the toxic response was entirely dependent on the magnitude of dose (Stern & Korn 2001). It became apparent, however, that certain subgroups were more sensitive that the rest e.g., children, elderly and the infirm. Aside from this, certain less-easily identifiable subgroups might be more sensitive, based on genetic differences in toxicokinetic processes. Following this line of argument, there might conceivably be genetic differences in methylmercury metabolism in a population, which could modify the risk of toxicity.

It is well accepted that a tripeptide called glutathione (GSH) is involved in methylmercury metabolism in the human body (WHO 1990; Clarkson 2002). GSH is a broad-spectrum defense mechanism, and protects the body against electrophilic substances in general. The glutathione S-transferase enzymes (GSTs) catalyzes the complexation of glutathione with these substances, and is in turn produced by a family of *GST* genes (Cnubben et al 2001). Genetic polymorphisms have been detected in these genes, the most interesting of which are the existence of null alleles in the *GST* genes Mu and Theta, and the presence of a mutation in *GST* Pi causing an amino acid switch from ile[105] to val[105] (Hayes & Strange 2000). The null allele for Mu produces an inactive enzyme, and has been associated with an increased risk for cancer in the lung, bladder, colon and breast (Autrup 2000; Benhamou et al 2002). The null allele for Theta similarly produces an inactive enzyme, and has been associated with multiple clusters of primary tumors in basal cell carcinoma (Strange & Fryer 1999; Strange et al 2001). Polymorphisms in Pi, on the other hand, have been associated with reduced risk of asthma in individuals possessing the val[105]/val[105] genotype (Strange & Fryer 1999; Strange et al 2001). In these three examples, the effect of *GST* genotype on the risk of cancer and asthma was attributed to the role of GST in detoxifying pollutants which would otherwise trigger disease.

Given that metabolism and detoxification of methylmercury (an electrophile) is mediated by glutathione complexation, and that GSTs are involved in the complexation of electrophilic substances with glutathione, could it also be the case that genetic polymorphisms in *GST* genes would result in differences in sensitivity to

methylmercury? While there is yet no support for this in the literature, such an assumed relationship could conceivably result in higher risk of toxicity for those persons harboring the null genotypes. This is the third and final question that the present paper sought to answer.

Objectives

The general objective of the present study was to describe the interaction between mercury toxicity from fish consumption and nutritional benefits from fish-derived ω3 PUFA, and then evaluate the expected effect of genetic polymorphisms in *GSTs* on the system. The specific objectives were as follows:

1. To construct a model that will relate methylmercury toxicity from fish consumption to nutritional benefits from ω3 PUFA

2. Using the model, to estimate the risks of methylmercury toxicity in a Swedish study population, vis-à-vis nutritional benefits from fish.

3. To determine whether hypothetically increasing the fish consumption could maximize nutritional benefits without increasing the risk of methylmercury toxicity.

4. To determine if genetic polymorphisms in *GSTs* could hypothetically increase the risk of methylmercury toxicity and thus limit the possibility of encouraging fish consumption.

Hypothesis

The relationship of fish consumption to methylmercury toxicity could be described as follows. Increasing fish consumption results in increased levels of methylmercury in the body, which in turn causes increased risk of toxicity. A rational person is therefore expected to decrease his/her fish intake once becoming aware that he/she may be getting exposed to toxic levels of mercury. Thus, the increased risk of toxicity would serve as negative feedback, and lead the person to decrease fish consumption. This chain of reasoning is shown diagrammatically in Figure 1, and consists of links A, B and C.

The relationship of fish consumption to nutritional benefits could be described thus: increasing fish consumption results in increased intake of ω3 PUFA, which in turn leads to increased nutritional benefits. A rational person is therefore expected to increase his/her fish intake once becoming aware of the health benefits of ω3 PUFA coming from fish. Thus, the increased nutrition would serve as positive feedback in order to further increase fish consumption. This chain of reasoning is shown diagrammatically in Figure 1, and consists of links D, E and F.

The expected behavior of fish consumption over time is shown in the inset in Figure 1. Initially, fish consumption continually increases, as nutritional benefits accrue from increasing ω3 PUFA intake. There comes a point, however, where increasing toxicity from methylmercury discourages any further increase in fish consumption, and fish consumption plateaus at the level where the trade-off between toxicity and nutrition is deemed acceptable.

The relationship of genetic polymorphisms in the system described above lies on the negative effect of *GST* deletion or substitution genotypes on the production of the active GST enzyme. GST enzyme, along with glutathione, is hypothesized to contribute to the metabolism of methylmercury, and ultimately serves to decrease the amount of methylmercury in the body. This is represented in Figure 1 as links G, H, I and J.
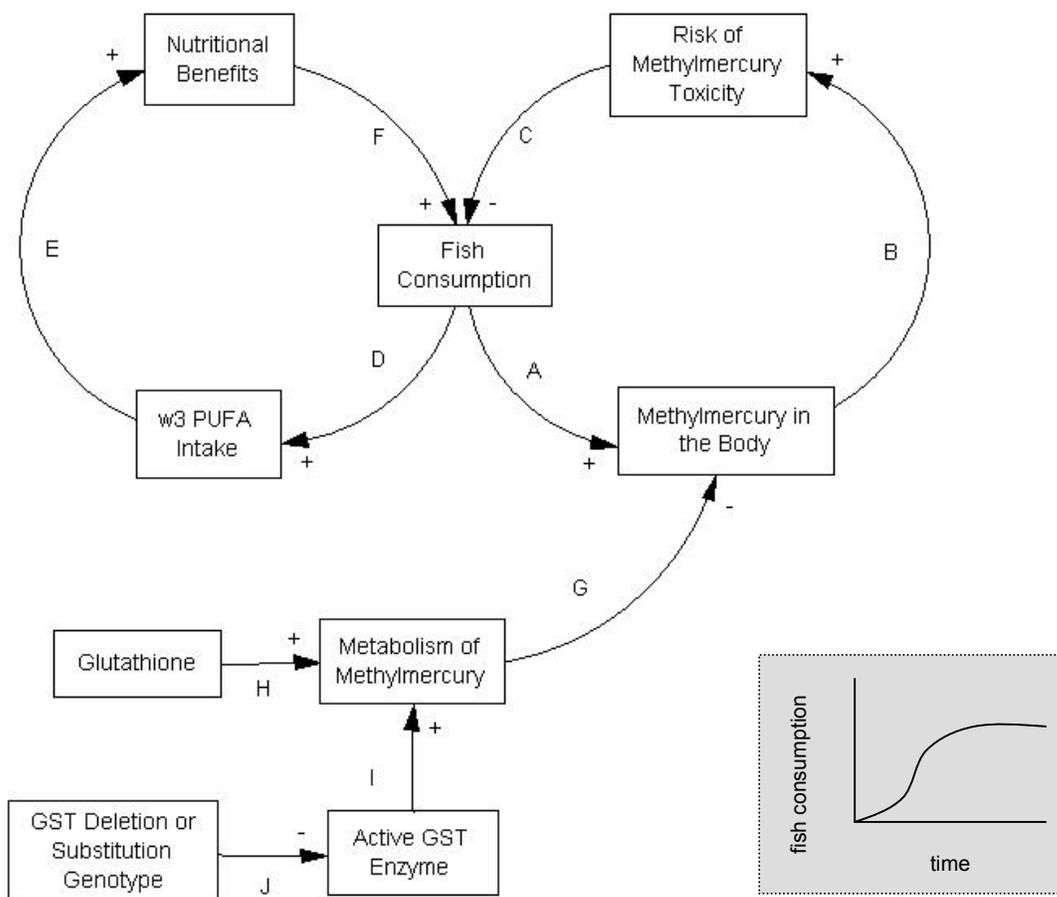
Figure 1. The proposed relationship between fish consumption, mercury toxicity, nutritional benefits from ω3 polyunsaturated fatty acids (PUFA) and genotype of glutathione S-transferases (*GST*). The representation above is a causal loop diagram. The boxes represent factors and variables, and the arrows represent causal links, or relationships. A positive sign (+) on the arrow indicates that a change in the cause variable results in a change in the same direction on the effect variable. Conversely, a negative sign (-) on the arrow indicates that a change in the cause variable results in a change in the opposite direction on the effect variable. The arrows are labeled from A to J, and are discussed in the text. *Inset*: expected behavior over time of the variable fish consumption.

Scope and limitations

The study originated from a proposal that the *GST* genotype of a Swedish population might result in differences in the linear regression of the blood concentration of mercury against the blood levels of ω3 PUFA between different genotypes. The scope of the project was initially delineated as the experimental determination of the relationship between *GST* genotype, blood PUFA levels and blood mercury concentration. It became impractical to perform this experimental work within the allocated time frame, and the scope was shifted to modeling the relationship between fish consumption, mercury toxicity and nutritional benefits. The effect of *GST* polymorphisms on this relationship was retained in order to locate the relevance of experimental work in the system. The present study was done as part of a *GST* genotyping project at the Department of Occupational and Environmental Medicine of Lund University Hospital.

The following have been placed outside the boundaries of the present study:

1. The effect of the model on the sustainability of fishing and fish consumption. The reader is instead referred to Pauly et al (2002) for an excellent discussion.

2. The effect of decreasing anthropogenic emissions of mercury and other related pollution-mitigation strategies on the toxicity of mercury from fish. It was assumed that the levels of mercury in food fish was a constant, inasmuch as it would take until after the year 2360 for mercury levels in fish (pike) to decrease to guideline levels (Håkanson 1996).

3. The experimental determination of the relationship between *GST* polymorphisms and the risk of methyl-mercury toxicity. The exclusion of experimental work from this thesis study was a matter of necessity, as insufficient time was available to perform the analysis. This relationship was instead explored hypothetically by modeling scenarios representing possibilities regarding the effect of *GST* polymorphisms on the risk of toxicity.

4. The effect of mercury from other sources, such as mercury vapor and amalgam fillings. Exposure of the Swedish population to ambient mercury vapor is assumed to be negligible, due to the effectiveness of past and present efforts to eliminate the use of mercury in industry and commercial goods in Sweden (Von Rein & Hylander 2000). Exposure from amalgam fillings are believed to be "well below those associated with overt toxic effects or even with subtler neurobehavioral and renal effects" (Clarkson 2002, p21).

5. The effect of other pollutants in fish, such as PCBs. Another LUMES thesis is being prepared on the topic of PCBs in fish, and a synthesis between that study and the present one would have to wait until later.

The paper is structured as follows:

1. The hypothesis is substantiated with studies from the literature which support the assumed links of causality. Where no definite causality is evident (as in the case of *GST* polymorphisms) studies showing the effect of *GST* polymorphisms on metabolism of other chemical toxicants are used to glean the possible range of effect that may be expected. This should not imply an unsubstantiated acceptance of the empirical link between *GST* polymorphisms and methylmercury toxicity. Rather, this approach is used to construct hypothetical scenarios for analysis.

2. A preliminary evaluation of the extent to which the literature supports the hypothesis is presented prior to the description of the methodology. This provides the basis for subsequent model selection and scenario generation.

3. The use of a pharmacokinetic model to evaluate scenarios is presented in the methodology. The selection of the model from literature and its subsequent modification is described, along with sensitivity testing. The model is then used to evaluate a number of scenarios.

4. The results of the scenarios are presented and subsequently discussed in terms of the objectives of the study. Finally, conclusions are drawn, along with recommendations regarding future empirical work.

Review of Literature

Literature support for the hypothesis

Link A: Methylmercury in the body increases with increasing fish consumption.

Humans are exposed to methylmercury primarily from the diet, and fish is its most important dietary source (WHO 1990; US-EPA 2001; Clarkson 2002). Almost all of the ingested methylmercury is absorbed into the bloodstream, from where it is rapidly distributed to various tissues in the body. Mercury accumulates in scalp hair, at concentrations 250 times greater than in blood. This ratio of hair to blood concentration is, however, subject to a wide range of variation, and the coefficient of variation has been pegged at 70% (Clewell et al 1999). Three studies relate fish consumption with mercury levels in the body, as described below.

1. A sample of 135 Swedish women showed significant association between methylmercury levels in serum and fish consumption, assessed by dietary history (Bergdahl et al 1998). The mean plasma methylmercury level was 2.4 nmol/L, and the mean fish consumption was 302 g/week. The women in the study had a mean inorganic mercury concentration of 2.5 nmol/L, which was associated with the number of amalgam surfaces, but not with the amount of fish intake.

2. Total mercury in erythrocytes (Ery-Hg) was significantly higher in Swedish subjects who reported at least one fish meal per week, compared to those who reported less than one fish meal per week (Hallgren et al 2001). Median Ery-Hg was 3.3 to 3.6 ng Hg/g erythrocyte in subjects with a low fish intake and 5.0 to 5.6 ng Hg/g erythrocyte in subjects with a high fish intake. Total mercury in erythrocytes was thus used as a marker of fish intake in their study, with the implicit assumption that fish intake was the most significant source of mercury, since methylmercury itself was not measured.

3. Organic mercury in erythrocytes was correlated with self-reported fish intake in 68 Latvians from a fishing village. Erythrocyte organic mercury was significantly correlated with both fatty- and lean fish intake. *High fish consumers* had a median fatty fish intake of 19 meals per month (mostly salmon and herring), median lean fish intake of 8 meals per month (mostly pike), and median erythrocyte organic mercury of 87.1 nmol/L. *Intermediate fish consumers* had a median fatty fish intake of 4 meals per month, median lean fish intake of 2 meals per month, and median erythrocyte organic mercury of 35.7 nmol/L. *Low fish consumers* had a median fatty fish intake of 0.3 meals per month, median lean fish intake of zero, and median erythrocyte organic mercury of 7.4 nmol/L (Hagmar et al 1995).

Link B: The risk of toxicity increases with increasing amounts of methylmercury in the body.

The major toxic effects of methylmercury occur in the central nervous system (CNS). In the adult CNS, methylmercury exposure is followed by a latent period of weeks or months, after which paresthesia appears; afterwards, ataxia, dysarthria, visual field constriction and hearing loss may ensue. It remains a matter of debate whether methylmercury itself causes toxic effects, or inorganic mercury derived from methylmercury metabolism does. Prenatal exposure of the fetal CNS to methylmercury, on the other hand, can have effects ranging from delayed achievement of developmental milestones to severe brain damage. A dose-response relationship between methylmercury exposure in the mother and consequent CNS damage in the developing fetus has been established (Clarkson 2002), and three large studies investigated prenatal methylmercury toxicity in Iraq, the Seychelles, and the Faroes. These are discussed below.

1. The methylmercury poisoning episode in Iraq of 1971-72 allowed the derivation of a dose-response relationship between maternal exposure and toxic effects on the child. Mercury levels in the mother's hair were measured, and correlated with the time when the child started to walk or speak. The children were also subjected to a neurological examination, which yielded a dose-response relationship between prenatal exposure and both developmental milestones and neurological score. Results showed that methylmercury exposure at maternal hair levels of 70 ppm correspond to a 30% risk for the fetus of developing abnormal neurologic signs. Downward extrapolation of this data showed that hair levels of 10-20 ppm imply a 5% risk of developing abnormal neurologic signs. Thus a NOAEL of 11 ppm mercury in hair was derived from the Iraqi data (Table 2; WHO 1990; US-EPA 1997; Myers et al 2000a).

2. The Seychelles Child Development Study (SCDS) examined the association between low-level methylmercury exposure from a maternal diet rich in fish, and subsequent child development. Most Seychellois consumed oceanic fish on a daily basis throughout their lives. A prospective longitudinal study involving a cohort of 779 children was initiated, which was characterized by the following: (a) mercury exposure during pregnancy was accurately measured, (b) the study was double-blind, (c) the study design was prospective and longitudinal, (d) a large number of possible neurodevelopmental endpoints was examined, and (e) compliance of the cohort was very high (Myers et al 1995b). Total mercury levels in maternal hair ranged from 0.5 ppm to 26.7 ppm, with a median of 5.9 ppm. No definite association of maternal mercury exposure with child development was discernible at 6½, 19, 29, 66 and 108 months of age. The infants from the Seychelles were essentially normal with respect to the performance of various cognitive, perceptual, memory, motor and language tasks. A free-standing NOAEL of 15 ppm in hair was thus derived from the Seychelles data (Table 2; Davidson et al 1995; Myers et al 1995a & b; Davidson et al 1998; ATSDR 1999; Davidson et al 2000; Myers et al 2000b).

3. A longitudinal, prospective study in the Faroes examined the association between low-level methylmercury exposure *in utero* from maternal diet and subsequent child development. A cohort of 1022 newborn Faroese children was followed up from birth, when the umbilical cord blood was obtained for determination of total mercury. This yielded a median of 121 nmol/L mercury. Maternal levels of hair mercury were also measured, yielding a median of 22.5 nmol/g. The total mercury levels in cord blood were closely correlated with mercury levels in mother's hair at the time of delivery, and unrelated to amalgam fillings (Weihe et al 1996). The children were subjected to various examinations at 7 years of age, which included physical, neurophysiological, and neuropsychological testing. *Physical examination* showed the children to be generally in good health. *Neurophysiological testing* showed a tendency in some peak latencies of the Visual Evoked Potential (VEP) to decrease at increased mercury exposures, and significant delays for peaks III and V of the Brainstem Auditory Evoked Potential (BAEP) with increasing exposure. *Neuropsychological Testing* was done to measure several areas of brain function, and showed significant correlation of prenatal mercury exposure with impairment in the tests for attention, language and memory. (Grandjean et al 1997). A NOAEL of 12 ppm in maternal hair was derived from the results (Table 2; Dourson et al 2001). The effect of methylmercury on neuropsychological functioning was shown to be unaffected by simultaneous exposure of the study cohort to PCB (Budtz-Jorgensen et al 1999).

Link C: Fish consumption decreases with increasing risk of methylmercury toxicity.

Fish consumption limits are calculated by the US-EPA based on the measured mercury levels in fish, using the following assumptions: (a) body weight of 70 kg, (b) fish meal size of 227 g, and (c) RfD of 0.1 μg/kgBW/day. These calculations showed that eating 16 fish meals a month could be allowed when fish mercury levels were less than 0.06 ppm. This decreases with increasing fish mercury levels, becoming zero when fish levels were greater than 1.9 ppm (US-EPA 2001). The calculations hinged on the numerical value of the RfD, which allowed

fewer fish meals a month for any given fish mercury level, compared to the WHO's TDI of 0.48 μg/kgBW/day (Egeland & Middaugh 1997). This RfD value was based on the Iraqi data, which was used to estimate a daily intake of 1.1 μg/kgBW/day corresponding to a hair level of 11 μg/g (11 ppm). An uncertainty factor of 10 was then applied to account for uncertainties in the data set. The US-EPA interpretation of the RfD is that mercury exposure at or below the RfD could be expected to be safe, and exposures above the RfD would not necessarily imply the presence of risk. Rather, the risk following exposures above the RfD is uncertain, but risk could be expected to increase with increasing exposure (US-EPA 1997).

There are two kinds of risk that need to be addressed regarding mercury toxicity in food advisories: (1) the health risks directly associated with methylmercury, and (2) the indirect social, economic and cultural risks mediated through the disruption of eating patterns and lifestyle. On the whole, direct health impacts have been difficult to prove except in cases of very severe pollution, while indirect health effects from social, economic and cultural disruption had been significant. This was especially true in populations that depended on fish as subsistence food. In indigenous communities, for instance, contamination of the environment with an invisible pollutant such as methylmercury disrupted the traditional lifestyle, especially since both the hazard and the ensuing regulatory actions were perceived to be outside community control. Some responded to reports of high mercury levels by totally refraining from eating fish. The perception that fish was contaminated led to a dietary shift, which was associated with a four-fold rise in the prevalence of diseases such as diabetes. In cases such as these, issuing an advisory against eating fish resulted in economic, social and cultural disruption, and the health of inhabitants have been affected disproportionately greater than what could be expected from the scientifically-measured risk from mercury toxicity (Wheatley 1997; Wheatley & Wheatley 2000).

Link D: The intake of ω3 PUFA increases with increasing fish consumption.

Fish is the direct source of the very long chain ω3 PUFA in the human diet (Jonnalagadda et al 1995; Stone 1996; Ollis et al 1999). These so-called marine or fish-derived PUFA are eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). There are some ω3 PUFA, such as linolenic acid (LNA), that do not come from fish. In this paper, the term ω3 PUFA will be used to refer to the fish-derived ω3 PUFA i.e., EPA, DPA and DHA. EPA and DHA are the predominant forms of marine ω3 PUFA (Hughes 1995) and are consequently the ones most commonly reported in the literature (Jonnalagadda et al 1995; Elmståhl et al 1999; Hallgren et al 2001; Albertazzi & Coupland 2002), although some studies report values for DPA as well (Ollis et al 1999; Harel et al 2001).

Swedish men from Malmö had a median fish intake of 34 to 53 g/day, while Swedish women ate 32 to 45 g/day. The resulting median intake of EPA was 0.1 to 0.2 g/day for men, and 0.1 g/day for women; while the median intake of DHA was 0.3 to 0.4 g/day for men and 0.2 to 0.3 g/day for women (Elmståhl et al 1999). EPA, DHA and DPA in the diet of Australians likewise came mainly from fish, although a significant proportion came from meat, due to the large quantities consumed. Their mean intake of fish was 28 g/day, which corresponded to a daily intake of EPA+DHA+DPA of 0.20 g/day (Ollis et al 1999). Similarly, EPA and DHA in the diet of North Americans came mainly from fish and shellfish, with a mean intake of EPA+DHA at 0.1 g/day for both male and female, whether children or adult (Jonnalagadda et al 1995). Another study showed that the percentage of EPA and DHA in the fatty acid component of plasma was significantly higher in Swedish subjects who reported at least one fish meal per week, in comparison with those who reported less than one fish meal per week. Median plasma DHA+EPA was 5.4 to 5.6% in subjects with a low fish intake and 6.1 to 6.5% in subjects with a high fish intake (Hallgren et al 2001).

Link E: Nutritional benefits increase with increasing intake of ω3 PUFA.

Nutrition for the human brain needs a balanced intake of PUFA, specifically DHA and arachidonic acid (a non-fish-derived ω6 PUFA), which are necessary for growth and development of the fetal and infant brains, and must be adequately supplied from the diet during pregnancy and lactation (Broadhurst et al 2002). Likewise, these fatty acids are necessary for sustaining normal brain function in adults. A deficiency of long chain ω3 PUFA (e.g., DHA) has been linked to various psychological and behavioral disorders, and intake of ω3 fatty acids was considered to be protective from mental ill health. Furthermore, marine ω3 PUFA (EPA and DHA) were considered to be protective against ischemic heart disease, and lowered the risk of death, myocardial infarction and stroke (Das 2002). As such, an increasing percentage of plasma EPA and DHA in Swedish subjects from Västerbotten was significantly associated with decreased risk for the first-ever myocardial infarction (Hallgren et al 2001). Aside from this, marine PUFA have anti-atherosclerotic properties, as well as neuro-protective actions, and PUFA from a marine diet reduced the incidence of osteoporosis in Greenland Eskimo women (Das 2002)

Due to its desirable nutritional effects, marine PUFA have been used to treat certain diseases. Supplementation of ω3 PUFA was associated with improvement in rheumatoid arthritis and psoriasis (Hughes 1995). Likewise, supplementation with EPA and DHA was associated with an increase in hip bone mass density among post-menopausal women (Albertazzi & Coupland 2002).

Link F: Fish consumption increases with increasing nutritional benefits.

This link came from the assumption that humans would rationally maximize nutritional benefits from food. However, nutritional awareness does not always translate into action. For instance, American adolescents showed awareness that ω3 PUFA is healthy and may prevent heart disease (Harel et al 2001). Most adolescents were also aware that ω3 PUFA comes from marine fish. However, their actual fish consumption did not vary with this awareness, and self-reported fish consumption ranged from once a week (36%), once a month (29%), less than thrice a year (18%), to never (17%). The consumption of ω3 PUFA was calculated from 24-hour dietary recall to be 0.037 to 0.043 g/day of EPA+DHA+DPA. This level of intake was lower than the recommendations for ω3 PUFA intake, which prescribes 0.3 to 0.4 g/day EPA+DHA+DPA.

The amount of fish oil in the human diet that is necessary to maintain optimal nutrition and health is still undetermined; however, the British Nutrition Foundation Task Force on Unsaturated Fatty Acids recommended that 0.5% of total energy intake should come from long chain ω3 PUFA (Hughes 1995). They recommended eating 35 g/day of fish, which would give 1.25 g/day EPA+DHA. The mean consumption in Western populations is however less than this, at 0.25 g/day EPA+DHA (Albertazzi and Coupland 2002).

Link G: Methylmercury in the body decreases with its increasing metabolism.

Methylmercury in the body is eliminated predominantly through the feces as inorganic Hg^{2+}, through a process which begins with secretion of methylmercury from the liver into the bile, mainly in the form of a glutathione conjugate (WHO 1990; Clarkson 2002). A fraction of methylmercury in the bile is then acted upon by intestinal bacteria to form Hg^{2+}, which is mostly excreted in the feces. Most of the secreted methylmercury, as well as about 10% of Hg^{2+}, is reabsorbed from the intestines into the bloodstream, which brings it back to the liver, to be excreted anew in the bile. This cycle of secretion and reabsorption, referred to as enterohepatic circulation, provides a continuous supply of methylmercury for the intestinal bacteria to convert to excretable inorganic Hg^{2+}. This process of methylmercury metabolism and elimination has been described using several models i.e., a single compartment model, a physiologically-based pharmacokinetic (PBPK) model, and a biologically-based model, and these are discussed below.

1. The single compartment model represents the accumulation and excretion of methylmercury in humans, and is considered a useful working model for comparing blood or hair levels to daily intake (WHO 1990). It has been shown empirically that at continuous exposure, a steady state (where intake equals excretion) will be attained in approximately one year. The accumulated amount of methylmercury in the body, A, will plateau at a level given by the equation:

$$A = a / b \qquad \text{(equation 1)}$$

where a is the amount taken up by the body daily, and b is the elimination rate constant (equal to 0.693 divided by the half-life $t_{\frac{1}{2}}$). The whole body half-life ranges from 52 to 93 days, and has an average value of 70 days—which corresponds to $b = 0.01$/day. The steady-state mercury concentration in blood, C (μg/L), is related to the average daily dietary intake, d (μg), by the equation:

$$C = f \cdot d / b = [(0.95) (0.05) d] / [(0.01 \text{ day}^{-1}) (5 \text{ L})] \qquad \text{(equation 2)}$$

where f is the fraction of daily intake taken up by the body, equal to 0.95. Equation 2 assumes a blood volume of 5 L, and that 5% of the absorbed amount goes to the blood. The half-life of methylmercury in blood ranges from 39 – 70 days, with a mean of 50 days. The mercury concentration in hair can be calculated as 250 times the blood concentration, although there is appreciable inter-individual variation in this ratio. The half-life in hair thus follows that in blood, but with a wider variation. This ranges from 35 to 100 days, with a mean of 65 days.

The single-compartment model was used by the US-EPA to relate hair concentration to blood concentration from the Iraqi data, and blood concentration to intake (Stern 1997). In their form of the model, the daily oral intake, D, is given by:

$$D = \{ [(1 / R) \cdot H \cdot b \cdot V] / [A \cdot F] \} / W \qquad \text{(equation 3)}$$

where R is the ratio of hair concentration to blood concentration, equal to 0.250 (μg/g)/(μg/L); H is the benchmark concentration of Hg in hair (NOAEL), equal to 11 μg/g; b is the elimination rate constant in blood of 0.014/day; V is the average blood volume of 5 L; A is the fraction of the ingested dose that is absorbed, at 0.95; F is the fraction of the absorbed dose that is distributed to the blood compartment, at 0.05; and W is the average body weight of 60 kg. Uncertainty in this model was shown to reside mostly in the variables R, b and V. Uncertainty in R came from the small size of the Iraqi data set, which limited the determination of the variability and distributional form of R. Uncertainty in b was due to the existence of individuals with long half-lives. It was not possible to determine whether these were present in all populations, or were specific to the Iraqi data. Uncertainty in V came from an inability to discern its probability distribution. Additional data on the distributional form of R, b and V would therefore greatly reduce uncertainty in the model (Stern 1997).

2. The physiologically-based pharmacokinetic (PBPK) model describes the details of methylmercury pharmacokinetics in the body, by separately modeling the individual body compartments, such as red blood cells, plasma, kidney, urine, richly-perfused tissue, fat, slowly-perfused tissue, hair, cerebral blood, brain, liver, gut, intestine, feces, placenta, fetal plasma, fetal red blood cells, fetal brain, and fetal body (Clewell et al 1999; Young et al 2001). The PBPK model assumes that distribution in the blood is limited by plasma flow, except for the transport across the placenta, blood-brain barrier, and red blood cell membrane, which are limited by diffusion. The use of PBPK models has grown in recent years, and the following has been cited as some of its strengths relative to the single-compartment model: (a) it can incorporate inter-individual variability in physiological characteristics (Clewell et al 1999); (b) it provides an excellent tool in extrapolating animal data to humans (Young et al 2001); and (c) it allows the incorporation of genetic polymorphisms in metabolizing enzymes into the model (Haber et al 2002). The

PBPK model thus allows direct analysis of the sources of inter-individual variability, which cannot otherwise be done using the single-compartment model.

3. A biologically-based dynamical model relates mercury species in blood, hair and urine to the absorbed dose and tissue burden (Carrier et al 2001a & b). It is simpler and has fewer parameters than the PBPK model, which was justified by the lack of detailed knowledge of internal parameters in humans. It describes the rate of change in the amounts of organic and inorganic mercury in a body compartment as the difference between rates of uptake and loss, without detailing the physiological processes involved (Figure 2; Table 3). Methylmercury concentrations in internal organs is assumed to have a fixed partition coefficient with blood, due to the rapid rates of distribution between the blood and these organs. Metabolism, demethylation and conjugation are assumed to occur mainly in the liver, utilizing organic mercury from the blood. The biological model represents an intermediate level of complexity between the single-compartment and PBPK models.
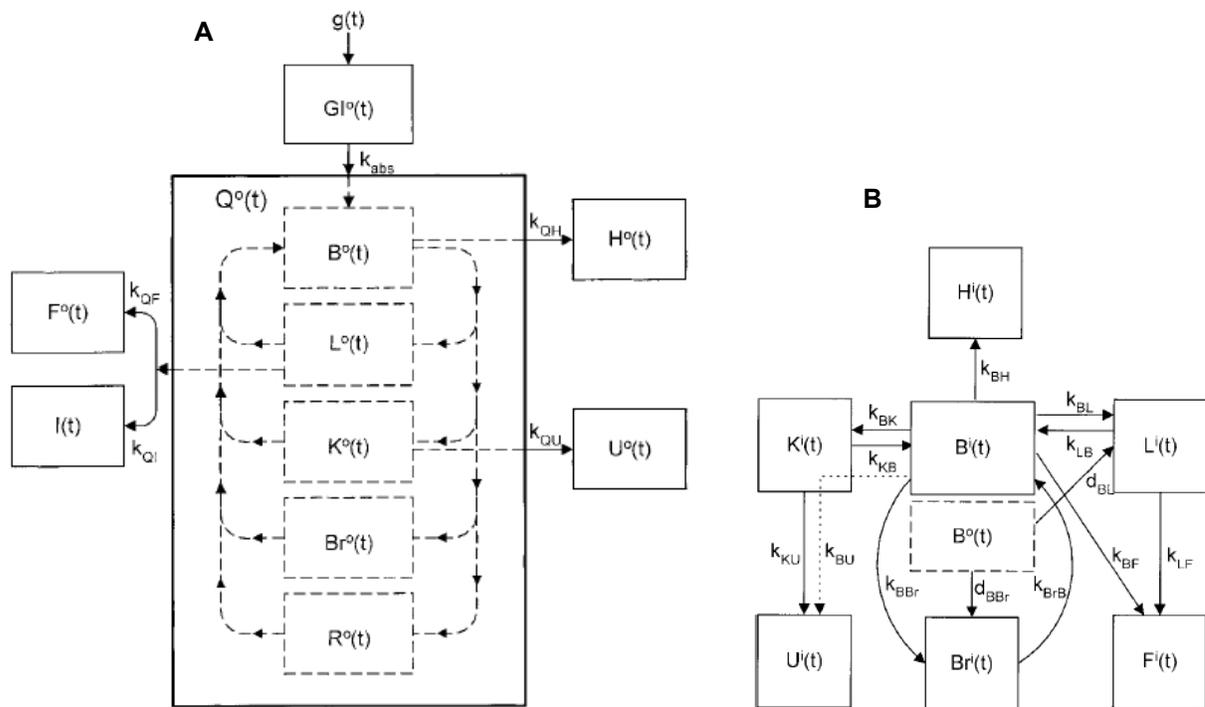
Figure 2. Biological model of methylmercury distribution and elimination in humans from Carrier et al (2001b). Diagram A represents the kinetics of methylmercury, while diagram B represents the kinetics of the inorganic mercury that is derived from methylmercury metabolism. Boxes with solid borders represent body compartments, while boxes with dashed borders represent sub-compartments. Arrows indicate mass flow between compartments. The symbols and abbreviations in the model are reproduced in Table 3. Methylmercury from dietary intake accrues to a single body compartment, because methylmercury is rapidly distributed within the body. Methylmercury concentration in the different sub-compartments equilibrate instantaneously, and could be adequately described by proportionality constants to the blood. Methylmercury elimination proceeds from first-order demethylation in the liver—defined by the metabolism rate constant k_{QI}—which produces inorganic mercury within the body. The inorganic mercury is distributed between body compartments at much slower rates than methylmercury. Inorganic mercury kinetics are thus represented by discrete tissue compartments. Mercury is subsequently excreted into the feces, urine and hair compartments.

Table 3. Symbols and abbreviations used in the biological model of methylmercury distribution and elimination in humans (from Carrier et al 2001b).

Variables and Parameters	Description
Organic mercury variables	
$g(t)$	Oral dose which can describe time varying inputs
$GI^o(t)$	Burden of organic mercury in the gastrointestinal tract as a function of time
$Q^o(t)$	Whole body burden of organic mercury excluding hair and excreta as a function of time
$B^o(t)$	Burden of organic mercury in blood as a function of time
$L^o(t)$	Burden of organic mercury in liver as a function of time
$K^o(t)$	Burden of organic mercury in kidney as a function of time
$Br^o(t)$	Burden of organic mercury in brain as a function of time
$R^o(t)$	Burden of organic mercury in the rest of the body as a function of time
$H^o(t)$	Cumulative burden of organic mercury in hair as a function of time
$U^o(t)$	Cumulative burden of organic mercury in urine as a function of time
$F^o(t)$	Cumulative burden of organic mercury in feces as a function of time
$I(t)$	Whole body and excreta burden of inorganic mercury as a function of time
Organic mercury constants	
K	Constant ratio $Q^o(t)/B^o(t)$
k_{abs}	Oral absorption rate constant
k_{QI}	Metabolism rate constant of organic mercury to inorganic mercury
k_{QF}	Whole body to feces transfer coefficient of organic mercury
k_{QU}	Whole body to urine transfer coefficient of organic mercury
k_{QH}	Whole body to hair transfer coefficient of organic mercury
k_{elim}	Whole body elimination rate constant of organic mercury
Inorganic mercury variables	
$B^i(t)$	Burden of inorganic mercury in blood as a function of time
$L^i(t)$	Burden of inorganic mercury in liver as a function of time
$K^i(t)$	Burden of inorganic mercury in kidney as a function of time
$Br^i(t)$	Burden of inorganic mercury in brain as a function of time
$H^i(t)$	Cumulative burden of inorganic mercury in hair as a function of time
$U^i(t)$	Cumulative burden of inorganic mercury in urine as a function of time
$F^i(t)$	Cumulative burden of inorganic mercury in feces as a function of time
Inorganic mercury constants	
d_{BL}	Blood to liver transfer coefficient combined with liver metabolism rate constant of organic mercury
d_{BBr}	Blood to brain transfer coefficient combined with brain metabolism rate constant of organic mercury
k_{LB}	Liver to blood transfer coefficient of inorganic mercury
k_{BK}	Blood to kidney transfer coefficient of inorganic mercury
k_{KB}	Kidney to blood transfer coefficient of inorganic mercury
k_{KU}	Kidney to urine transfer coefficient of inorganic mercury
k_{BH}	Blood to hair transfer coefficient of inorganic mercury
k_{BU}	Blood to urine transfer coefficient of inorganic mercury
k_{BF}	Blood to feces transfer coefficient of inorganic mercury
k_{LF}	Liver to feces transfer coefficient of inorganic mercury
k_{BBr}	Blood to brain transfer coefficient of inorganic mercury
k_{BrB}	Brain to blood transfer coefficient of inorganic mercury

Link H: The metabolism of methylmercury increases with more glutathione.

The metabolism of methylmercury is facilitated by the tripeptide glutathione (GSH). GSH functions as a reaction partner for the detoxification of various compounds in the body. It is supported in this role by several enzymes, namely glutathione peroxidases which reduce peroxides in the body (Dringen 2000), and GSTs which render toxins more water soluble and generally less biologically active (Knapen et al 1999). GSH is consumed in the detoxification of xenobiotics, through the formation of glutathione S-conjugates by GST, followed by subsequent excretion of these conjugates from the cell (Dringen 2000).

GSH is the major defense mechanism employed by cells against electrophilic substances such as methylmercury. It is generally believed that GSH complexes directly with methylmercury, thereby both lessening toxic activity and facilitating excretion out of the cell (Sarafian et al 1996). A large fraction of the methylmercury in liver cells is bound to glutathione as a glutathione conjugate ($CH_3Hg^.SG$), which is then excreted into the bile in order to eliminate it from the body (Dutczak & Ballatori 1994). While GSH is considered to be the major cellular defense mechanism against methylmercury, other mechanisms involving the metallothioneins, the heat shock proteins, and heme oxygenase also play a role (see Sarafian et al 1996).

Link I: The metabolism of methylmercury increases with more active GST enzymes; and
Link J: GST enzyme activity decreases with the deletion or substitution genotypes.

The GSTs are enzymes that catalyze the nucleophilic attack of the sulfur atom of glutathione (GSH) on electrophilic substrates. The natural substrates of these enzymes are basically unknown, and their characterization relies mostly on chemical substrates such as chloronitrobenzenes and halogenated hydrocarbons (Mannervik & Danielson 1988). Using these substrates, it has been shown that the isozymes of the polymorphic GST enzyme family possess differences in enzyme activity. The Mu class isozymes, for instance, show different rates of enzyme activity against the substrate trans-stilbene oxide (tSO). A null allele for Mu produces no active enzyme and thus confers no activity against the substrate. On the other hand, Pi class isozymes that have val^{105} possess seven-fold greater activity against polycyclic aromatic hydrocarbons (PAH) compared to the other Pi isozymes. Paradoxically, these val^{105} isozymes show weaker activity against another substrate, 1-chloro-2,4-dinitrobenzene (CDNB). As another example, the Theta class enzymes have an active form which metabolize the substrates dibromoethane, dichloromethane, ethylene oxide and methyl bromide. A null allele produces inactive enzyme and therefore confers no activity against these substrates (Board et al 1998; Bruhn et al 1998; Hayes & Strange 2000).

The differences in activity of GST isozymes against organic chemical substrates led to the investigation of the effect of these polymorphisms—especially the deletion genotypes—on diseases caused by toxic substances, such as cancers, asthma and allergies. These studies are summarized as follows:

1. Deletion of the Mu gene *GSTM1* was associated with increased risk for lung, bladder, colon and breast cancer in the homozygous null (Autrup 2000). The association of the null allele with lung cancer is ascribed to the role of GST enzymes in detoxifying carcinogens in tobacco smoke (Benhamou et al 2002).

2. Polymorphisms in the Pi gene *GSTP1* were associated with differences in asthma phenotype. The genotype frequency of *GSTP1 val^{105}/val^{105}* was reduced in atopic individuals. This genotype is thus associated with reduced risk of asthma. The asthma-protective effect of *GSTP1* is attributed to its role in detoxifying products of oxidant stress associated with airway inflammation (Strange & Fryer 1999; Strange et al 2001).

3. The biological effects of polymorphisms in the Theta gene *GSTT1* are rather difficult to predict, because the enzyme detoxifies substances such as monohalomethanes and ethylene oxides, but activates other substances such as methylene chloride. This double nature of *GSTT1* could explain the fact that there was no clear association of *GSTT1 Null* with carcinogenesis (Autrup 2000). One study showed that deletion of *GSTT1* is associated with differences in basal cell carcinoma (BCC) phenotype, since the frequency of *GSTT1 Null* was significantly greater in BCC patients with multiple clusters of primary tumors. The molecular basis behind this was not clear, but may involve the detoxification of products from UV radiation (Strange & Fryer 1999; Strange et al 2001).

No publications directly examined the role of GST enzymes in methylmercury metabolism; and therefore, these links remain speculative. However, one study investigated the relationship of *GST* polymorphisms with allergic sensitization to the mercury compound thimerosal (Westphal et al 2000). In that study, deletion of *GSTM1* and/or *GSTT1* was associated with allergic sensitization to thimerosal. *GSTM1 Null* was significantly more frequent in sensitized persons compared to healthy controls; similarly, *GSTT1 Null* was slightly more frequent in the sensitized group. The combined deletion genotype for *GSTM1* and *GSTT1* was, furthermore, significantly more frequent in sensitized patients. These results are consistent with the hypothesis that decreased enzyme activity in *GSTM1 Null* and *GSTT1 Null* caused a decreased capacity to metabolize and detoxify thimerosal and/or its mercury products. As a caveat, mercury in thimerosal exists as ethylmercury, which was previously assumed to be toxicologically similar to methylmercury. However, this assumption may not be entirely correct (Clarkson 2002).

Preliminary evaluation of the hypothesis

The methylmercury toxicity loop: links A, B & C

The literature supports Link A in that increasing fish consumption does increase the amount of methylmercury in the body, and that fish consumption is the major source of methylmercury. Total mercury concentration in blood has been used as a proxy measure for methylmercury coming from the diet (Hallgren et al 2001), which seems justified by the fact that inorganic mercury concentration in blood is up to two orders of magnitude less than methylmercury (see section on sensitivity testing, later in the methodology).

Literature support for Link B seems quite straightforward. A dose-response relationship for methylmercury exposure and toxicity has been established for high-level exposure (Clarkson 2002). For low-level exposure, the critical effect was stipulated to be impairment of fetal brain development from in utero exposure, and the NOAEL for prenatal toxicity is expressed in terms of total mercury concentration in maternal hair. The Iraq and Faroes studies report a dose-response threshold for low-level toxicity, although the Seychelles study failed to detect any response. From these three studies, the NOAEL has been variously set at 11, 12 and 15 ppm (Table 2), and this introduces uncertainty regarding which value best defines the risk of toxicity.

Link C is supported by literature as being a negative feedback, although the magnitude of its effect is uncertain. On the one hand, the modulating effect of methylmercury toxicity on fish consumption is based on the assumption that a person's fish intake shouldn't result in the ingestion of methylmercury in excess of the guideline value. Therefore, fish consumption should decrease proportionately with increasing methylmercury content of the food fish, in order to avoid increasing the risk of toxicity (WHO 1990; US-EPA 2001). On the other hand, fish consumption was demonstrated to decrease disproportionately to the actual risk of toxicity, and some people stopped consuming fish in response to advisories to merely reduce fish intake (Wheatley 1997; Wheatley & Wheatley 2000). Thus, Link C could either mean that increasing risk of methylmercury toxicity causes a *reduction* of fish consumption, or that increasing risk of methylmercury toxicity causes *cessation* of

fish consumption. Aside from this, the guideline values for methylmercury intake have been variously estimated at 0.48, 0.1 and 0.3 μg/kgBW/day (Table 1). This introduces further uncertainty regarding which guideline value best protects against risk of toxicity.

On the whole, the literature supports the hypothesis that links A, B and C constitute a negative feedback loop. The uncertainties associated with this loop do not change this negative feedback relationship, but might cause it to be more strongly negative than expected.

The ω3 PUFA nutrition loop: links D, E & F

The literature supports Link D in that increasing fish consumption does increase the intake of ω3 PUFA, and that fish is the major source of ω3 PUFA (Jonnalagadda et al 1995; Stone 1996; Ollis et al 1999), although the shape of the relationship between fish consumption and PUFA intake—whether linear, sigmoid or otherwise—has not been reported. Literature support for Link E is, likewise, quite straightforward, in that ω3 PUFA intake led to a host of nutritional benefits. Link F is supported by literature as a positive feedback, although the magnitude of its effect is much weaker than expected. On the one hand, the promotive effect of nutritional benefits on fish consumption would imply that a person would rationally eat more fish as he/she realizes greater nutritional benefits from doing so. On the other hand, fish consumption has been shown to be less than nutritionally optimal (Albertazzi and Coupland 2002), and did not increase with awareness of its beneficial effects (Harel et al 2001).

On the whole, the literature supports the hypothesis that links D, E and F constitute a positive feedback loop. It is, however, a weak positive feedback. Therefore the encouraging effect of this loop on fish consumption would be less important than the negative effect exerted by the risk of methylmercury toxicity.

Metabolism of methylmercury and effect of GST polymorphisms: links G,H,I & J

Link G is well-supported by the literature, and can be numerically described by a number of pharmacokinetic models. These range in complexity from one-compartment models (WHO 1990; US-EPA 1997), the biological model (Carrier et al 2001a & b), and PBPK models (Clewell et al 1999; Young et al 2001). All three types of models for methylmercury metabolism had been validated using experimental data from humans and other mammalian species, and therefore represent increasingly detailed representations of the same system. The choice of one model over another would not, therefore, result in model uncertainty (sensu Stern 1997).

The role of glutathione (GSH) in the metabolism of methylmercury, described by Link H, is well- established in literature (WHO 1990; Clarkson 2002). The role of the GSTs in methylmercury metabolism (Links I & J) is, however, not supported by any literature. Only indirect support for this comes from the study linking *GST* polymorphisms to sensitization to the mercury compound thimerosal. Validation of these links, therefore, would have to come from experimental investigation of the supposed relationship.

Despite this uncertainty, it is still possible to assume a relationship between *GST* polymorphisms and methylmercury metabolism. At one extreme, the *GST* genotype may have insignificant effect on methylmercury metabolism, resulting in a identical risk of toxicity for persons with the variant genotype compared to the rest of the population. At the other extreme, the *GST* genotype may have significant effect on methylmercury metabolism. Persons harboring variant genotypes (esp. the gene deletions that produce inactive enzymes) would therefore have impaired methylmercury metabolism and, consequently, greater risk of toxicity. These two extremes of possibility can be incorporated in the hypothesis as alternative scenarios: one assuming no effect of

genotype, and the other assuming an effect in the harmful direction. This approach was used in the present study, by assuming that each person would have the average half-life value of mercury in the blood (about 50 days) in the case that differences in GST enzymes, and ergo *GST* polymorphisms, have no impact on methylmercury metabolism. Alternatively, it was assumed that persons with deletion and/or mutant genotypes for GST would have a long half-life (more than 100 days, which has been reported in the literature, Birke et al 1972) in the hypothetical case that *GST* polymorphisms have appreciable impact on metabolism.

As a caveat, it should be borne in mind that studies showing statistically-significant associations between *GST* polymorphisms and cancer tended to show only a small magnitude of effect on meta-analysis i.e., an odds ratio of 1.17 for increased risk of lung cancer with *GSTM1 null* (Benhamou et al 2002). The large effect of GST polymorphism on methylmercury metabolism that is being assumed in the present study (viz. doubling the half-life) was made for the purpose of generating alternative scenarios for discussion, and should not be construed as reflective of actual empirical findings. The study was limited by the absence of literature directly linking *GST* with methylmercury, and it was not possible to integrate the effect of polymorphisms into the pharmacokinetic model (see Haber et al 2002 for a very good discussion of how this has been done for methylene chloride).

Methodology

Human data set of mercury concentration and ω3 PUFA levels

A human data set containing total mercury concentrations in red blood cells (RBC) and corresponding levels of ω3 PUFA in plasma, was available from a sample population from northern Sweden. It contained 233 individuals, with corresponding measurements of RBC total mercury (in both nanograms per gram RBC and nanomoles per gram RBC), and percentage PUFA composition for both the cholesterolester and phospholipid fractions of the blood. This data set was obtained from the University of Umeå as part of a collaborative project with the Department of Occupational and Environmental Medicine of Lund University Hospital. It represented an anonymous sample which is later to be subjected to *GST* genotyping. Dietary exposure to methylmercury in the sample was assumed to be chronic in nature, in the absence of contrary evidence; hence, the RBC mercury concentration in the data set was assumed to represent steady-state levels.

Simplification of the hypothesis into a working model

The quantitative relationship between methylmercury intake from fish consumption, accumulation in the body, and risk of toxicity was described using the biological model of Carrier et al (2001a & b)(Figure 2), which was used in this study for two reasons: (1) it contained sufficient detail to relate the RBC mercury concentration in the Swedish data set with the level of methylmercury intake and hair concentration, and (2) it gave the same behavior compared to the more complex PBPK models (data not shown).

The relationship between fish consumption, ω3 PUFA intake, and nutritional benefits was not incorporated in the model, due to the lack of a quantitative dose-response relationship comparable to the methylmercury model. Instead, a range of scenarios with increasing levels of fish consumption was analyzed, and it was assumed that the high fish consumption scenarios represented greater nutritional benefits.

The metabolism of methylmercury, mediated by glutathione, was an integral part of the biological model and did not need to be constructed separately. The effect of *GST* polymorphisms, on the other hand, was not explicitly incorporated in the model, due to the uncertainty regarding the magnitude of its effect. Instead, the deletion and substitution genotypes were assumed to represent a longest half-life of methylmercury available in the literature. This represented the worst-case scenario, where GST enzyme is assumed to have a significant effect on methylmercury metabolism, and deletion and/or substitution resulted in impaired metabolism. This approach was facilitated by the fact that Carrier et la (2001b) had calculated several values for the metabolism rate constant of organic mercury to inorganic mercury (k_{QI}). For the purposes of this study, their average value of $k_{QI} = 0.01347$, corresponding to a blood half-life of 50 days, was adopted to represent the hypothesis of no effect of *GST* polymorphisms on methylmercury metabolism. An alternative value of $k_{QI} = 0.005672$, corresponding to a blood half-life of 116 days (from Birke et al 1972), was used to represent the hypothesis of a harmful effect of *GST* deletion and/or substitution.

Implementation of the Modified Biological Model

The biological model of Carrier et al (2001a & b) was implemented on a Pentium II personal computer using STELLA Version 5.1.1 for Windows (High Performance Systems Inc., 1998). STELLA is a graphics-oriented modeling software which solves a series of differential equations and provides an output in the form of graphs and tables. The differential equations of Carrier et al (2001a & b) were rewritten into STELLA, and the

correctness of the computer implementation was checked against their figures. Several modifications were made in the model to allow direct usage of the data set, and the final version of the methylmercury pharmacokinetic model will hereafter be referred to as the Modified Biological Model. The modifications were as follows:

1. The data set contained measurements of total mercury in RBC as nanograms mercury per gram RBC. The model, however, calculates methylmercury concentration in whole blood as micrograms per milliliter. Several equations were incorporated in the model in order to convert RBC mercury to blood methylmercury. First, an RBC:plasma partition coefficient ($P_{RBC:plasma}$) of 12 was assumed (Clewell et al 1999), such that the RBC concentration is equal to 12/13 of the whole blood concentration. Second, RBC mass was converted to RBC volume using the factor 1.1104 g/ml RBC (Beutler 1994). Third, RBC volume was assumed to be 27.5 milliliters per kilogram body weight (ml/kgBW), and blood volume was assumed to be equal to 67 ml/kgBW. These represent an average between male and female normal values (Petersdorf et al 1983). The resulting series of calculations are given in Appendix B in the section on "Partitioning of methylmercury in blood to the RBC".

2. Methylmercury intake was standardized to micrograms methylmercury per kilogram body weight per day (μg/kgBW/day), in order to have the same units as the guideline values in Table 1. Body weight was assumed to be 70 kg (after WHO 1990, and US-EPA 2001)

3. Mercury concentration in hair was calculated from blood methylmercury concentration using a range of values for the hair:blood partition coefficient ($P_{H:B}$) generated by Monte Carlo simulation. This was done to address the large variability surrounding the estimated value of $P_{H:B}$ (WHO 1990; Stern 1997), and was greatly facilitated by the fact that Clewell et al (1999) has derived a lognormal distribution of $P_{H:B}$ from earlier studies, with a mean of 248.7 and coefficient of variation (CV) equal to 0.70. For each run of the Modified Biological Model, STELLA generated a random sequence of 100 $P_{H:B}$ values conforming to the specified lognormal distribution. One hundred estimates for the hair mercury concentration were thus computed for a given blood concentration, which represent a range of possible values, given the large level of inter-individual variability in $P_{H:B}$. No more than 100 Monte Carlo simulations were obtainable within STELLA due to limitations of the software. As will be seen later, this produced a rather rough appearance in the output distributions of hair concentrations, and led to some undesired variability in the extreme high values of hair concentration.

Sensitivity testing of the Modified Biological Model

The Modified Biological Model was subjected to sensitivity testing, in order to evaluate the effect of the assumptions and modifications described above. Monte Carlo simulation was not performed during sensitivity testing; instead, the mean value of $P_{H:B} = 248.7$ was used for these runs. Sensitivity testing showed the following:

1. Hair mercury concentration was insensitive to whether only methylmercury in blood or both methylmercury and inorganic mercury in blood were considered in its calculation. At an inputted intake of 0.1 μg/kgBW/day, the calculated hair concentration in the model was 2.07 ppm when only methylmercury concentration was used, and 2.08 ppm when the sum of methylmercury and inorganic mercury concentrations was used. The insensitivity was due to the fact that methylmercury concentration in blood (8.32×10^{-3} μg/ml) was greater than inorganic mercury concentration (2.78×10^{-5} μg/ml) by two orders of magnitude. Thus, methylmercury concentration in the blood compartment—rather than total mercury—was used to calculate the expected hair concentrations in the model.

2. Hair mercury concentration was insensitive to sexual differences in blood volume and RBC volume. The model was run using male values of 69 ml/kgBW blood and 30 ml/kgBW RBC, and afterwards female values of 65 ml/kgBW blood and 25 ml/kgBW RBC (Petersdorf et al 1983). The hair concentrations of mercury were 2.0 ppm for males and 2.1 ppm for females at a hypothetical dose of 0.1 µg/kgBW/day. Thus, the average of the male and female normal values for blood volume and RBC volume were used in the model calculations.

3. Hair mercury concentration was sensitive to variation in the hair:blood partition coefficient ($P_{H:B}$). The mean $P_{H:B}$ value of 248.7 yielded 2.0 ppm mercury in hair after a hypothetical chronic dose of 0.1 µg/kgBW/day. Using a $P_{H:B}$ value of one standard deviation below the mean yielded 0.6 ppm mercury, while a $P_{H:B}$ value of one standard deviation above the mean yielded 3.5 ppm mercury. The sensitivity of hair concentration to the value of $P_{H:B}$ justified the use of a probability distribution, rather than a point estimate, to represent $P_{H:B}$. This was the same approach followed by Clewell et al (1999).

4. Hair mercury concentration was sensitive to variation in the rate of methylmercury demethylation in the liver, represented by the parameter k_{QI} (Figure 2). Using the average k_{QI} of 0.01347/day (Carrier et al 2001b) yielded 2.1 ppm mercury in hair after a hypothetical chronic dose of 0.1 µg/kgBW/day. Using an alternative k_{QI} of 0.005672/day, on the other hand, yielded 4.6 ppm mercury in hair. The prolonged k_{QI} value was thereafter used to represent the worst-case assumption that presence of *GST* deletion and substitution genotypes would cause impaired metabolism.

Model simulation

The final version of the Modified Biological Model takes two input values i.e., the daily intake of methylmercury (in µg/kgBW/day) and the metabolism rate constant of organic mercury to inorganic mercury (k_{QI}). It uses this input to calculate the blood methylmercury concentration (and also the RBC mercury concentration), from which it calculates 100 values for the corresponding hair mercury concentration using Monte Carlo simulation. For each of the scenarios given below, model simulations were carried out in STELLA using Euler's method of integration. The model was run for 600 model days, in order to achieve steady state. The time increment (dt) value was set at 0.1, as it gave the same steady-state values of mercury concentration in hair and RBC as relatively finer values of dt. The results of each scenario thus consisted of a frequency distribution representing 100 estimated values for the hair mercury concentrations associated with the given level of methylmercury intake from the diet. These frequency distributions were plotted as a histogram, and the 95th percentile and 99th percentile values of hair mercury concentration were calculated for each scenario using SPSS Version 11.0 for Windows (SPSS Inc., 2001).

Scenario analysis

Five scenarios were explored in the Modified Biological Model, as follows:

1. The first scenario calculated the daily intake of methylmercury corresponding to the maximum RBC mercury concentration in the Swedish data set. This was done in order to estimate the maximum level of exposure that the population sample might be being subjected to. This scenario was executed in several steps. First, RBC mercury concentrations in the data set were arranged in ascending order to extract the uppermost values. Second, the Modified Biological Model was run with an arbitrary sequence of ascending levels of daily methylmercury intake as input, until the RBC mercury concentration equaled that of the

uppermost observed values from the data set. This yielded the level of daily methylmercury intake which corresponds to the uppermost values of RBC mercury concentration. Third, the frequency distribution of expected hair mercury concentrations was generated from the model using Monte Carlo simulation, and subsequently plotted as a histogram using SPSS. The 95th and 99th percentile values of the estimated hair mercury concentration were also calculated with SPSS. Lastly, the 95th and 99th percentile values of hair concentration were compared to the NOAEL. If the estimated hair concentration exceeded the NOAEL, then the level of methylmercury intake would result in a significant risk of toxicity. If, on the other hand, it did not exceed the NOAEL, the risk of toxicity could be considered negligible. The 95th percentile value of hair concentration was interpreted as follows: "given the specified level of methylmercury intake, there is a 5% chance that the resulting hair mercury concentration will be greater than X ppm," where X is the 95th percentile value in ppm. Similarly, the 99th percentile value of hair concentration was interpreted as: "given the specified level of methylmercury intake, there is a 1% chance that the resulting hair mercury concentration will be greater than Y ppm," where Y is the 99th percentile value in ppm. The choice of whether to use the 95th or 99th percentile estimates of hair concentrations in comparing with the NOAEL is discussed later in the paper. In this first scenario, the metabolism rate constant k_{QI} was set at the average value of 0.01347, since this scenario is not concerned yet with *GST* polymorphisms.

2. The second scenario calculated the frequency distribution of expected hair mercury concentrations at a level of intake equal to the US-EPA reference dose (RfD), which is 0.1 µg/kgBW/day. This served as a basis of comparison for the risk of methylmercury toxicity in the Swedish data set. Again, the metabolism rate constant k_{QI} was set at the average value of 0.01347, since this scenario is not concerned yet with *GST* polymorphisms.

3. The third scenario explored the impact of increasing fish consumption on the risk of methylmercury toxicity. Fish consumption was increased in this scenario in an attempt to maximize the associated nutritional benefits. The new level of fish consumption was arbitrarily defined as one that would result in a methylmercury intake of 0.3 µg/kgBW/day. This corresponded to the minimal risk level (MRL) set by the Agency for Toxic Substances and Disease Registry (ATSDR), and was therefore not expected to result in undue risk of toxicity. As in the previous scenarios, the resulting frequency distribution of expected hair mercury concentrations was compared to the NOAEL. The metabolism rate constant k_{QI} was set at the average value of 0.01347, since this scenario is not concerned yet with *GST* polymorphisms.

4. The fourth scenario explored the impact of increasing fish consumption to a level corresponding to less than the MRL. The frequency distribution of expected hair mercury concentrations was again compared to the NOAEL to quantify the risk of toxicity. The metabolism rate constant k_{QI} was set at the average value of 0.01347, since this scenario is not concerned yet with *GST* polymorphisms.

5. The fifth scenario simulated the hypothetical effect of *GST* polymorphism on methylmercury metabolism, by using a value of $k_{QI} = 0.005672$ to reflect a half-life of 116 days. This scenario represents the worst-case assumption that the presence of GST deletion and/or substitution genotypes would cause impaired methylmercury metabolism. The results of this scenario were intended to show a worst-case situation, in the hypothetical case that *GST* genotype would indeed have a significant impact on methylmercury metabolism. Two levels of fish intake were simulated in this scenario: (a) 0.1 µg/kgBW/day, representing the approximate level of intake in Scenario 1, and (b) 0.2 µg/kgBW/day, representing the level of methylmercury intake that could be expected if the sample population should consume twice as much fish as usual (assuming a constant methylmercury concentration in the food fish). As before, a frequency distribution of expected hair mercury concentrations was generated from this scenario, and subsequently compared to the NOAEL.
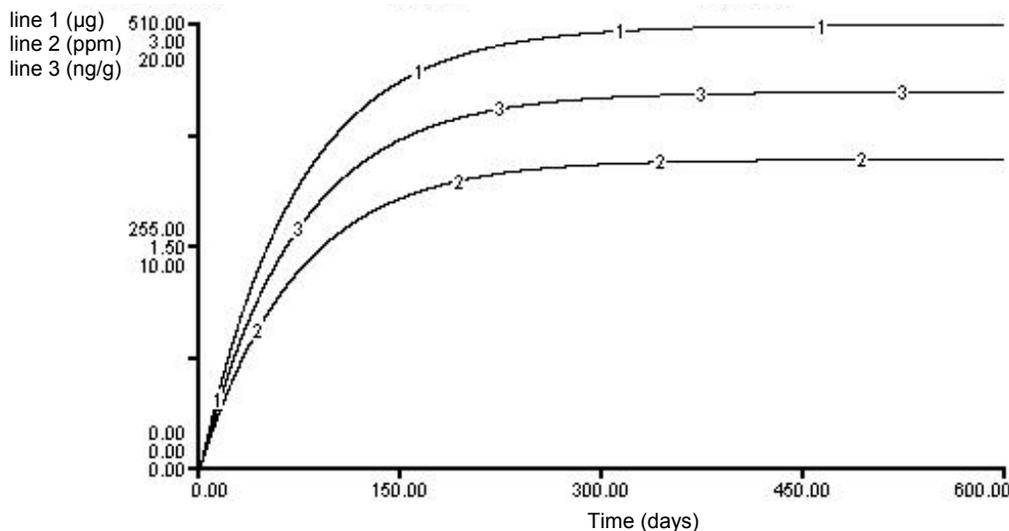
# Results

Baseline behavior of the Modified Biological Model

The Modified Biological Model described the accumulation of methylmercury in the body, given a daily intake of methylmercury as input (in μg/kgBW/day), and calculated the resulting mercury concentrations in hair (in ppm) and in RBC (in ng/g; see Figure 3). The model behavior was comparable to the described behavior of the more widely-used single-compartment model (WHO 1990; US-EPA 1997; ATSDR 1999), as follows:

1. The body burden of methylmercury in the model reaches steady state after about a year, and this is reflected by the plateauing mercury concentrations in blood and hair (Figure 3).

2. The body burden at steady state in the model is equal to 72 times the daily intake of methylmercury. This is equal to the value predicted from Equation 1, when the whole-body elimination rate constant (k_{elim}) of 0.01380/day from Carrier et al (2001b) is used (Table 3).

3. The steady-state blood concentration of methylmercury at a hypothetical chronic dose of 0.1 μg/kgBW/day is predicted by the Modified Biological Model at 8.3 μg/L. The corresponding blood concentration predicted by the single compartment model is 6.6 μg/L, calculated from Equation 2. The difference between the two models was due to different assumptions on the fraction of daily intake taken up by the body (*f*), the fraction of the absorbed amount going to the blood, the elimination rate constant *b*, and blood volume. Substituting the value of these parameters in Equation 2 with those used in the Modified Biological Model yielded a steady-state blood concentration of 8.2 μg/L in the single compartment model.

The Modified Biological Model calculated a range of values for hair mercury concentration from any given level of methylmercury intake, corresponding to the lognormal distribution of the hair:blood partition coefficient ($P_{H:B}$) derived from Monte Carlo simulation (Figure 4).

Figure 3. Body burden of methylmercury (line 1, in micrograms) after a hypothetical chronic dose of 0.1 μg/kgBW/day, and the resulting mercury concentrations in hair (line 2, in parts per million), and red blood cells (RBC; line 3, in nanograms per gram RBC).
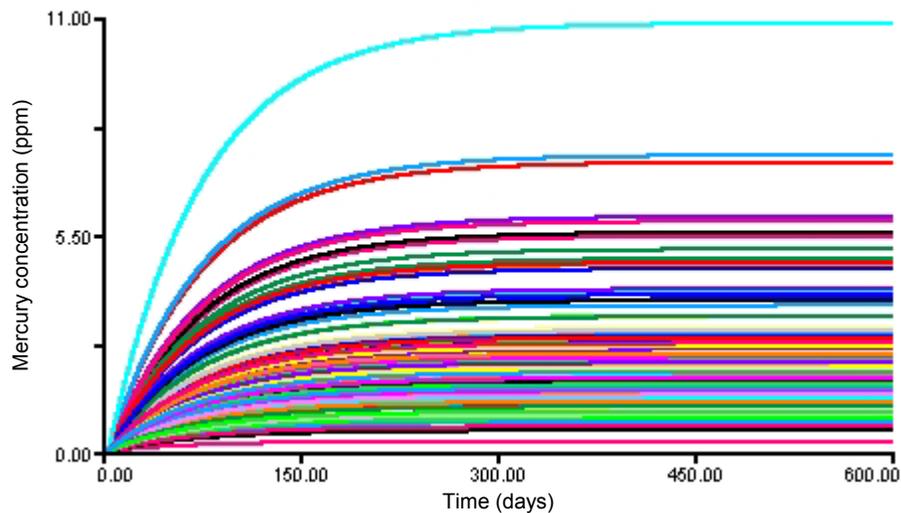
Figure 4. Range of mercury concentrations in hair (ppm) associated with a hypothetical chronic dose of 0.1 μg/kgBW/day. The graph-lines represent hair mercury concentrations calculated from a range of estimates for the hair:blood partition coefficient ($P_{H:B}$), derived from Monte Carlo simulation (n=100). $P_{H:B}$ was assumed to be lognormally distributed with a mean of 248.7 and CV = 0.70.

Scenario 1. Methylmercury exposure in the Swedish data set

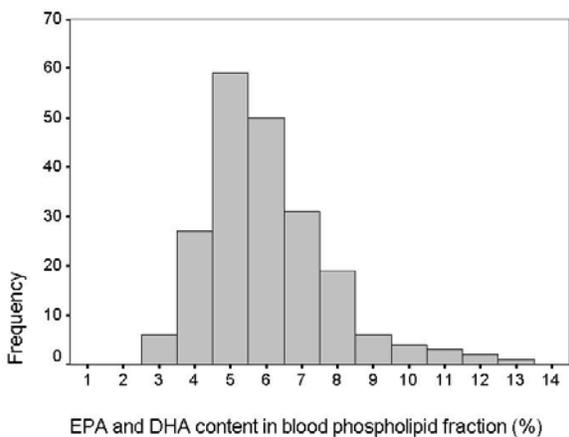The distribution of RBC mercury concentration from the Swedish data set has a mean = 5.1 ng/g and standard deviation = 5.2 ng/g (Figure 5). The data conformed to a lognormal distribution (Kolmogorov-Smirnov test, P=0.20), and contained two extremely high values of 30.9 ng/g and 66.8 ng/g. The distribution of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content in blood phospholipid fraction from the Swedish data set has a mean = 6.1% and standard deviation = 1.6% (Figure 6). The data also conformed to a lognormal distribution (Kolmogorov-Smirnov test, P=0.20). The scatter plot of RBC mercury concentration versus EPA and DHA content in blood phospholipid is shown in Figure 7. The two variables are significantly correlated (Pearson coefficient = 0.26; P<0.001), indicating that methylmercury exposure probably came from the same source as ω3 PUFA i.e., fish consumption. The extreme values of RBC mercury concentration were culled from the data set, since the scatter plot indicated that they might be subject to a different manner of exposure compared to the rest of the data set.

The maximum RBC mercury concentration in the Swedish data set was 17.5 ng/g, after culling the two outliers. The model was used to calculate the daily methylmercury intake that would give this RBC concentration at steady state, which was 0.1037 μg/kgBW/day. This was rounded off to 0.1 μg/kgBW/day. The model also calculated a distribution of hair mercury concentrations associated with this level of methylmercury intake (Figure 8). The 95th and 99th percentiles of hair mercury concentration corresponding to the maximum methylmercury intake in the data were 5.4 and 7.3 ppm, respectively (Scenario 1a in Table 4). Both of these are below the NOAEL, regardless of which NOAEL value was used (Table 2). Therefore, the Swedish sample in the data set could be said to have a negligible risk of toxic effects from methylmercury exposure.

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As an aside, the outliers were subjected to the same analysis above. The lesser of these extreme values (30.9 ng/g mercury) corresponded to a daily intake of 0.2 μg/kgBW/day, which gave a distribution of hair concentrations with 95th and 99th percentile values of 8.9 and 11.9 ppm, respectively (Figure 9A; Scenario 1b in Table 4). The 95th percentile value is less than any NOAEL, and may be considered safe. The 99th percentile value, on the other hand, is in a gray area, as it is less than the Faroes NOAEL but greater than the Iraq NOAEL. It may be stipulated that the Faroes NOAEL represents a better guideline than the Iraq NOAEL, owing to the larger sample size and superior study design. Thus, it may be considered that this outlier has negligible risk of toxicity. The other extreme value (66.8 ng/g mercury) corresponded to a distribution of hair concentrations with 95th and 99th percentile values of 24.9 and 33.6 ppm, respectively (Figure 9B; Scenario 1c in Table 4). This is definitely above any NOAEL, and implies that this person was being exposed to mercury levels great enough to entail significant risk of toxic effects. It must be emphasized at this point that this outlier was culled out from analysis only because he/she did not seem to be exposed in a similar manner as the rest of the sample. It may be inferred from Figure 4 that this person might be exposed to other sources of mercury aside from food (e.g., exposure in the workplace). Culling out him/her should not mean that his/her health risks were not important, only that the assumption in the model that methylmercury exposure came mainly from eating fish may not apply to him/her. In any case, additional data needs to be collected from this outlier to substantiate his/her exclusion from the data.
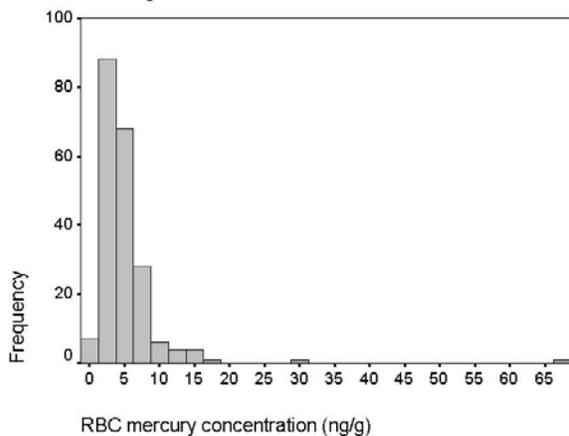
Figure 5. Distribution of mercury concentration in red blood cells (RBC) from the Swedish data set. Note the presence of two extreme values at 30.9 ng/g and 66.8 ng/g.

Figure 6. Distribution of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content in the phospholipid fraction of blood from the Swedish data set.
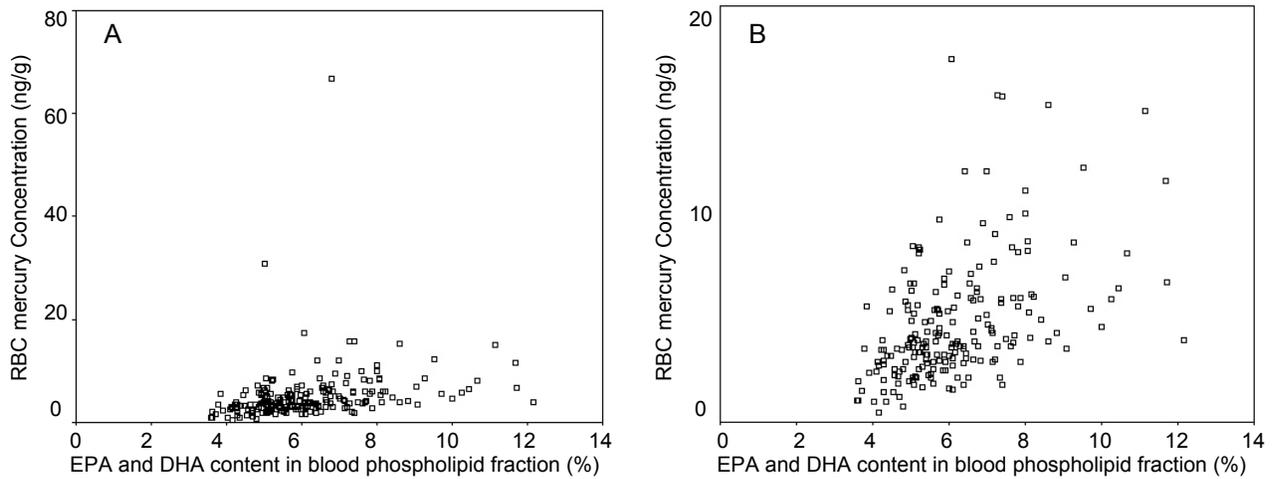
Figure 7. Scatter plot of mercury concentration in red blood cells (RBC) versus EPA and DHA content in blood phospholipid fraction. The two variables are significantly correlated (Pearson coefficient = 0.258, P<0.001). Note that two extreme values of RBC mercury concentration (30.9 and 66.8 ng/g) do not follow the same trend as the rest of the data (A). Culling out these values gives the scatter plot in (B).

Mercury Concentration in Hair (ppm)
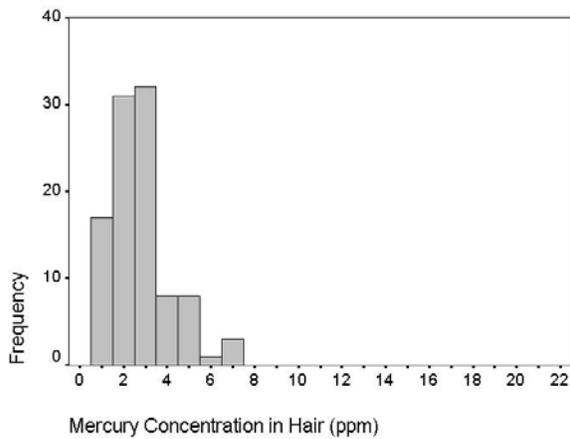
Figure 8. Distribution of hair mercury concentrations associated with a chronic dose of 0.1 μg/kgBW/day, the daily intake corresponding to the maximum RBC mercury concentration in the Swedish data set (17.5 ng/g) after culling two extremely high values. Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Table 4. Expected levels of mercury in red blood cells (RBC) and hair at various given levels of chronic methylmercury intake. Hair concentrations are presented as the 95th and 99th percentile of expected values from Monte Carlo simulation (n=100). The average value of kQI = 0.01347 was used in scenarios 1 to 4, while the alternative value of kQI = 0.005672 was used in scenario 5 to simulate a hypothetical effect of *GST* deletion and/or substitution genotypes on methylmercury metabolism.

Scenario	Mercury Intake (µg/kgBW/day)	RBC Concentration (ng Hg/g RBC)	Hair Concentration (ppm) 95th Percentile	99th Percentile
1a	0.1	17.5	5.4	7.3
1b	0.2	30.9	8.9	11.9 [a]
1c	0.4	66.8	**24.9** [c]	**33.6** [c]
2	0.1	16.9	5.8	10.9
3	0.3	50.6	14.0 [b]	**22.3** [c]
4	0.2	33.7	11.0	13.5 [b]
5a	0.1	37.7	11.6 [a]	14.1 [b]
5b	0.2	75.5	**20.4** [c]	**32.5** [c]

[a] hair concentrations exceeding the Iraq NOAEL of 11 ppm

[b] hair concentrations exceeding the Faroes BMD of 12 ppm

[c] hair concentrations exceeding the Seychelles NOAEL of 15 ppm, indicated by bold numbers
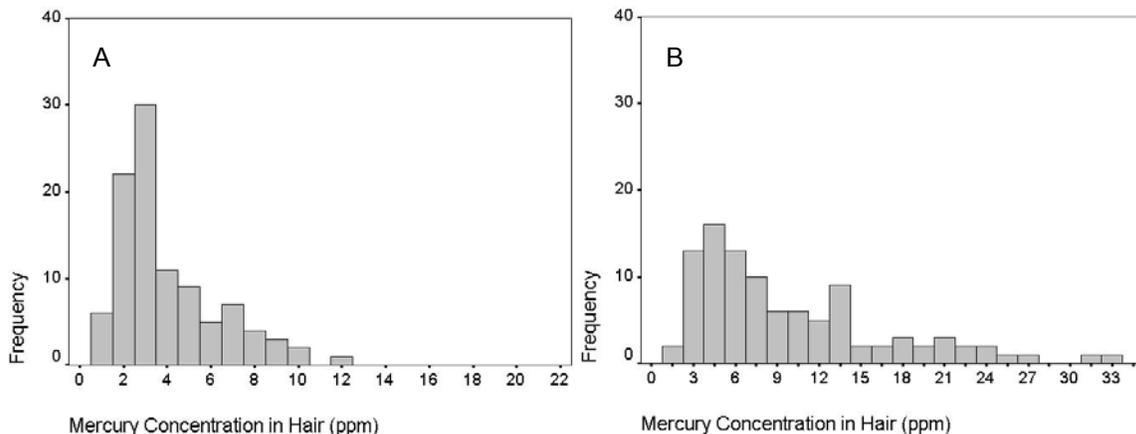
Figure 9. Distribution of hair mercury concentrations associated with methylmercury intakes of 0.2 µg/kgBW/day (A) and 0.4 µg/kgBW/day (B), corresponding to the two extreme values of RBC mercury concentration culled from the data (30.9 ng/g and 66.8 ng/g, respectively). Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Scenario 2: The US-EPA reference dose
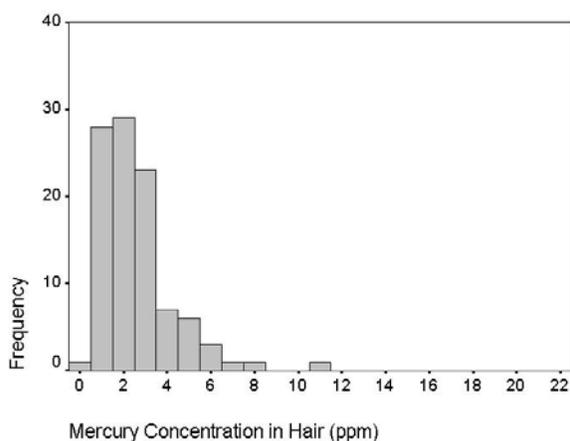
The distribution of hair mercury concentrations associated with methylmercury intake equal to the US-EPA reference dose (RfD) yielded 95th and 99th percentile values of 5.8 and 10.9 ppm, respectively (Figure 10; Scenario 2 in Table 4), which are both less than the Iraq NOAEL. This result was rather expected, since the Iraq NOAEL was used by the US-EPA in its derivation of the RfD. This scenario thus serves the purpose of validating the Modified Biological Model against the single-compartment model used by the US-EPA in deriving the RfD. As shown in this scenario, the present approach of using the biological model of Carrier et al (2001b) combined with Monte Carlo simulation to represent the uncertainty in the hair:blood partition coefficient ($P_{H:B}$) gave the same estimate of risk, when compared to the US-EPA's methodology of using a single compartment model using a fixed value of $P_{H:B}$ followed by the application of an uncertainty factor of 10 (US-EPA 1997). This gives greater confidence for using the Modified Biological Model.

It must be mentioned, however, that the random nature of Monte Carlo simulation introduced some degree of error in the resulting distributions of hair mercury concentration. Keeping in mind that the level of methylmercury intake in Scenario 1 (0.1 µg/kgBW/day) is equal to the RfD, then the shape of Figure 8 is expected to be identical to Figure 10. Likewise, the 95th and 99th percentile values, respectively, are expected to be identical in Scenarios 1a and 2 (Table 4). This is however not the case, and the divergence is most obvious in the 99th percentile hair mercury concentrations. This can only be attributed to the fact that Monte Carlo simulation in STELLA generated different sets of random values for $P_{H:B}$ for each of these scenarios. And although both sets of random $P_{H:B}$ values conformed to the same lognormal distribution, generating only 100 $P_{H:B}$ values was not enough to ensure reproducibility of the results, esp. at the extreme high ends of the distribution. This is most apparent at the 99th percentile hair mercury concentration, due to the fact that it represents the single highest hair concentration out of 100 (see Figure 4). And since the tail of a lognormal distribution extends to positive infinity, the single highest estimate of $P_{H:B}$ can assume any extremely high value and still conform to the specified lognormal distribution. In contrast, the 95th percentile hair mercury concentration is subject to a smaller amount of random error (5.4 ppm in Scenario 1a versus 5.8 ppm in Scenario 2, in Table 4). Thus, it is preferable to use the 95th percentile hair concentration in evaluating the risk of methylmercury toxicity against the NOAEL value. The fact remains, however, that 100 Monte Carlo simulations is insufficient for the task at hand, and the use of software that would allow a greater number of Monte Carlo runs should be explored in future.

Figure 10. Distribution of hair mercury concentrations associated with a chronic methylmercury dose of 0.1 µg/kgBW/day, which corresponds to the US-EPA reference dose. Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Scenario 3: Increasing fish intake

As shown by Scenarios 1 and 2, the level of exposure of the Swedish sample population to methylmercury from fish was at or below the US-EPA reference dose, with the result that they incurred negligible risk of toxic effects. The present scenario then explored the possibility of increasing fish intake, in an attempt to provide more ω3 PUFA from the diet. And since methylmercury was assumed to come mainly from the diet, increasing fish consumption also results in an increased intake of methylmercury. The new level of fish intake was arbitrarily set to give a methylmercury intake of 0.3 µg/kgBW/day. This results in a range of hair concentrations with a 95th percentile value of 14.0 ppm (Figure 11; Scenario 3 in Table 4). This value is in a gray area, as it is less than the Seychelles NOAEL but greater than the Faroes NOAEL (Table 2). There is no clear guidance from the literature regarding which of these two studies is better than the other, since both utilized a powerful study design (double-blind, prospective, longitudinal study) on large cohorts (1022 and 779 mother-child pairs, respectively). This point will be discussed later in the paper.
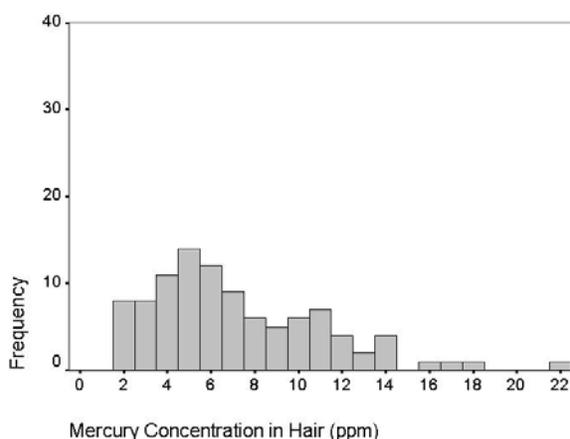
Figure 11. Distribution of hair mercury concentrations associated with a chronic methylmercury dose of 0.3 µg/kgBW/day, which corresponds to the ATSDR minimal risk level (MRL). Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Scenario 4. Increasing fish intake by a smaller increment

Since tripling the fish intake led to an equivocal estimate of toxic risk in Scenario 3, the present scenario explored the effect of a smaller increment in fish intake. The new level of fish intake was arbitrarily set to produce a methylmercury intake of 0.2 µg/kgBW/day, which resulted in a range of hair concentrations with a 95th percentile value of 11.0 ppm. (Figure 12; Scenario 4 in Table 4). This is below any NOAEL value, and may be considered safe unequivocally. Thus, increasing fish consumption two-fold incurred negligible risk of toxic effects.

This scenario addresses the concerns aired by Egeland and Middaugh (1997) that the Rfd may unduly restrict fish consumption and forego the associated nutritional benefits. As the present results show, there exists sufficient space to increase fish consumption to double the present amount without incurring undue risk of toxic effects from mercury. These results are consistent with the analysis of Clewell et al (1999), who used a PBPK model to demonstrate that the uncertainty factor of 10 applied to the RfD could be reduced to a factor 4.5, in order to raise the RfD value to 0.2 µg/kgBW/day.
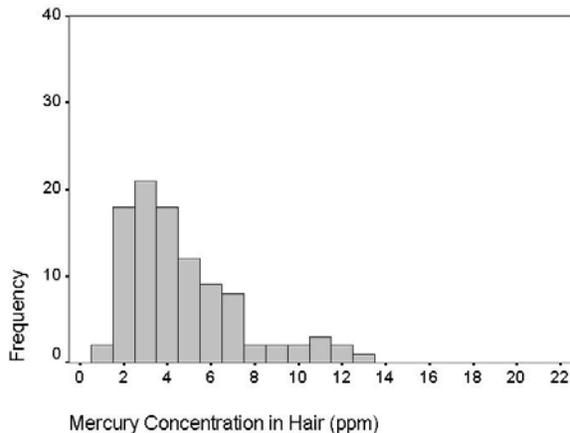
Figure 12. Distribution of hair mercury concentrations associated with a chronic methylmercury dose of 0.2 µg/kgBW/day. Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Scenario 5. Modeling the putative effect of *GST* polymorphisms

This scenario was based on the assumptions that (1) since complexation with glutathione is an important step in methylmercury metabolism and subsequent excretion, the GSTs, which are responsible for producing glutathione conjugates of various electrophiles, might be involved in the process as well; (2) since genetic polymorphisms in *GST* lead to differences in susceptibility to environmentally-induced diseases such as cancer and asthma, *GST* polymorphisms might also cause differences in susceptibility to methylmercury toxicity; and (3) if the active forms of GST were assumed to have a role in methylmercury metabolism, those genotypes with deleted or substituted *GST* genes would be expected to cause impairment in methylmercury metabolism and thus lead to greater susceptibility to toxic effects. The effect of genetic polymorphisms, if any, would therefore be in the harmful direction, and could be represented by a worst-case scenario with a lengthened elimination half-life. This scenario was translated to a k_{QI} value of 0.005672 in the Modified Biological Model, which results in a half life of 116 days (Carrier et al 2001b).

The distribution of hair mercury concentrations associated with a methylmercury intake of 0.1 µg/kgBW/day yielded a 95th percentile value of 11.6 ppm when k_{QI} = 0.005672 (Figure 13A; Scenario 5a in Table 4). This is below both the Faroes and Seychelles NOAEL, and thus carries negligible risk of toxic effects. Since the maximum intake in the Swedish data set is equal to this level (Scenario 1), the implication is that even at the worst case, the present level of methylmercury exposure would not result in increased risk of toxic effects to those persons harboring the *GST* deletion and/or substitution genotypes.

On the other hand, the distribution of hair mercury concentrations associated with the increased methylmercury intake of 0.2 µg/kgBW/day yielded a 95th percentile value of 20.4 ppm when k_{QI} = 0.005672 (Figure 13B; Scenario 5b in Table 4). This is well above any NOAEL, and thus carries a significant risk of incurring toxic effects. This implies that at the worst case, doubling the fish intake to twice the present levels might result in an increased risk of methylmercury toxicity to those persons harboring the *GST* variant genotypes. Therefore, it becomes relevant to empirically determine the existence—and measure the magnitude—of any effect that *GST* polymorphisms might have on the metabolism of methylmercury. This scenario serves as a caveat to Scenario 4.

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In the best case, therefore, namely that *GST* polymorphisms has no measurable effect on methylmercury metabolism, the risks of toxicity associated with doubling the fish consumption would be negligible as described in Scenario 4. However, invoking the precautionary principle means that the absence of an effect of *GST* polymorphisms must first be experimentally demonstrated, before any actions to encourage more fish consumption (for better nutrition) would be initiated.
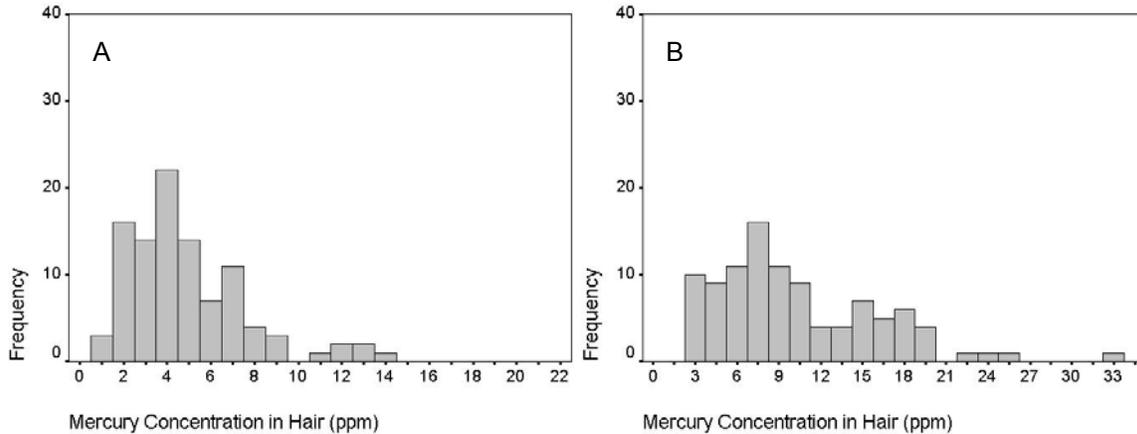
Figure 13. Distribution of hair mercury concentrations associated with a chronic methylmercury dose of 0.1 μg/kgBW/day (A) and 0.2 μg/kgBW/day (B), when k_{QI} = 0.005672, representing impaired metabolism. Y axis represents frequencies derived from Monte Carlo simulation (n=100).

Discussion

Risks of methylmercury toxicity in the Swedish sample population

The first objective of the study was to analyze whether or not having a less stringent mercury exposure guideline than the RfD carried a higher risk for mercury toxicity in the Swedish population. The typical U.S. consumer is described to be in no danger of consuming harmful levels of methylmercury in fish, and should not limit fish consumption out of concern for mercury poisoning (US-EPA 2001). This advice is considered to be applicable to people who consume less than 10 g/day of fish, at fish mercury concentrations not exceeding 0.15 ppm. The median fish intake of the Swedish consumer, in contrast, ranges from 32 to 53 g/day fish (Elmståhl et al 1999), and the Swedish critical limit for mercury allows levels as high as 0.5 ppm in fish tissue (Von Rein & Hylander 2000). Does this mean that the Swedish is exposed to inordinately high risks of mercury toxicity?

The results of Scenario 1 showed that the Swedish sample population faces negligible risk of methylmercury toxicity from food fish. This should not be interpreted to mean that the Swedish population in general faces negligible risk, since the study sample was limited to people from the northern part of Sweden, and was not meant to be representative of the entire Swedish population. Still, these results were within the range reported by dietary studies, which estimated the mercury intake from the Swedish diet to be from 0.65 μg/day (Jorhem et al 1998) to 1.8 μg/day (Becker & Kumpulainen 1991)—equal to 0.09 μg/kgBW/day and 0.26 μg/kgBW/day, respectively, for a 70-kg person. Following this line of reasoning, it may be inferred that the present low levels of RBC mercury in the study sample might have been due to either (1) a decreased consumption of the mercury-contaminated fish species, or (2) a decrease in the mercury content of the normally-consumed food fish. If Håkanson (1996) is to be believed that mercury levels in contaminated fish species would not decrease to safe levels until after three centuries, the conclusion would be that the low level of mercury exposure in the sample population must have been due to decreased consumption of contaminated fish species. Furthermore, the blood load of methylmercury in Swedish women was shown to have decreased from the late 1960s to the early 1980s, which was attributed to decreased consumption of contaminated fish (Bergdahl et al 1998). Ergo, the results imply that Swedish food advisories proscribing the consumption of potentially-contaminated fish had been effective in protecting the sample population from mercury toxicity (Pederby 1995).

It must be remembered that food advisories in Sweden are based on a critical limit of 0.5 mg/kg in fish, which is a proxy value representing a critical limit of 40 μg/day in humans (Sverdrup 2000). While this corresponds to a mercury intake limit of 0.57 μg/kgBW/day (for a 70-kg average person), the end result of the fish advisories had been to decrease mercury exposure to much lower levels. In the study population at least, this resulted in mercury exposure that was equivalent to the much more stringent US-EPA RfD of 0.1 μg/kgBW/day (compare Scenarios 1 and 2). This behavior is consistent with the earlier assertion about Link C (Figure 1) that the negative feedback of an increasing perception of risk of mercury toxicity on fish consumption could be disproportionately greater than the actual magnitude of risk. This is, of course, desirable behavior, since it means that the guidelines have produced error on the side of safety.

Increasing fish intake

Given that the Swedish do not seem to be facing an inordinate risk of mercury toxicity from fish, and that fish consumption is associated with numerous health benefits, the next question was whether or not fish consumption could be increased to maximize nutritional benefits without incurring an unacceptably high risk of methylmercury toxicity. As shown by the results in Scenarios 3 and 4, fish consumption could be increased to levels that would double or maybe even triple the levels of mercury intake (from the present level of 0.1

μg/kgBW/day), without incurring significant risks of toxic effects. Thus, Table 4 shows that at double the methylmercury intake from fish (Scenario 4), the 95th percentile of expected hair concentration is below the NOAEL, and at triple the methylmercury intake from fish (Scenario 3), the 95th percentile of expected hair concentration is below the Seychelles NOAEL, although this is above the Faroes NOAEL.

There is, however, a price to pay for increasing fish consumption. As shown in Scenario 5, the present low level of methylmercury intake results in negligible risk of toxicity even for those members of the population which may be more sensitive than the rest of the population (presumably because of an inherited impairment in methylmercury metabolism). Thus, on the worst-case assumption that bearers of *GST* deletion and/or substitution genotypes have a longer mercury half-life in the body, intake of 0.1 μg/kgBW/day gives rise to hair mercury concentrations with a 95th percentile value below both the Faroes and Seychelles NOAELs (Scenario 5a in Table 4). However, these persons could be expected to have hair mercury concentrations with a 95th percentile value greater than the NOAEL if they increase their fish consumption to levels that would give twice the amount of mercury in the diet (Scenario 5b in Table 4). Any recommendation to increase fish consumption beyond present levels would have to be tempered by this caveat. There is presently no empirical evidence that *GST* variant genotypes confer impaired methylmercury metabolism. However, there are enough reasons to suspect the link, based on the putative role of GST on glutathione conjugation of electrophiles, and the established role of glutathione in detoxifying the electrophile methylmercury. The onus then is to falsify the hypothesis that the link exists—through empirical investigation—before recommending that fish intake be increased. This seems to be a reasonable application of the precautionary principle.

Choosing between several estimates of the NOAEL

The presence of significant risk of toxic effect in the various scenarios of methylmercury intake was analyzed in the preceding discussion in terms of three distinct estimates of the NOAEL (Table 2). This introduces uncertainty over which level of the NOAEL is most preferable, and the following discussion emphasizes the point that a precautionary principle provides too little guidance to settle the issue. The three values of the NOAEL given in Table 2 represent different measures of the same critical endpoint i.e., neurodevelopmental toxicity in the fetus coming from prenatal exposure. The differences lay in their origin from three separate field studies, as follows:

1. The Iraq NOAEL of 11 ppm was derived from a retrospective study on a small sample (81 mother-child pairs) that was acutely exposed to methylmercury in poisoned grain. The data suffered from confounders, since: (a) interviews were done through interpreters, (b) birth dates were not remembered exactly in the Iraqi culture, (c) the background prevalence of neurological abnormalities were unknown, and (d) social and economic covariates which could affect child development were not investigated (Myers et al 2000a). Despite these limitations, the Iraqi data was used by the US-EPA in its derivation of the RfD, since statistical analysis of the Faroes and Seychelles data were still pending at the time (US-EPA 1997).

2. The Faroes NOAEL of 12 ppm was derived from a more powerful study design (a double-blind, prospective, longitudinal study) that investigated long-term dietary exposure in a large cohort (1022 children), using a wide array of physical, neurophysiological and neuro-psychological tests (Weihe et al 1996; Grandjean et al 1997). As such, the United States National Academy of Sciences recommended using the Faroes NOAEL in future revision of the RfD (Dourson et la 2001). However, the potential confounding effect of PCB co-exposure was considered a serious limitation (Clewell et al 2000), despite the study group's claims to the contrary (Budtz-Jorgensen et al 1999).

3. The Seychelles NOAEL of 15 ppm was derived from a double-blind, prospective, longitudinal study that investigated long-term dietary exposure in a large cohort (779 children), again using a wide array of neurodevelopmental tests (Myers et al 1995b). This study is considered to be relatively free from confounders, and was used by ATSDR to derive its minimal risk level (MRL).

Due to the limitations of the Iraqi study, the choice of which NOAEL value to use in the present paper could be narrowed down to either the Faroes or Seychelles NOAEL. A precautionary approach in this case could be interpreted to mean choosing the more conservative Faroes value. However, doing so could limit the possibility of allowing the population to maximize nutritional benefits from eating more fish (Scenario 3). As shown in Table 4, the 95th percentile of hair mercury concentrations associated with the dose of 0.3 μg/kgBW/day is 14.0 ppm—which would be considered safe based on the Seychelles NOAEL, but not so by the Faroes NOAEL. Therefore, if the main interest lay in maximizing nutrition (as in the present study), then it might be preferable to accept the Seychelles NOAEL as final guide, given that there seems to be no general consensus in the literature regarding how to choose between these two NOAEL values. This means that a precautionary approach does not always provide the best or final guide, especially in a situation involving a trade-off between risk and benefit (see Fairman et al 1998, p18, for a more thorough discussion).

Modeling the effect of genetic polymorphisms

This paper attempted to model the putative effect of *GST* polymorphisms on methylmercury metabolism by assuming two scenarios, viz. one scenario of no effect, represented by using the average k_{QI} value corresponding to a half-life of 50 days, and an alternative scenario of a worst-case effect, represented by using the alternative k_{QI} value corresponding to a half-life of 116 days. This approach is admittedly much too simplified, and was the result of a lack of detailed information regarding the mechanism of effect of GST enzymes on methylmercury metabolism. The intent of the above modeling was to show that *GST* polymorphisms could potentially affect the estimation of risk, and should therefore be explored in greater detail to elucidate how, and by which mechanisms, it might impact methylmercury metabolism. And since the worst-case scenario half-life has actually been observed in reality (Birke et al 1972), it only remains to be established whether *GST* polymorphisms could actually affect metabolism to this great extent, or have much lesser impact. Preliminary data from our laboratory have shown no clear trends regarding the effect of *GST Mu*, *Pi* or *Theta* on the RBC mercury concentration of 166 individuals from the Swedish data set. It remains to be seen whether this is due to an insufficient sample size to detect such differences, or whether this indicates that *GST* polymorphism has indeed no impact on methylmercury. More work is currently underway to answer this question.

It must be kept in mind that the GSTs are but one of the many enzymes acting on glutathione (Knapen et al 1999; Dringen 2000), and that the glutathione system is but one of many mechanisms utilized by mammalian cells as a defense against mercury toxicity, although it is considered to be the most important (Sarafian et al 1996). The relative contribution of such GSH-associated enzymes as glutathione peroxidases, and of other cellular defense mechanisms such as metallothioneins, heat shock proteins, and heme oxygenases has to be evaluated experimentally before the impact of genetic polymorphisms can be integrated into the pharmacokinetic modeling of methylmercury. This fault is by no means unique to the present paper. Haber et al (2002), for instance, pointed out that insufficiency of data regarding (1) the relative contribution of multiple enzyme systems and (2) the effect of large numbers of low-frequency alleles remain common gaps which increase uncertainty in this kind of modeling.

Lastly, it must be remembered that genetic polymorphisms is but one source of overall variation in the human population with respect to methylmercury susceptibility. Non-genetic sources such as age and infirmity could also contribute to increased susceptibility to methylmercury exposure. The general trend in the literature is,
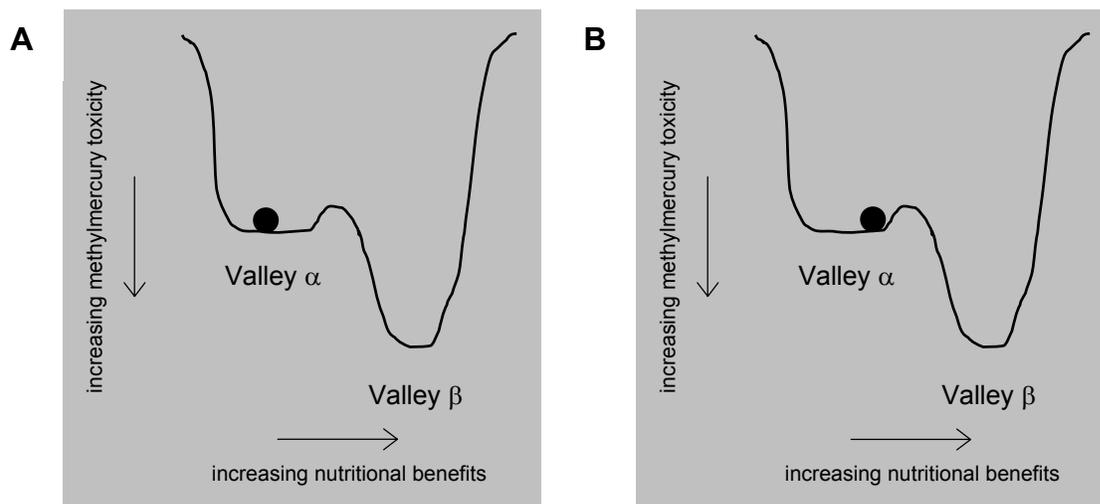
however, to start integrating data on genetic polymorphisms in the estimation of risk, especially since the two large studies on methylmercury toxicity (viz. Faroes and Seychelles) effectively excluded a large number of covariates from confounding their data. In particular with the Seychelles, the presence of as yet unidentified intrinsic differences in the study population with respect to methylmercury susceptibility has been suggested as one of the possible reasons why the data failed to show any discernible effect even in the upper ranges of exposure (Stern & Korn 2001). One of the postulated sources of variability is the presence of genetic polymorphisms. Another one could be intrinsic differences in the rate of physiological processes in the body (Haber et al 2002). The present trend in the literature is to use PBPK models in order to account for inter-individual physiological variability, while at the same time attempting to integrate the relative contribution of genetic polymorphisms in key enzyme systems, as well as the population frequency of these polymorphic alleles, into the PBPK model. While this approach is fraught with numerous sources of uncertainty at present, it is felt that this limitation only sets the stage for future empirical work which will help decrease the uncertainty (Haber et al 2002).

A systems approach to mercury toxicity

This paper has shown that the risk of mercury toxicity from food fish can be analyzed as a system of three main parts i.e., a strong negative feedback loop mediated by the toxic effects of mercury exposure, a weak positive feedback loop mediated by nutritional benefits from fish-derived ω3 PUFA, and a genetic component mediated by a putative role of GST in methylmercury metabolism and detoxification (Figure 1). The present state of the system for the Swedish sample population could be described as having negligible risk of mercury toxicity from fish consumption (Figure 14A). This is due to previous efforts by government agencies to set a critical limit on the mercury content of fish, accompanied by food advisories about which fish were safe to eat. This was also due to the fact that the population tended to heed this advice and modify their diet accordingly.

The paper also demonstrated that sufficient space existed to encourage the population to consume more fish, in view of the expected nutritional benefits to be gained from ω3 PUFA. The caveat, however, was that an empirical study is needed to show that such action would not affect their risk of mercury toxicity. On the assumption that genetic differences in mercury susceptibility might be mediated by the enzymes GSTs, such empirical study is needed to falsify the assumption that persons with deletion and/or substitution genotypes for these enzymes carry an increased risk of toxicity at high levels of fish intake. This constitutes an application of the precautionary principle on the study system.

The desired new state of the system is represented in Figure 14B. This future state is described as having increased fish consumption, while maintaining a negligible risk for mercury toxicity. The hill illustrated in the figure blocks the ball and keeps the system from shifting to a new state where increased fish consumption also leads to increased risk of toxicity. This hill represents those protective mechanisms that protect against incurring toxic effects. On the institutional level, these represent regulatory efforts by government agencies to define a critical limit for mercury and convert this information to fish advisories that are then communicated to the public. On the personal level, these represent the perception that fish consumption carries a risk of fish toxicity, which leads people to heed the government's regulatory advice regarding fish consumption.

A

increasing methylmercury toxicity

Valley α

Valley β

increasing nutritional benefits

B

increasing methylmercury toxicity

Valley α

Valley β

increasing nutritional benefits

Figure 14. Ball-and-topography analogy depicting two alternative stable states of the system under study, with respect to both methylmercury toxicity and nutritional benefits from PUFA. Valley α represents a state of low risk of mercury toxicity resulting from a low fish consumption, which also results in relatively small nutritional benefits from fish-derived PUFA. Valley β represents a state of greater nutritional benefits from PUFA resulting from greater fish consumption, which also results in higher risk of toxicity from mercury. The ball depicts the present state of the system, and could roll from one valley to another in much the same manner as a rubber ball rolls on the ground. Frame A above shows the state of the system as deduced from the Swedish data set i.e., low methylmercury toxicity resulting from a controlled intake of fish. Frame B depicts a new desirable state of the system, where fish consumption is increased in order to maximize nutritional benefits from PUFA, but not to the extent that the ball rolls down to Valley β and results in increased mercury toxicity. The hill blocking the ball, thereby keeping it from rolling into Valley β, represents the sum of all protective mechanisms—both personal and institutional—which prevent the human population from being exposed to levels of methylmercury that would cause toxic effects. The ball-and topography analogy was adapted from Holling et al (1995).

Setting a new critical limit

The remaining part of this paper explores the implications of the above modeling—or systems—approach of analyzing the risks from methylmercury toxicity on the proposal to set a new and stricter Swedish critical limit for mercury exposure. The Ad Hoc Expert Meeting on Setting Critical Limits for Heavy Metals in Bratislava proposed two alternative uptake levels for mercury in the human, depending on the definition of risk as being based either on a precautionary principle (Pp) or a maximum acceptable damage (MDp) approach (Sverdrup and Ashmore 2001). The resulting critical limits will be 10 μg/day (Pp) and 22 μg/day (MDp), which correspond to 0.14 μg/kgBW/day (Pp) and 0.31 μg/kgBW/day (MDp), respectively, at an average body weight of 70 kg. If it is assumed that mercury exposure occurs chiefly through the diet, then the Pp critical limit would correspond to a level only slightly above the US-EPA RfD. The distribution of hair mercury concentrations associated with methylmercury intake equal to the Pp critical limit yields a 95th percentile value of 7.8 ppm (data not shown). This is below the NOAEL, and poses the same negligible risk of toxicity as the RfD. Given the similarity between the Pp critical limit and the RfD, then it may also be said that the Pp critical value might severely

restrict fish consumption and its associated nutritional benefits. And similar to the RfD, the possibility of slightly relaxing the Pp critical limit should be explored, since Scenario 4 has demonstrated that ample room exists to increase fish consumption without incurring an unduly high risk of mercury toxicity. Once again, the caveat is that an empirical study is needed to show that relaxing the critical limit would not increase the risk of mercury toxicity, by showing that even sensitive members of the population would only incur negligible risk.

Additionally, setting a more stringent critical limit for mercury in Sweden would require the same broad public debate that accompanied the setting of the present threshold value during the 1960's. At that time, fishermen voiced out a concern that setting a critical limit would cause undue economic loss (Lidskog & Uggla 2000). Today, that concern is still a valid part of the discussion, since there are still about 3 000 professional fishermen who presumably wouldn't want the stricter guidelines to reduce their profits (Von Rein & Hylander 2000). Apart from them, such future debate should represent those parties interested in maintaining and/or improving the nutrition of the Swedish population. This is an important component of the issue, since (1) fish consumption yields various nutritional benefits; (2) the perception of mercury toxicity strongly discourages fish consumption, while an awareness of its nutritional benefits only weakly encourages fish consumption; and (3) sufficient space exists to relax the proposed Pp critical limit in order to maximize nutrition without increased risk of toxicity.

An added concern is that a restrictive critical limit on mercury intake might potentiate social and cultural problems resulting from economic dislocation and/or loss of traditional lifestyle (Wheatley 1997; Wheatley and Wheatley 2000). This is arguably not expected to pose much of a problem in Sweden, given that the affected parties (e.g., fishermen and associated occupations) would be involved in social discussion. Furthermore, there exist both sufficient forms of alternative employment and sources of food fish, unlike the situation in indigenous communities of subsistence fishermen. Finally, close monitoring should follow the implementation of any new food advisories, to assess its impact not only on the risk of toxicity, but also on human nutrition and any social dislocation. This means that future proposals to redefine the Swedish critical limit based on a more stringent definition of risk should be accompanied by studies that will closely measure the actual results of the resulting food advisories on fish consumption. While it is perhaps desirable to define a critical level based on a precautionary principle, great care must be taken to ensure that its final application does not cause fish consumption to decrease to too low levels, which might compromise getting a proper nutrition.

Summary and Conclusions

The problem of methylmercury toxicity in humans was analyzed in terms of a dynamic system composed of three components: (1) a strong negative feedback loop mediated by the toxic effects of mercury exposure; (2) a weak positive feedback loop mediated by nutritional benefits from fish-derived ω3 PUFA; and (3) a genetic component mediated by a putative role of GSTs in methylmercury metabolism and detoxification. The system was implemented in silico using a modified biological model of mercury pharmacokinetics. There was sufficient basis in the literature to allow the uncertainty in the hair:blood partition coefficient to be represented as a lognormal distribution, which was implemented in the model using Monte Carlo simulation. The model was used to predict the expected distribution of hair mercury concentrations, given a certain level of daily methylmercury intake from fish consumption. A comparison of the 95th percentile value of the expected hair mercury concentration with the NOAEL derived from the Iraq, Faroes and Seychelles studies allowed a numerical evaluation of the risks associated with each given level of exposure.

The Swedish sample population faced negligible risk of mercury toxicity, since the level of daily intake predicted from the maximum RBC mercury concentration in the sample corresponded to a 95th percentile of expected hair concentrations less than the NOAEL. This is within the range reported by dietary studies in the literature, and implies that the present critical limit of 0.5 mg/kg mercury in fish sufficiently protected this population from harmful levels of exposure. Furthermore, the resulting level of mercury intake (0.1 µg/kgBW/day) was disproportionately lower than the calculated levels corresponding to the established critical limit (0.57 µg/kgBW/day). This implies that the population reacted conservatively to fish consumption advisories, and limited their consumption much more than was originally intended.

Sufficient space existed to recommend increasing fish intake, in order to increase the population's intake of fish-derived ω3 PUFA and maximize their health benefits. Increasing the fish intake to levels that would give twice or even thrice the present mercury intake would not result in an increased risk of mercury toxicity. However, some segments of the population may possess genetic polymorphisms that might slow down their metabolism of methylmercury, and consequently increase their risk of toxicity. While this link between genetic polymorphisms in the GST enzymes and methylmercury toxicity has not been empirically established, the precautionary principle posits that the absence of this link has to be demonstrated before any attempts were made to encourage increased fish intake. This provides a rationale for experimental work, which is now being conducted by the author and his associates in the laboratory.

Lastly, future revision of the Swedish critical limit to a more stringent value based on a precautionary principle may result in overly restrictive fish consumption advisories that lessen the intake of ω3 PUFA from fish. This is because the population's perception of risk from mercury toxicity discourages fish consumption much more than the desire for proper nutrition encourages it. The possibility of relaxing the proposed guideline value is therefore suggested, with the caveat that the absence of toxic risk must again be demonstrated even for genetically more sensitive subgroups in the population.

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Appendix A: STELLA Diagram of the Modified Biological Model
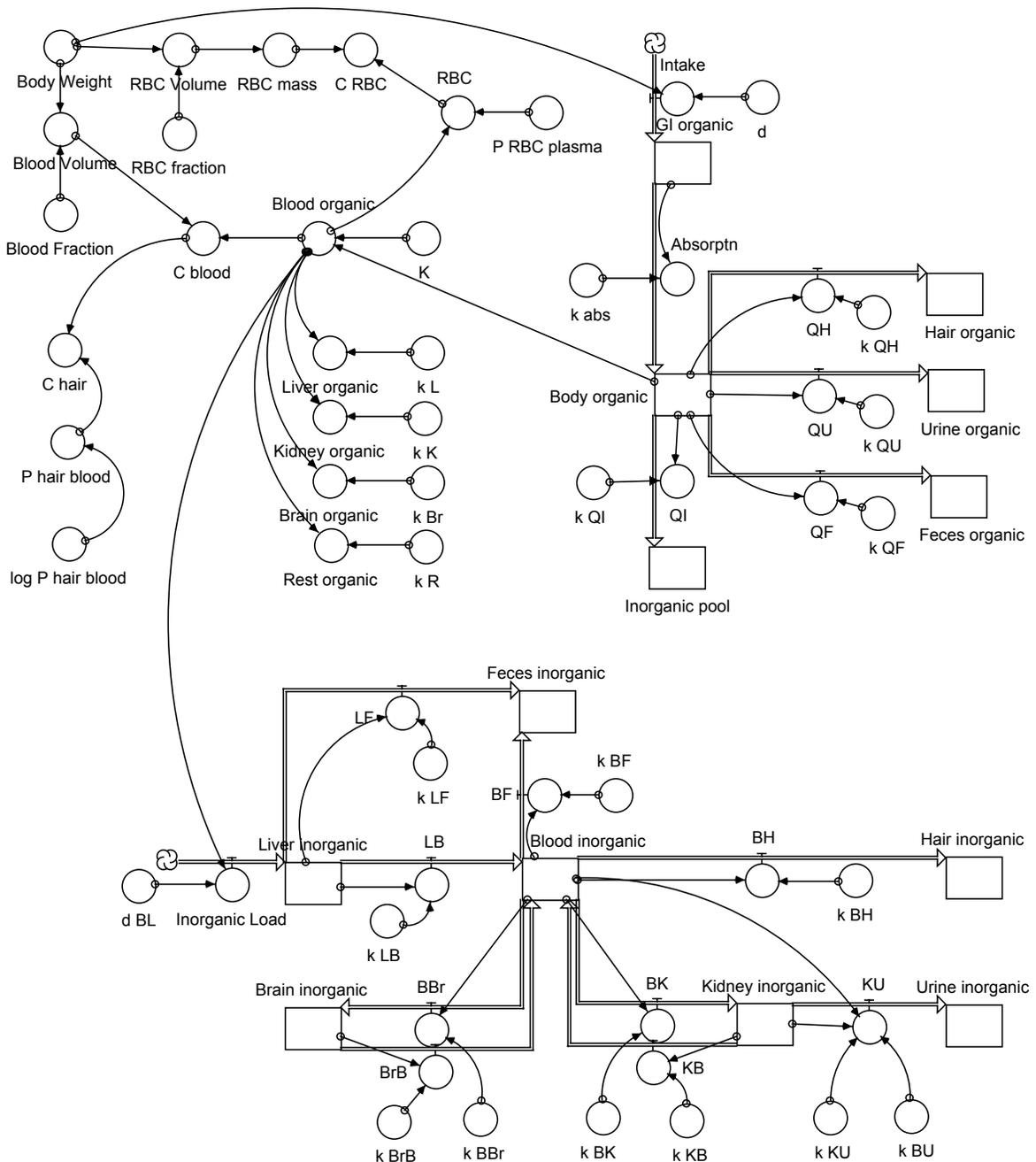
The diagram above is STELLA's graphical representation of the Modified Biological Model, and was included to allow the interested reader to test the model on his/her computer. It consists of three general parts: (1) a network of boxes and arrows on the upper right-hand side, which represent the kinetics of organic mercury in Figure 2a; (2) a network of boxes and arrows on the bottom part, which represent the kinetics of inorganic mercury in Figure 2b; and (3) a network of circles and arrows on the upper left-hand side, which represent the various calculations which allow the model to calculate both the RBC and hair mercury concentration from blood methylmercury. The STELLA code corresponding to the above figure is given in Appendix B.

Appendix B: STELLA Code of the Modified Biological Model

{Body Compartments of Organic Mercury}

Body_organic(t) = Body_organic(t - dt) + (Absorptn - QH - QU - QF - QI) * dt

INIT Body_organic = 0
    INFLOWS:
        Absorptn = k_abs * GI_organic   {intestinal absorption}
    OUTFLOWS:
        QH = k_QH * Body_organic   {body to hair outflow}
        QU = k_QU * Body_organic   {body to urine outflow}
        QF = k_QF * Body_organic   {body to feces outflow}
        QI = k_QI * Body_organic   {metabolism to inorganic mercury}
GI_organic(t) = GI_organic(t - dt) + (Intake - Absorptn) * dt

INIT GI_organic = 0
    INFLOWS:
        Intake = d * Body_Weight   {daily intage from the diet}
    OUTFLOWS:
        Absorptn = k_abs * GI_organic   {absorption by the body}
Feces_organic(t) = Feces_organic(t - dt) + (QF) * dt

INIT Feces_organic = 0
    INFLOWS:
        QF = k_QF * Body_organic   {body to feces inflow}
Hair_organic(t) = Hair_organic(t - dt) + (QH) * dt

INIT Hair_organic = 0
    INFLOWS:
        QH = k_QH * Body_organic   {body to hair inflow}
Urine_organic(t) = Urine_organic(t - dt) + (QU) * dt
    INIT Urine_organic = 0
    INFLOWS
        QU = k_QU * Body_organic   {body to urine inflow}

{Body Compartments of Inorganic Mercury}

Inorganic_pool(t) = Inorganic_pool(t - dt) + (QI) * dt

    INIT Inorganic_pool = 0
    INFLOWS:
        QI = k_QI * Body_organic   {metabolism to inorganic mercury}
Blood_inorganic(t) = Blood_inorganic(t - dt) + (LB + KB + BrB - BF - BH - BK - BBr) * dt

INIT Blood_inorganic = 0
    INFLOWS:
        LB = k_LB * Liver_inorganic   {liver to blood inflow}
        KB = k_KB * Kidney_inorganic   {kidney to blood inflow}
        BrB = k_BrB * Brain_inorganic   {brain to blood inflow}
    OUTFLOWS:
        BF = k_BF * Blood_inorganic   {blood to feces outflow}
        BH = k_BH * Blood_inorganic   {blood to hair outflow}
        BK = k_BK * Blood_inorganic   {blood to kidney outflow}
        BBr = k_BBr * Blood_inorganic   {blood to brain outflow}
Brain_inorganic(t) = Brain_inorganic(t - dt) + (BBr - BrB) * dt
    INIT Brain_inorganic = 0
    INFLOWS:
        BBr = k_BBr * Blood_inorganic   {blood to brain inflow}
    OUTFLOWS:
        BrB = k_BrB * Brain_inorganic   {brain to blood outflow}
Kidney_inorganic(t) = Kidney_inorganic(t - dt) + (BK - KB - KU) * dt

    INIT Kidney_inorganic = 0
    INFLOWS:
        BK = k_BK * Blood_inorganic   {blood to kidney inflow}

```
        OUTFLOWS:
            KB = k_KB * Kidney_inorganic            {kidney to blood outflow}
            KU = ( k_KU * Kidney_inorganic ) + ( k_BU * Blood_inorganic ) {outflow to urine}
Liver_inorganic(t) = Liver_inorganic(t - dt) + (Inorganic_Load - LB - LF) * dt

INIT Liver_inorganic = 0
        INFLOWS:
            Inorganic_Load = d_BL * Blood_organic {metabolism to inorganic mercury}
        OUTFLOWS:
            LB = k_LB * Liver_inorganic              {liver to blood outflow}
            LF = k_LF * Liver_inorganic              {liver to feces outflow}
Feces_inorganic(t) = Feces_inorganic(t - dt) + (LF + BF) * dt

INIT Feces_inorganic = 0
        INFLOWS:
            LF = k_LF * Liver_inorganic              {liver to feces inflow}
            BF = k_BF * Blood_inorganic              {blood to feces inflow}
Hair_inorganic(t) = Hair_inorganic(t - dt) + (BH) * dt
        INIT Hair_inorganic = 0
        INFLOWS:
            BH = k_BH * Blood_inorganic            {blood to hair inflow}
Urine_inorganic(t) = Urine_inorganic(t - dt) + (KU) * dt

INIT Urine_inorganic = 0
        INFLOWS
            KU = ( k_KU * Kidney_inorganic ) + ( k_BU * Blood_inorganic )
```

{Subcompartments of Organic Mercury}
```
        Blood_organic = Body_organic / K
        Brain_organic = k_Br * Blood_organic
        Kidney_organic = k_K * Blood_organic
        Liver_organic = k_L * Blood_organic
        Rest_organic = k_R * Blood_organic
```

{Pharmacokinetic Parameters from Carrier et al 2001b}

K = 12.987	k_abs = 5.5440	k_BBr = 0.0028	k_BF = 3.9917
k_BH = 0.1400	k_BK = 17.1234	k_Br = 0.007134	k_BrB = 0.0520
k_BU = 0.06994	k_K = 0.1140	k_KB = 0.0010	k_KU = 0.006949
k_L = 0.1490	k_LB = 0.8940	k_LF = 1.5476	k_QF = 9.0668e-5
k_QH = 2.3825e-4	k_QI = 0.01347	k_QU = 0	k_R = 2.0254
d_BL = 0.1750			

{Input Variables}
```
        d = 0.1          {daily dose of methylmercury in micrograms per kilogram body weight}
```

{Output Variables}
```
        C_blood = Blood_organic / Blood_Volume     {blood concentration in micrograms per ml}
        C_RBC = RBC / RBC_mass                      {RBC concentration in micrograms per gram}
        C_hair = C_blood * P_hair_blood             {hair concentration in ppm}
```

{Partitioning of methylmercury in blood to the RBC}
```
        Body_Weight = 70                            {assumed average body weight in kilograms}
        Blood_Fraction = 67                         {males 69 ml/kgBW; females 65 kg/kgBW }
        Blood_Volume = Blood_Fraction  * Body_Weight

        RBC_fraction = 27.5                         {males 30 mL/kgBW; females 25 mL/kgBW }
        RBC_Volume = RBC_fraction * Body_Weight
        RBC_mass = RBC_Volume * 1.1104 {g/ml }

        P_RBC_plasma = 12                           {RBC:plasma partition coefficient}}
        RBC = P_RBC_plasma * Blood_organic  / ( P_RBC_plasma + 1 )
```

{Partitioning of mercury into hair]
```
        P_hair_blood = EXP(log_P_hair_blood)        {hair:blood partition coefficient}
        log_P_hair_blood = 5.516247                 {natural logarithm of the hair:blood partition coefficient}
```

{end of STELLA code}