4 Health Risks from Eating Contaminated Fish

4.1 Introduction

Assessing and quantifying the potential risks to human health from eating contaminated fish is essential to evaluating both the target risks from consuming contaminated fish and the countervailing risks that may result from consumers following fish advisory advice. Adverse health effects from contaminants in fish range widely and may include cancer, developmental and reproductive toxicity, and other systemic effects. The occurrence and severity of the effects will depend upon the amount to which a person is exposed, and characteristics of the individual, including genetic makeup and life stage.

Traditionally, risk assessors have calculated estimates of an individual or population's risk for getting cancer from exposure to chemicals, while for non-cancer endpoints, a reference dose or concentration (for contaminants in air) is identified at which one would not expect to see adverse effects in a population (including sensitive subgroups). Cancer slope factors are estimates of risk that are derived from dose-response data from laboratory animal or human epidemiology studies. Traditionally, a linearized multi-stage model has been used to extrapolate from what is observed at high experimental concentrations to lower environmental exposure levels. This cancer potency is estimated as the 95% upper confidence limit of the slope of the dose-response curve in the low dose region. This is an upper estimate of risk and the actual risk may be much lower or even approach zero. EPA proposed revised cancer guidelines in 1996 (U.S. EPA 1996) and additional proposed guidance in 1998 (U.S. EPA, 1998), which recommend that the mode of action be considered. The guidance recommends that a linear extrapolation should be used if the chemical is believed to act via a genotoxic mode of action, if the mode of action is expected to be linear at low doses, or (as a default) if no mode of action data are available. The guidance also recommends that a non-linear approach to extrapolation to low doses should be used when sufficient information on mode of action warrants. For non-cancer effects, a single estimate of a "safe" dose is identified from animal or human data, using the No Observed Adverse Effect Level (NOAEL) divided by uncertainty factors to account for extrapolation from animals to humans, variability in the human population, and deficiencies in the database of studies on the substance. The resulting RfD is defined as "an estimate (with uncertainty perhaps spanning an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (U.S. EPA 1999).

These cancer risk estimates and reference doses have been used to estimate consumption rates of contaminated fish which would be without appreciable risk to humans following exposure over a lifetime (U.S. EPA, 1997b). However, in order to compare risks to risks and risks to benefits for both cancer and non-cancer endpoints, we thought it necessary to express both types as a risk estimate. A method to determine how likely it is that adverse effects will occur above the RfD (or with a hazard index (HI) greater than 1 for a mixture of chemicals) is needed. In this document we use a method for estimating non-cancer risk above the reference dose (RfD) developed by the U.S. EPA and the ChemRisk Division of McLaren/Hart (Price *et al*., 1997). This is an evolving area of research, however, which needs further development.

To assist in illustrating the framework, six target substances were chosen to develop estimates of risk above the RfD (see Table 4-1). The six were selected based upon the frequency of detection in a national study of chemical residues in fish (U.S. EPA 1992), and the number of states that have issued advisories for that substance. Of these six substances appropriate data for developing these non-cancer estimates were available for methylmercury and chlordane.

Table 4-1. Frequency of residue presence in fish, and the number of states that have issued advisories for the chosen chemicals.[1]

Chemical Name	Number of states with advisories	Percent of sites sampled were compound was detected in fish[2]
DDT and Metabolites	9	99%
Methylmercury	27	92%
Dioxin (TCDD)	22	70%
PCBs	31	91%
Chlordane	24	61%
Chlorpyrifos	--	26%

[1] Source: U.S. EPA (1992).

[2] Fish were collected from 388 sites throughout the United States. Sites included target areas near point and nonpoint sources, sites representative of background levels, and USGS NASQAN sites. A subset of 103 sites were sampled during the National Dioxin Study (U.S. EPA, 1987) that had been analyzed only for 2,3,7,8 TCDD, but were reanalyzed for all dioxin/furan congeners and other xenobiotic compounds.

This chapter briefly explains the available methods for estimating risk above the RfD and resulting risk information for the six selected chemicals. More information on traditional risk assessment methods and calculating risks from contaminants in fish is found in EPA's *Guidance for Assessing Chemical Contaminant Data for use in Fish Advisories: Volume 2* (U.S. EPA 1997b).

4.2 Calculating Risk above the Reference Dose for Noncancer Endpoints

The RfD approach and measures of hazard such as the Margin of Exposure (MOE) and Hazard Index (HI) are useful to determine unsafe doses or aid in the evaluation of mixtures and comparison of chemicals; however, these approaches provide no guidance on risk. The framework developed in this document looks at both the noncancer and carcinogenic risks from chemicals together to balance these with the health benefits of consuming fish. This necessitates using a method to estimate health risk for noncancer endpoints when the RfD or "safe" level is exceeded.

Unlike for carcinogenicity, there is no noncancer dose-response model commonly accepted for estimating risks above the RfD. For the purposes of illustrating this framework, we estimated risk using the approach developed by the U.S. EPA and ChemRisk (Price *et al*., 1997). This approach was selected because of its ease of use and fidelity to existing EPA sources of information. Two other possible approaches are briefly presented below. Estimating noncancer risk is an area that needs further research and development.

4.2.1 EPA/ChemRisk Model

This approach is the result of a four-year collaboration between the ChemRisk division of McLaren/Hart and the U.S. EPA (Price *et al.*, 1998; Swartout *et al.*, 1998b). It builds on the existing RfD framework, requires minimal new information, and quantitatively deals with uncertainty and variation in response. The basic premise is that there is generally more information available than is used to derive the RfD and that the same method that estimates the RfD can be applied to other points on the dose-response curve for humans. This method postulates a conservative linear threshold model for the dose-response relationship of noncarcinogenic agents. The approach uses Monte Carlo simulation of uncertainty factor distributions to develop a family of potential dose-response curves reflecting the uncertainty in interspecies extrapolation, interindividual variability, extrapolation from subchronic to chronic responses, extrapolation from LOAEL to NOAEL, and database uncertainty.

The approach is based on three concepts:

- Use the RfD as a model of a zero (or minimal) risk (ED_0- effective dose zero) in humans.
- Use the current system of uncertainty factors to predict the dose that causes an effect in a typical human (ED_{50}).
- Use a linear model as an upper bound to the actual dose response for doses between the ED_{50} and ED_0.

To model the ED_0, one can use the definition of the RfD where ED_0 is equal to the NOAEL, or Benchmark Dose, divided by the uncertainty factors for various extrapolations (e.g., intra- and interspecies extrapolation). These uncertainty factors are defined as distributions.

The concept of estimating the dose that causes an effect in a "typical individual" is not usually considered in noncancer risk assessment; in this approach the dose causing a response in a typical individual is conceptually similar to a chronic ED_{50}. To calculate the ED_{50} for humans one could take the ED_{50} in animals (ED_{50a}) and divide by the uncertainty factor for interspecies extrapolation (UF_A). In this case the intraspecies uncertainty factor (UF_H) is not applied. This UF_A is traditionally viewed as representing interspecies differences in the NOAEL, not in the ED_{50s}. The ED_{50a} should be based on all adverse effects in the test animals, not just the critical effect.

The third concept -- use of a linear model -- assumes that the fraction of the population that responds at doses between ED_{0h} and ED_{50h} is a linear function of dose in excess of the ED_{0h}. This assumption will be conservative for compounds with sublinear dose-response curves.

In this model the uncertainties in factors such as UF_A and UF_H are expressed as distributions. The model predicts the dose response by randomly selecting values for UF_A and UF_H and calculating a response to the dose. The process is repeated several thousand times and a range of response values is produced for each dose. There is a limitation to this approach in that the

assumption that a linear response is conservative only holds for doses below the ED_{50}. The proposed model cannot be used, therefore, to predict the dose that causes responses greater than 50 percent.

The predicted response should be viewed as a conservative (health protective) estimate of the probability of one or more adverse effects occurring in an exposed individual. Due to limited evidence of concordance between effects in animals and humans, however, the effect in humans should not be assumed to be the same. The linear response will result in an overestimate of risk. This approach has several strengths: it can produce quantitative estimates of risk; the analysis is independent of the actual dose-response curve, and the analysis is conservative (linear model assumption).

Because of the uncertainty in the level of risk at the RfD, this model should be used to estimate doses associated with low risk above the RfD with some caution. An estimate of a dose causing a 5% response (ED_{05}), however, would not be expected to change much. The assumptions upon which this approach is based are not without controversy. Many toxicologists and risk assessors are not comfortable with the assumption that the RfD is a measure of the ED_0, while others believe that the RfD is a subthreshold dose. For certain uses, this model is insensitive to whether the RfD compares to zero risk or merely very low risk ($ED_{0.0001}$). This proposed method produces a constant measure of risk, is consistent with the RfD, and differentiates between uncertainty and variability.

To illustrate this framework, calculation of risks above the RfD were attempted for the six selected chemicals (DDT, methylmercury, dioxin, PCBs, chlordane, and chlorpyrifos). The risks are based on the critical effect given in EPA's Integrated Risk Information System (IRIS). Dose response modeling was used to estimate the dose causing a 50% response (ED_{50}). Probability distributions were employed for each uncertainty factor (UF) used by EPA in setting the RfD. Where an uncertainty factor of ten was used in the RfD derivation, the reference distribution developed by Swartout *et al*. (1998b) to represent the uncertainty factor was used. Where a value of three was used, the square root of the values from the Swartout *et al*. (1998b) distribution was used. In cases where another factor was used, an alternative distribution was used (e.g., methylmercury). The specific distributions used are detailed in the sections on individual chemicals below.

The results of this analysis is an estimate of the dose response for a substance between the threshold (or minimal risk level, such as the ED_{01}) and the ED_{50}. The model assumes that the dose response is linear over this range, and where a compound has a sub-linear response this approach will over-estimate the risk. The approach also characterizes the uncertainty in the dose response that occurs because of the uncertainty in the estimates of the threshold and the ED_{50}. In this analysis, this uncertainty is presented in terms of the median estimate of a response for a given dose (i.e., the response that has a good chance of being above or below the true risk) and the 90 percent confidence limits for the response.

Analyses of risks above the RfD for chlordane and methylmercury were completed. The critical studies and UFs for chlorpyrifos are also presented below; however, the analysis could not be

completed due to unavailability of necessary data from the critical studies. Risks above the RfD could not be estimated for dioxin, DDT, and PCBs due to limitations in the available data. Details regarding the limitations of the critical study/effect data are provided below.

4.2.2 Other Approaches to Calculate Risk above the RfD

Another method to potentially estimate risk above the RfD is to adapt the use of a benchmark dose (BMD). EPA (1995) has defined the BMD as a statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect compared to background. The BMD method attempts to use more of the available dose-response information by fitting a mathematical model to the data and then determining the dose associated with a specified response rate of an adverse effect. The resulting BMD value is used as a substitute for the no effect level and divided by appropriate uncertainty factors to estimate a RfD

A number of decisions need to be made in applying the BMD method to estimate RfDs and likewise apply to estimating risks above the RfD. For example, for risk above the RfD decisions must be made on which mathematical model to use, what confidence limit to use, and what effect to model. Furthermore, the choice of uncertainty factor must also be incorporated into the estimation of risk and the model should have some way to approximate the RfD as zero risk.

A third method that has been proposed for quantitative dose-response analysis for noncancer toxicity data is that of categorical regression. This involves statistical regression on severity categories of overall toxicity (Hertzberg and Miller 1985; Hertzberg and Wymer 1991; Hertzberg 1991). By assigning severity categories, all adverse effects may be taken into account rather than focusing on the critical effect only, as in the previous two approaches. In addition, toxicity data from multiple studies can be used in this approach. [Categorical regression also has the added advantage of incorporating a severity ranking into its determination, thereby avoiding unnecessary criticism of the scale severity that we highlight in Chapter 6.]

The results of the regression can then be used to estimate risk above the RfD, by providing information about increasing toxicity with increasing dose rate. Categorical regression may be a preferred approach for calculating risk above the RfD because it uses more data than the other approaches. However, the approach is data and resource intensive and has not been done for the most significant fish contaminants. We recommend that the categorical regression approach be used for common pollutants found in fish in order to better estimate risks and allow consideration of benefits.

Confidence in this approach was enhanced by the close proximity of the data to the RfD. Confidence in using categorical regression or BMD modeling to estimate the risk above the RfD is increased when the RfD is based on human data (and thus a small uncertainty factor was used). Greater caution would be needed in the estimation of risks further from doses at which data exist.

BMD and categorical regression are newer dose response modeling techniques for use with noncancer toxicity data. One can with caution extend the modeling below the data to regions above the RfD – and the closer the extrapolation is to the data, the more confidence one has in the results.

4.3 Dose Response Information for the Six Selected Target Substances

The framework relies upon estimates of risk for both cancer and non-cancer endpoints. For illustration in this document, EPA risk estimates from the IRIS have been used (along with the estimates of risk above the RfD calculated for this project, which were based on IRIS RfDs and their corresponding principal studies).

Volume 2 of U.S. EPA's series on *Guidance for Assessing Contaminant Data for Use in Fish Advisories* provides guidance on chemical contaminant data for use in fish advisories and on the development of risk-based meal consumption limits for 25 high-priority chemical contaminants, referred to as target analytes (U.S. EPA, 1997b). These 25 target analytes were identified by EPA's Office of Water as significant based on documented occurrence in fish and shellfish, persistence in the environment, potential for bioaccumulation, and oral toxicity to humans. Volume 2 contains a toxicological profile summary for each of the target analytes is provided and consumption limit tables for adults and children are presented. Instructions for modifying the consumption limit tables to reflect local site-specific conditions for populations of concern are given. Separate tables are provided for women of reproductive age for methylmercury and polychlorinated biphenyls (PCBs). Additional information on risk assessment methods, population exposure, fish consumption patterns consumption surveys, risk reduction through use of various preparation and cooking procedures, and risk characterization is presented. Unless otherwise noted the toxicity data in this chapter are summarized from Volume 2.

In the development of the following framework, a hypothetical example was used which included estimates for risks above the RfD for methylmercury and chlordane. The details of these estimations are found below, and were derived using methods summarized below. In addition, cancer risk was estimated for chlordane since an EPA slope factor is available. Several of the other chemicals have cancer slope factors, but for none of the others have risk above the RfD calculations been developed.

4.3.1 DDT and Metabolites (DDE and DDD)

DDT is an organochlorine pesticide that in experimental animals causes cancer, liver damage, and to a lesser extent leukocytosis and decreased hemoglobin levels. Adverse developmental and immunological effects have been shown as well as estrogen-like effects on the developing reproductive system with chronic exposure. Prenatal exposure in experimental animals also evokes latent effects such as altered learning ability and permanent structural changes in the brain. Immunological effects have been also observed after short exposures. Some groups of people may be at greater risk, including children, those with cardiac disease, diseases of the nervous system or liver, and nursing infants (due to increased exposures).

EPA's IRIS classifies DDT (and its metabolites DDE and DDD) as B2, probable human carcinogens by the U.S. EPA. This classification is based upon studies in various mouse strains and studies in rats. IRIS reports a medium confidence RfD of 0.0005 mg/kg-day for liver lesions based upon a 1950 dietary study with rats. For cancer, the slope factor for oral exposure to DDT is 0.34 per (mg/kg)/day. This corresponds to a risk specific dose (RSD) of 0.00003 mg/kg-day at

the 1 in 100,000 risk level. These values should be used for the sum of the 4,4', and 2, 4' isomers of DDT, DDE, and DDD.

To estimate risk from exposures above the RfD, the IRIS RfD was examined. This RfD was derived from a dietary study in weanling rats in which animals (25/sex/group) were fed commercial DDT at levels of 0, 1, 5, 10, or 50 ppm for 15-27 weeks (Laug *et al*., 1950). The critical effect was defined by EPA as liver lesions described as hepatocellular hypertrophy, especially centrilobularly, increased cytoplasmic oxyphilia, and peripheral basophilic cytoplasmic granules (based on H and E paraffin sections). The NOAEL and LOAEL were defined as 0.05 mg/kg-day (1 ppm) and 0.25 mg/kg-day (5 ppm), respectively. A total UF of 100 was used representing values of 10 each for interspecies extrapolation and interindividual variation.

Unfortunately, dose response modeling for noncancer endpoints could not be performed and risks above the RfD for DDT could not be quantified because sufficient data were not available from the critical study (Laug *et al*., 1950) regarding the incidence of animals with liver lesions in each dose group. It is also important to note that the liver changes observed in this study were subsequently suggested to be adaptive in nature and not representative of actual liver toxicity (Ortega, 1966). The noncancer risks for DDT may be overestimated by using a RfD based on this study.

4.3.2 Methylmercury

Chronic exposure to methylmercury (MeHg) produces impairment of nervous system development in human fetuses, with exposure at sufficient levels evoking cerebral palsy-like symptoms. Prenatal exposure to lower doses shows more subtle retardation of infant development. In postnatal chronic exposure from fish consumption, neurological effects are also exhibited. Symptoms include visual and aural impairment, numbness in the extremities and around the mouth, impairment of fine motor functions such as writing, speaking, and walking, and mental disturbances. Chronic oral risk values have been developed by EPA (1999), ICF Kaiser (*ITER*, 1999) and the Agency for Toxic Substances and Disease Registry (ATSDR, 1999). Mice exposed to methylmercury developed kidney tumors in males but not females. However, carcinogenic effects in mice were observed in the presence of extensive tissue damage.

For methylmercury, the U.S. EPA's IRIS reports a medium confidence oral RfD for chronic exposure of 0.0001 mg/kg-day based upon neurological effects on 81 Iraqi children who had been exposed in utero. The mothers had consumed methylmercury-contaminated grain. The RfD is based on a level of exposure estimated by determining the mercury level in hair associated with the 95% lower confidence limit on a benchmark dose (BMDL) of 10%. The BMDL of 11 ppm maternal hair is adjusted by a dose conversion equation used to relate exposure level to the concentration of methylmercury in blood and hair, and an uncertainty factor of 10. The U.S. EPA has not evaluated the risk of carcinogenic effects because of insufficient data.

A site-specific RfD distribution (1-99 percentiles) of 0.0003 to 0.001 mg/kg-day, based upon a cohort study of mother-infant pairs in the Seychelles Islands has been developed by ICF Kaiser

and peer reviewed by an independent group of scientists (*ITER*, 1999). The RfD is based on a distribution of intakes associated with a BMDL of 10% of 21 ppm maternal hair, a physiologically-based pharmacokinetic (PBPK) model, and an uncertainty factor of 3.

The Agency for Toxic Substances and Disease Registry (ATSDR, 1999) updated its earlier Toxicological Profile on Mercury and developed a minimal risk level (MRL) of 0.0003 mg/kg-day based upon a cohort study of mother-infant pairs in the Seychelles Islands, a NOAEL of 0.0013 mg/kg-day, an uncertainty factor of 3, and a modifying factor of 1.5. ATSDR noted high confidence that the MRL of 0.0003 mg/kg-day "is protective of the health of all potentially exposed human populations" (ATSDR, 1999; p. 258). In its announcement of this new MRL, ATSDR advises fish consumers, states and other agencies not to revise their existing fish advisories based on the ATSDR updated profile.

Developing fetuses and individuals with impaired central nervous system (CNS), kidney, or liver function are particularly susceptible to adverse effects from exposure to methylmercury. Individuals with inadequate levels of zinc, glutathione, antioxidants and/or selenium are also at a higher risk.

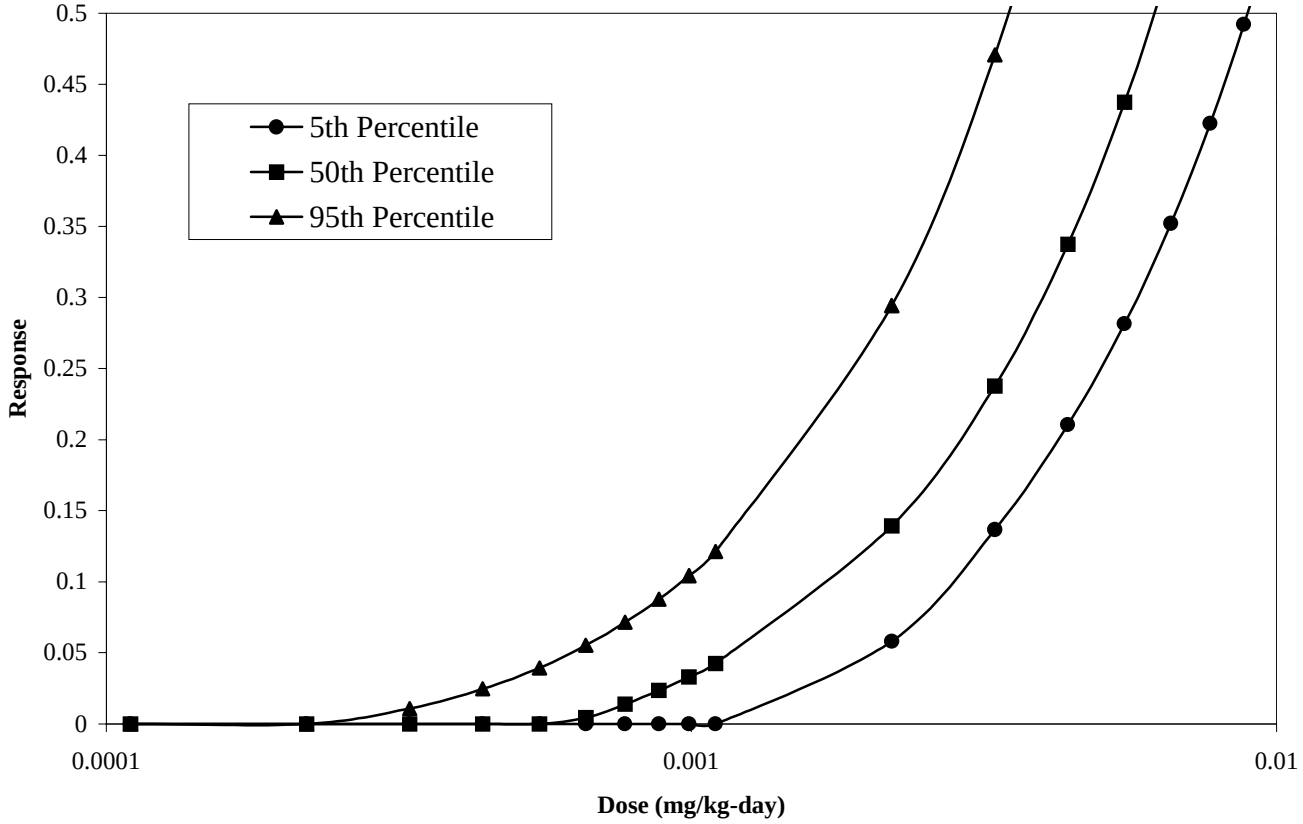
EPA issued interim guidance in April 1999 to EPA managers directing them to continue to use the RfD on IRIS until the National Academy of Sciences completes its report in 2000. Therefore, to calculate the risk above the RfD, the data behind the RfD on IRIS were examined. The methylmercury RfD is based on an evaluation of mother-child pairs from Iraq who were exposed to methylmercury in grains and bread (Marsh *et al*., 1987; Seafood Safety, 1991). The critical effect used by EPA to derive the RfD were developmental effects in infants, including delayed onset of walking, delayed onset of talking, mental symptoms, seizures, and neurological scores based on clinical evaluations. Continuous data from this study were placed in 5 different dose groups and incidence rates were determined. The RfD (0.0001 mg/kg-day) was based on a benchmark dose of 11 ppm methylmercury in maternal hair. A pharmacokinetic algorithm was used to transform the concentration in maternal hair to the daily intake of 1.1 ug/kg-day. The following quantal data were used to calculate the benchmark dose and the ED_{50}:

Dose (ppm in hair)	Incidence of Developmental Effects
1.37	5/27
10.0	3/14
52.5	6/13
163	8/12
437	14/15

The MLE (maximum likelihood estimate) of the ED_{50} for this data is 117 ppm MeHg in maternal hair or 0.0011 mg/kg-day. This value was derived using a Weibull model with the threshold set as zero (Swartout, 1998a).

A total UF of 10 was used for the IRIS RfD, which represents values of 3 each for human variability and database uncertainty, respectively. The UF of 3 for human variability represents the uncertainty in the ratio of daily intake to hair levels due to human variability in

Figure 4-1. Dose-Response Curves for Methylmercury



methylmercury pharmacokinetics. This uncertainty was characterized by EPA using a one-compartment pharmacokinetic model and data on interindividual variation in the model inputs (EPA, 1997a). Therefore, the distribution used to characterize this UF was based on specific data on methylmercury pharmacokinetics (Swartout, 1998a). The square root of the reference distribution was used to establish the distribution of the database UF.

Figure 4-1 shows the 5^{th}, 50^{th}, and 95^{th} percentile dose-response curves for methylmercury. Responses at these percentiles for given multiples of the methylmercury RfD are also presented in Table 4-2. Figure 4-1 and Table 4-2 suggest a much steeper dose-response curve for methylmercury than for chlordane (see below). As the table shows, at a dose only five times higher than the RfD, the 90% confidence limit suggests that as many as 4% of the population might respond. At 50 times the RfD, there is 50% probability that 44% of the population would respond, with 90% certainty that the response would range from 28% to greater than 50%.

Table 4-2: Methylmercury Responses at Multiples of the Reference Dose[1]

		Response		
Multiple of Reference Dose	Dose (mg/kg-day)	5th Percentile	50th Percentile	95th Percentile
1	0.00011	0.0%	0.0%	0.0%
5	0.00055	0.0%	0.0%	3.9%
10	0.0011	0.0%	4.3%	12.1%
50	0.0055	28.2%	43.7%	>50%
100	0.011	>50%	>50%	>50%

[1] Based on the RfD and underlying data as on EPA's IRIS (U.S. EPA, 1999). The use of other, more recent, data is also possible which may lead to a different estimation of the risk above the RfD.

4.3.3 Dioxin

Dioxin is a generic term that is used for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). However, seventeen 2,3,7,8-substituted dibenzo-*p*-dioxin compounds are grouped together in the interest of simplicity (U.S. EPA 1987). Dioxin is extremely toxic and targets multiple organ systems in experimental animals. Some of these effects have also been seen in humans. Effects observed in animal studies include teratogenicity, fetotoxicity, reproductive dysfunction, carcinogenicity, and immunotoxicity. Wide differences in the toxic responses to dioxin are seen among species. There is a great deal of concern over the health effects of TCDD because of its persistence in the environment, its potency as a carcinogen, and its potential for bioaccumulation (U.S. EPA 1987). Dioxin is currently under reassessment by the U.S. EPA; for the purposes of this report information from U.S. EPA (1987) is summarized below.

Dioxins have the highest cancer potency in animals of any chemicals evaluated by the U.S. EPA. U.S. EPA (1987) reports a cancer slope factor of 1.56×10^{5} per (mg/kg)/day, based on experimental animal results. This corresponds to an RSD of 6.4×10^{-11} mg/kg-day or 2×10^{-9} mg/ L drinking water at the 1 in 100,000 risk level. EPA at one time calculated a RfD of 0.000001 mg/kg-day. EPA is in the process of revising its assessment of the risk from dioxin exposure. This reassessment may likely change both the cancer and noncancer risk values for this chemical.

To calculate the risk above the reference dose, the acceptable daily intake (ADI) of 0.000000001 mg/kg/day from EPA's Ambient Water Quality Criteria Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (EPA, 1984) was examined. This ADI was based on a reproductive effects reported in a 3-generation rat study (Murray *et al*., 1979). A LOAEL of 0.001 ug/kg-day was determined for a reduction in the gestation index, decreased fetal weight, increased liver to body weight ratio, and increased incidence of dilated renal pelvis. This was based on a re-evaluation of the data from the original study (Nisbet and Paxton, 1982). A total uncertainty factor of 1000 was used, consisting of interspecies, interindividual, and LOAEL to NOAEL UFs of 10 each.

The gestation survival index was the only critical effect data available from the Murray *et al*., (1979) for which dose response modeling could be performed to generate an ED_{50}. Gestation survival data for the f1a and f1b generations (offspring of two separate matings of exposed parents) were combined prior to dose response modeling. Dose response modeling was performed for this data using the THRESH multistage model (ICF Kaiser, 1997). The gestation survival data failed the goodness of fit criteria for the model and the ED_{50} could not be estimated. Risks above this ADI could therefore not be determined for TCDD using this data.

4.3.4 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are mixtures of chlorinated biphenyl compounds manufactured by under the trade name Aroclor with a numeric designation indicating the chlorine content of the mixture. Manufacture and use of PCBs was banned 1979, however, PCBs are extremely persistent in the environment and biomagnify *via* the food chain (U.S. EPA, 1997b).

The majority of mutagenicity assays for PCBs have been negative, but positive cancer responses are seen with the higher chlorinated congeners (ATSDR, 1997). EPA classifies PCBs as Group B2, probable human carcinogens, based liver tumors seen in studies in rats exposed to various Aroclor mixtures.

Because environmental processes such as degradation and transportation alter the composition of environmental PCB mixtures compared with commercial mixtures, EPA has used a tiered approach for assessing cancer potency of PCB mixtures and developed several ranges of risk values. For the "high risk and persistence" tier, EPA calculated a central-estimate slope factor of 1.0 per (mg/kg)/day and an upper-bound slope factor of 2.0 per (mg/kg)/day, based on several studies of Aroclor 1254. This corresponds to Risk Specific Doses of 0.00001 and 0.000005 mg/kg-day at the 1 in 100,000 level. This range of risk values should be used when exposure is likely to be through the food chain, soil ingestion, dust or aerosol inhalation, or whenever there is potential for exposure in early life (U.S. EPA, 1999). Evaluating exposure and risk from contaminated fish would use this range of slope factors.

The U.S. EPA has established a chronic oral RfD for both Aroclor 1254 and Aroclor 1016. The medium confidence RfD for Aroclor 1254 is 0.00002 mg/kg-day based upon a LOAEL of 0.005 mg/kg-day for ocular and immunological effects in monkeys. The medium confidence RfD for Aroclor 1016 is 0.00007 mg/kg-day based on reduced birth weights in a monkey reproductive bioassay. The ATSDR also has established a minimal risk level (MRL) for Aroclor 1254 of 0.00002 mg/kg-day based upon a LOAEL of 0.005 mg/kg-day for an immunological endpoint (ATSDR, 1999).

To estimate risk above the RfD, the Aroclor 1254 RfD was examined (it is more toxic and the 1016 data did not appear adequate for modeling). This RfD is based on dermal/ocular and immunological changes in rhesus monkeys exposed to dietary concentrations of 5, 20, 40, or 80 ug/kg-day Aroclor 1254 for over 5 years (Tryphonas *et al*., 1989, 1991a,b; Arnold *et al*., 1993a,b). The lowest dose was designated as a LOAEL for these effects. A total uncertainty

factor of 300 was used based on an interindividual UF of 10, an interspecies UF of 3, a subchronic to chronic UF of 3, and a NOAEL to LOAEL UF of 3.

The incidence data for the dermal/ocular effects in each dose group is not available from the critical studies. Group mean data are available for some of the immune system parameters measured (i.e., decrease in antibody response to sheep erythrocytes). However, the data from each individual monkey was needed for conversion of the data to a quantal form that could be used in standard dose response modeling to generate the ED_{50}. Individual clinical records for each monkey could be reviewed to generate incidence data for the clinical health findings; however, this was beyond the scope of the current project. Thus, risk above the RfD was not estimated for aroclor 1254.

To date, U.S. EPA has not evaluated data on Aroclors 1242 or 1260; nor has EPA developed RfCs for any of the Aroclors. The Great Lakes Task Force has developed an interim position on the noncancer toxicity of PCBs in fish using an average of the RfDs for Aroclors 1016 and 1254. This value is 0.00005 mg/kg-day. As mentioned above, the U.S. EPA has also published a revised position on PCB cancer risk assessment on IRIS (EPA, 1999).

A problem with the available risk values is that they are based on the toxicity of commercial mixtures, and the environmentally-relevant mixtures have not been tested. While use of a sufficiently similar mixture may be appropriate, the question of what constitutes “sufficiently similar” for environmental mixtures of PCBs when compared to commercial mixtures needs to be answered at each individual site or situation. Toxicity equivalency factors have been discussed for different PCB congeners and some limited conclusions reached (EPA, 1997b). For the purposes of this report, however, we assume that the toxicity data on PCBs found on EPA’s IRIS is relevant for the estimation of potential risks from PCB mixtures found in fish.

The results of human epidemiology studies differ somewhat from the non-human primate toxicity studies in critical effect. PCB mixtures passed to neonates from *in utero* exposure and breast milk have been shown to cause developmental defects including cognitive deficits that persisted at least until 4 years of age in human infants (ATSDR and U.S. EPA, 1997).

A number of epidemiological studies relating fish consumption with deleterious effects have been conducted over the past two decades. Several cohort studies in the Great Lakes Basin have been conducted (the New York State Angler Cohort, the Michigan Sports Fisherman Cohort, the Michigan Maternal/Infant Cohort, and the Wisconsin Maternal/Infant Cohort). These studies have focused upon possible adverse effects due to fetal PCB exposure from mothers who consume fish from the Great Lakes. There have been indications of alterations in birth size, gestational age, and neurological development in these studies. ATSDR and EPA recently reviewed studies of exposure to PCBs through fish consumption, particularly for fish from the Great Lakes. The two agencies prepared a paper entitled "Public Health Implications of Exposure to Polychlorinated Biphenyls (PCBs)" (ATSDR and U.S. EPA, 1999) which concluded that the "weight of evidence clearly indicates that populations continue to eat fish containing PCBs and that significant health consequences are associated with consumption of large amounts of some fish" (ATSDR and U.S. EPA, 1999, p. 2). Health effects include possible reproductive

function may be disrupted, neurobehavioral and development deficits in newborns and school-aged children exposed in utero, other systemic effects that are associated with elevated serum levels of PCBs, and increased cancer risks.

Swain (1991) compared all four of the cohorts and concluded that PCB exposure from fish could be correlated with alterations in neonatal health and health in early infancy with reasonable certainty (Swain, 1991). Swain (1991) found that effects in infant birth weight, maternal health condition, gestational age, composite activity ranking and McCarthy memory scale deficits had to be classified as indeterminate, but could not be negated. Swain (1991) also stated that the relationship between adverse effects on the health status of neonates and infants and PCB exposure in the Michigan cohort could be causally affirmed and that data from other geographic locals only tends to support this hypothesis. The available human data could not be used to estimate a risk above the RfD.

Contrary to the findings of most other studies, Dar *et al*. (1992) found increasing birth weight with increased fish consumption for women who gained less than 34 pounds during pregnancy. Dar *et al*. (1992) showed a positive correlation between maternal serum PCB levels, and fish consumption. However, PCB exposures were lower than those in the other studies (Dar *et al*., 1992).

In a recent study (Lonky et al. 1996), 395 infant-mother pairs that consumed Lake Ontario fish were compared to 164 pairs who did not. The exposed pairs were divided into high and low exposure groups and examined using the NBAS (Neonatal Behavioral Assessment Scale). The neonates from the high exposure group scored more poorly on the reflex, autonomic, and habituation clusters of the NBAS. These results confirm the findings of the Michigan maternal/infant Cohort.

It may be useful to look at these human data to estimate an RfD for PCB mixtures in fish. If possible, risk above this new RfD might be very useful in future evaluations of the framework.

4.3.5 Chlordane

Chlordane exposure affects the liver, nervous system, and immune system. Liver effects include hepatocellular hypertrophy (swelling), hepatic fatty degeneration, hepatocellular adenomas, and hepatic necrosis. Neurological effects include *grand mal* seizures and altered EEG results. Prenatal and postnatal chlordane exposure may have permanent effects on the immune system such as a reduction in the number of stem cells. Early childhood exposure to chlordane has been associated with prenatal and early childhood neuroblastoma and acute leukemia.

Chlordane is classified as a probable human carcinogen (B2) by the U.S. EPA based on oral studies in animals. IRIS reports an oral slope factor of 0.35 per (mg/kg)/day, which corresponds to a RSD of 0.00003 mg/kg-day at the 1 in 100,000 risk level. U.S. EPA's IRIS (1999) reports a medium confidence oral RfD of 0.0005 mg/kg-day for chlordane based on a 2-year mouse NOAEL of 0.15 mg/kg-day for hepatic necrosis.

To calculate risk above the RfD the IRIS RfD was examined. A chronic feeding study in mice was used to define the RfD for chlordane (Khasawinah and Grutsch, 1989). ICR mice (80/sex/group) were fed 0, 1, 5, or 12.5 ppm technical grade chlordane in the diet for 104 weeks. The critical effect is defined in IRIS as liver necrosis in male mice. Other cited effects include increased liver weight, liver cell hypertrophy and fatty degeneration of the liver. Hepatocellular adenomas were also observed at the high dose group. Hepatic necrosis was observed in male mice only at the following incidence rates:

Dose (mg/kg-day)	Incidence of Hepatic Necrosis in Male Mice
0	7/80
0.15	8/80
0.75	25/80
1.88	27/80

EPA has defined the NOAEL and LOAEL doses as 0.15 mg/kg-day and 0.75 mg/kg-day, respectively. A total uncertainty factor of 300 was used, which includes factors of 10 for interspecies extrapolation, 10 for interindividual variation, and 3 for lack of reproductive studies (database uncertainty).

The ED_{50} was calculated using the THRESH multistage model (ICF Kaiser, 1997). To estimate risks above the RfD, reference distributions were used for the interspecies and interindividual UFs. The square root of the reference distribution was used to establish a distribution for the database UF.

Figure 4-2 shows the 5^{th}, 50^{th}, and 95^{th} percentile dose-response curves for chlordane. The 50^{th} percentile curve can be viewed as the estimate of dose response that is equally likely to over estimate or under estimate response if the substance followed a linear response between the RfD and the ED_{50}. The remaining two curves can be viewed as the 90 percent confidence limits for the dose response. That is the true response has a 90 percent certainty of falling between the values. Responses at these percentiles for given multiples of the chlordane RfD are also presented in Table 4-3. As the figure and table show, at a dose of 0.005 mg/kg-day, or ten times the chlordane RfD, the median estimate is 0% with 90% certainty that less than one percent of the population would respond. Likewise, at 50 times the RfD, the median estimate is less than 2% with 90% certainty that less than 10% of the population would respond.

Figure 4-2. Dose-Response Curves for Chlordane

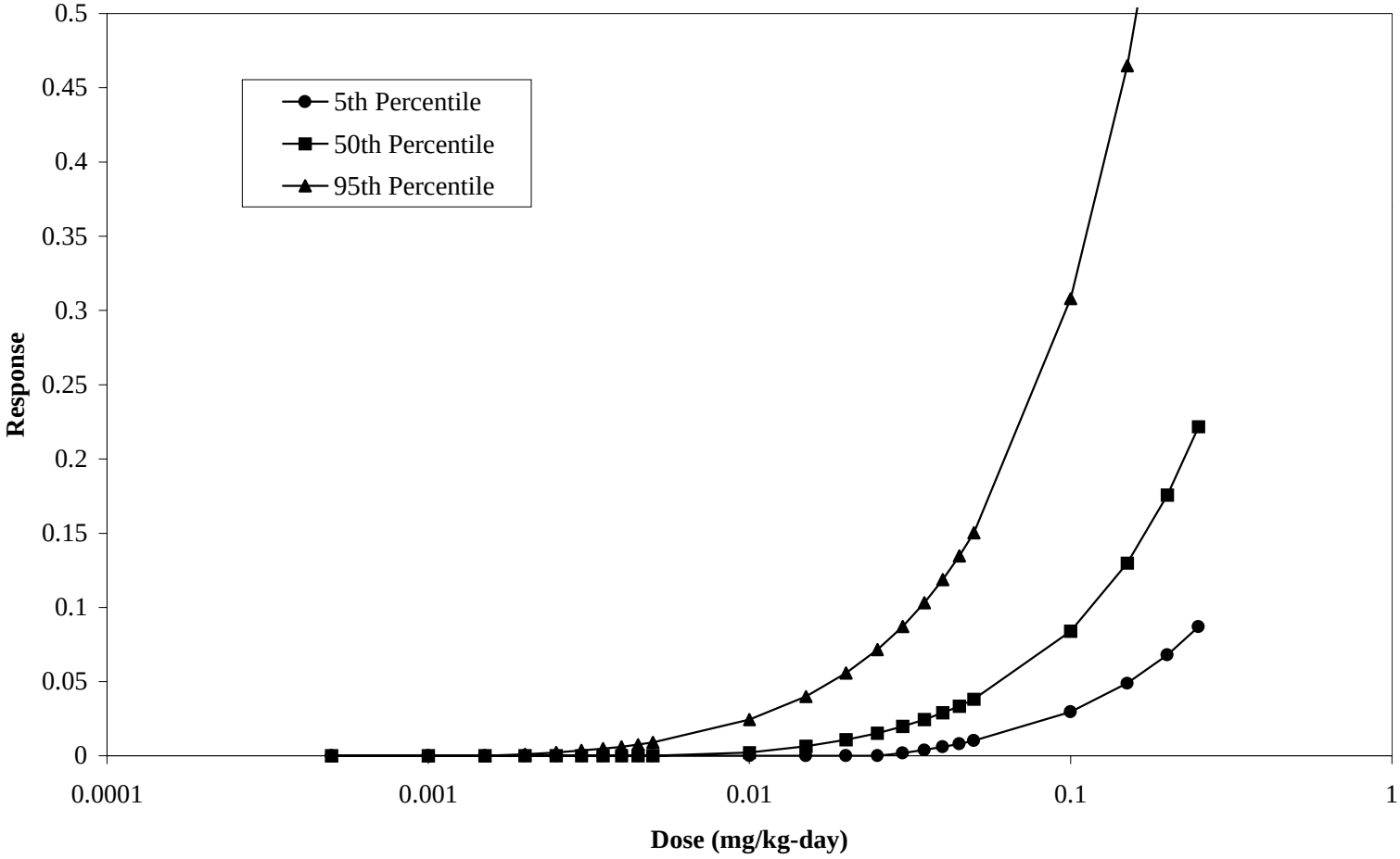


Table 4-3: Chlordane Responses at Multiples of the Reference Dose

		Response		
Multiple of Reference Dose	Dose (mg/kg-day)	5th Percentile	50th Percentile	95th Percentile
1	0.0005	0.0%	0.0%	0.0%
5	0.0025	0.0%	0.0%	0.2%
10	0.005	0.0%	0.0%	0.9%
50	0.025	0.0%	1.5%	7.2%
100	0.05	1.0%	3.8%	15.0%
500	0.25	8.7%	22.2%	>50%

4.3.6 Chlorpyrifos

Chlorpyrifos is known to cause cholinesterase inhibition in human blood serum. Other CNS effects do not occur at similar or lower doses than that causing cholinesterase inhibition. For this reason, some controversy exists as to whether cholinesterase inhibition in and of itself should be considered an adverse effect. In experimental animals, cholinesterase inhibition is seen at low doses and more severe neurotoxicity is seen at higher doses. It is also thought to be fetotoxic.

For chlorpyrifos, IRIS provides a medium confidence oral RfD of 0.003 mg/kg-day based on a NOEL of 0.03 from a 20-day human study reported in 1972 for plasma cholinesterase inhibition in adult males after 9 days of exposure. The U.S. EPA has not developed a risk value for the carcinogenicity of chlorpyrifos.

To estimate risk above the RfD, the IRIS RfD was examined. The critical study for the RfD was conducted in human volunteers that were treated with chlorpyrifos (4/dose group) at doses of 0, 0.014, 0.03, or 0.1 mg/kg-day (Dow Chemical Company, 1972). Doses were administered in a capsule for 20 days at the low and mid dose and 9 days at the high dose. Treatment at the high dose was discontinued after 9 days due to runny nose and blurred vision in one individual. Mean plasma cholinesterase (ChE) in the high dose group was 65% of control. No effect on RBC ChE was observed at any dose. Decreased plasma ChE after 9 days was considered the critical effect and EPA defined the NOAEL and LOAEL as 0.03 mg/kg-day and 0.1 mg/kg-day, respectively. A UF of 10 was used as the standard factor for the range of human sensitivity to ChE inhibition.

The detailed dose response data for the critical study is not available from IRIS. The Dow Chemical Company report (1972) presumably contains the pretreatment and 9-day plasma ChE level for each individual subject, but it was not available at the time our analysis was performed. Incidence data could be generated from this study by assuming that individuals with a greater than 20% decrease in the plasma ChE level at 9 days are adversely impacted (Dourson *et al.*, 1997). The quantal incidence data derived from this study could be used to calculate the ED_{50}.

The reference distribution for interindividual uncertainty would be used to estimate risks above the RfD.

It should be noted that the time scale of the effects of chlorpyrifos is much shorter than chlordane and methylmercury. Both of those compounds must accumulate in the body and reach a critical level in order to produce adverse effects in individuals or their offspring. In contrast, chlorpyrifos is rapidly metabolized and does not accumulate in humans to any great extent. The adverse effects on which the RfD is based are from short term (9-20 days) toxicity studies in humans. As a result, it may be appropriate to evaluate the risks based on acute exposures (the day the fish was consumed) rather than annual average doses.

4.4 Multigenerational Study of Great Lakes Salmon Fed to Rats

The vast majority of studies on health effects of contaminants involve single compounds due to the complexities of testing mixtures of chemicals. However, many human exposures, including exposure to contaminated fish, involve combinations of chemicals. To help address this issue, Health Canada initiated a multigenerational study of the health effects in rodents consuming diets containing Lake Ontario or Lake Huron chinook salmon (*Oncorhynchus.tsawytscha*) (Arnold *et al*., 1998a; Arnold *et al*., 1998b; Feely and Jordan, 1998; Tryphonas *et al*., 1998a; Tryphonas *et al*., 1998b; Pappas *et al*., 1998; Seegal *et al*., 1998; Iverson *et al*., 1998). Contaminant levels exceeded existing standards for commercial fish and seafood for PCBs, dioxins, mirex, chlordane, and mercury (Feely *et al*., 1998). Results of this study are summarized here. It is the only experimental dose-response study with environmentally relevant exposures to mixtures of chemicals of interest in fish.

The chinook salmon fillets used in the dietary formulations for this study were lyophilized and analyzed for concentrations of the following contaminants: polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs), dibenzofurans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, metals, volatile organics, chlorinated phenols, and benzenes (Feely and Jordan, 1998). Levels of contaminants were obtained before and after lyophilizing. Lyophilized salmon fillets were then incorporated into the normal rat diet in varying proportions (control, 5% salmon, 10% salmon, 15% salmon, 20% salmon for five different dose groups including one control. After 70 days on the diet males and females (F_0) were mated on a one-to-one basis within each group (Arnold *et al*., 1998b). The (F_1) pups were weaned from the dam after 21 days and then fed the diets for 13 weeks. Seventy days after weaning, one (F_1) male and one (F_1) female within each dose group were mated. The (F_2) pups were then treated similarly to the (F_1) pups. Randomly selected (F_0), (F_1), and (F_2) adults and neonates were necropsied (Arnold *et al*. 1998b). The study included a reversibility group (F_1-R) in which the rats were switched to the control diet after 13 weeks of exposure for 13 weeks (Tryphonas *et al*., 1998a). Increased relative liver and kidney weights were observed in both generations and both sexes fed diets containing 20% Lake Huron or Lake Ontario salmon (Arnold *et al*., 1998b). Tryphonas *et al*. (1998a) reported on additional effects. Reduced thymus weights were observed in the Lake Ontario (20%) female (F_1-R) reversibility group. Increased growth rates in the (F_1) male rats were observed in those consuming the Lake Huron diets compared with those consuming the Lake Ontario diets. Reduced, reversible, decreases in counts of red blood cells, white blood

cells, neutrophil, lymphocytes, and monocytes in the fish-fed (F_1) females were seen. This reduction in counts was greater in the Lake Ontario salmon fed females than the Lake Huron fed females. Red blood cell, white blood cells, and lymphocyte counts were decreased in the (F_2) male rats fed the Lake Ontario (20%) diets compared to the Lake Huron (20%) diets.

Although quantitative aspects of the immune system were affected by the treatments, no significant effect on its function was observed (Tryphonas *et al*., 1998b). No significant behavioral effects were observed in any of the treatments except for one effect observed in the 20% (F_1) Lake Ontario and the (F_2) Lake Huron males (Pappas *et al*., 1998). These males showed reduced performance in the reference/working memory version of the radial arm maze. Frontal cortex dopamine concentrations were significantly reduced in all of the fish fed rats (Seegal *et al*., 1998). Caudate nucleus dopamine levels were also reduced in all fish fed groups (Seegal *et al*., 1998). However, decreases in dopamine levels in the *substantia nigra* were only observed in the Lake Ontario (20%) fed rats (Seegal *et al*., 1998). Significant effects on all of the fish fed groups except the Lake Ontario 5% group in levels of norepinephrine concentrations were observed in the *substantia nigra* (Seegal, 1998). The same was true for 3,4-dihydroxyphenylacetic acid (Seegal, 1998).

Overall, the authors concluded that the consumption of the fish diets by rats of two consecutive generations resulted in a variety of effects that can be described as adaptive responses or of limited biological significance (Feeley *et al*., 1998). Exceptions to their general statement include potential modification of working and reference memory, an effect on thymus weights noted in the first generation and an effect of lymphocyte numbers in the second generation. All of these exceptions occurred at the highest dose. The authors concluded that the risk presented by the complex mixture of contaminants in salmon collected from two locations in the Great Lakes could be considered minimal, especially if sport fish consumption advisories are followed.

4.5 Breast Milk as a Source of Contaminants

Breast milk is the ideal source of nutrients for newborns. However, breast milk is also a route of excretion for some toxic substances and an extremely important route of exposure for the nursing child. Substances are secreted into the milk by simple diffusion (Klaassen, 1991). Several factors influence excretion of substances in breast milk.

(1) Three to four percent of human milk consists of lipids. Lipid soluble compounds diffuse into the mammary gland along with fats from the plasma.
(2) Milk is more acidic than plasma. Therefore, basic compounds are concentrated in the milk, while acidic compounds have lower concentrations in the milk than in the plasma (Findlay, 1983; Wilson, 1983).
(3) Compounds that are chemically similar to calcium such as lead and substances that form complexes with calcium are excreted into the milk (Klaassen, 1991).
(4) Differences in excretion between mammalian species depends upon the amount of lipid secreted into the mammary gland from the plasma vs. the amount of lipid synthesized *de novo* in the mammary gland.

Mercury is an example of an environmental contaminant that transfers to mothers milk. In mice, the transfer of inorganic mercury from plasma to milk is greater than the transfer of methylmercury to the milk. However, neonate uptake of methylmercury is greater than uptake of inorganic mercury. In humans exposed to mercury *via* dental amalgam and contaminated fish in Sweden, milk levels of mercury were approximately 30% of plasma levels. Exposure to methylmercury from recent consumption of fish was reflected in the plasma but not in the milk (Oskarsson *et al*., 1996)

4.6 Conclusions and Research Needs

For the framework to be most useful, noncancer risks above the RfD must be estimated for all significant critical effects of chemicals that contaminate fish, in particular, for the contaminant PCBs. For example, the case study of the Vietnamese immigrant women consuming Lake Ontario sportfish (discussed later) was severely hampered by our inability to estimate the risks above the RfD for PCBs; this was critical because some exceedances of the RfD were as much as 40-fold. Other chemicals need similar investigation.

RfDs are designed to be protective of the critical effect. This means that as long as doses remain below the RfD, neither the critical effect, nor any other adverse effect associated with the chemical is expected to manifest itself in the population. When doses exceed the RfD, as the framework assumes they could, then the critical effect may begin to manifest itself in the exposed population. The framework uses dose-response information on the critical effect to predict the increased incidence of the critical effect. But in addition to the critical effect, other effects may also be seen at higher doses. Some of these may be more severe than the critical effect. At present, EPA has not developed dose-response relationships for non-critical effects in humans. For the framework to fully characterize potential risks, and the net possible health benefit of eating contaminated fish, dose-response relationships for non-critical effects should also be developed.

Moreover, the method that we chose for determining these risks above the RfD (Price *et al*., 1997) should be more closely examined. This method has the advantages that it is more generally applicable than categorical regression and is less resource intensive. It can be used directly from the existing data as on EPA's IRIS. However, it is not the only approach to the problem of risk above the RfD, and as demonstrated, the method does not work for all chemicals.

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