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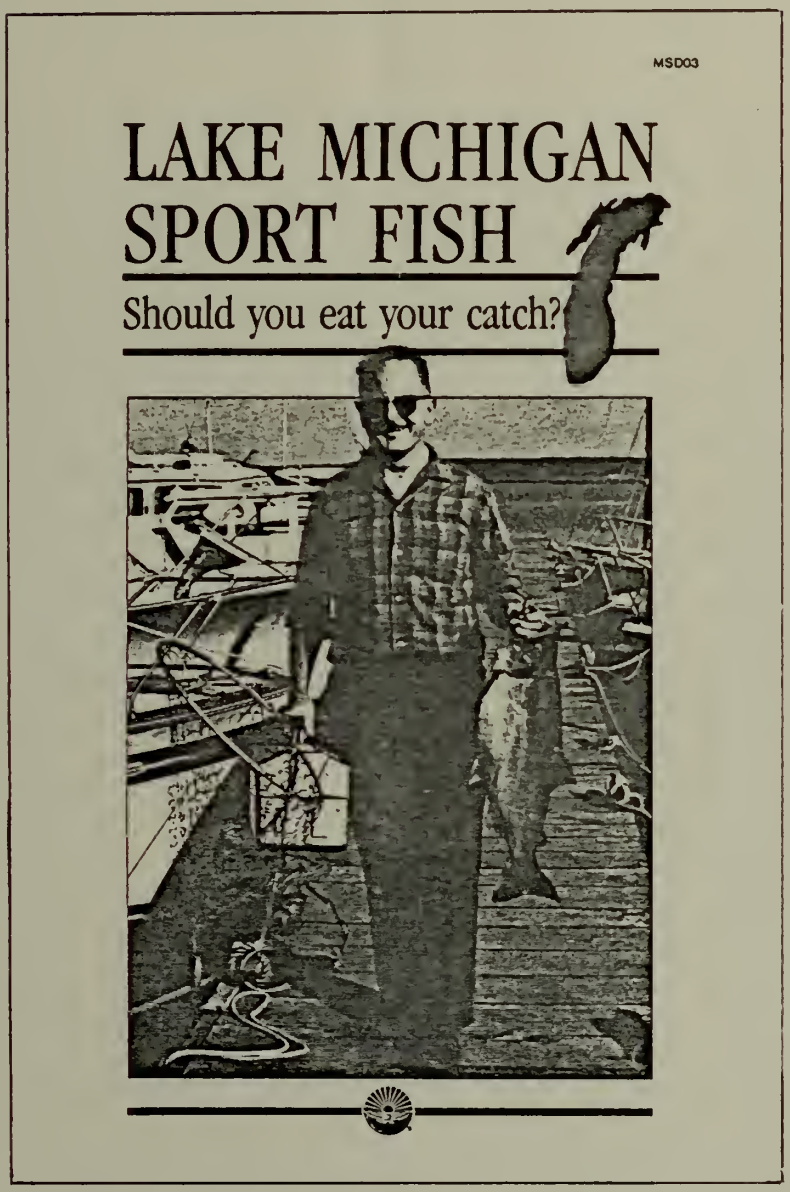
LAKE MICHIGAN SPORT FISH CONSUMPTION ADVISORY PROJECT
VOLUME I



August, 1989

Barbara S. Glenn, Project Manager
Jeffery A. Foran, Ph.D.

TECHNICAL SUPPORT DOCUMENT

LAKE MICHIGAN SPORT FISH CONSUMPTION ADVISORY PROJECT

NATIONAL WILDLIFE FEDERATION

Jay D. Hair, President
William W. Howard, Executive Vice President
Larry J. Schweiger, Vice President for Regional Programs

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* The Project Science Review Group:

  John Crellin, Ph.D.
  Harold Humphrey, Ph.D.
  Robert Kreiger, Ph.D.
  David Belluck, Ph.D.

* The Project Affiliate Advisory Committee:

  Al Rodgers, Chair, Illinois
  Herbert Krauch, Chair, Indiana
  John Eichinger, Chair, Michigan
  James Baldock, Chair, Wisconsin

* Kathy J. Towler, Staff Assistant, NWF Great Lakes Natural Resource Center

* Kris Olsson, NWF Great Lakes Natural Resource Center

* The University of Michigan graduate students who assisted in researching the hazard assessments:

  Jeff Ambroso
  Michael Cox
  David Croxton
  Costas Tsolakidis
  Jill Wiese

* The participants in the April 13, 1989 workshop on managing the risks of consuming contaminated sport fish:

Jim Baldock, Wisconsin Wildlife Federation
Jonathan Bulkley, Ph.D., School of Natural Resources, University of Michigan
Gale M. Carlson, Missouri Department of Health
Candace Chandler, American Fishing Tackle Manufacturer's Association
Tom Crane, Great Lakes Commission
John Crellin, Missouri Department of Health
David DeVault, U.S. EPA Great Lakes National Program Office
Mark DuCharme, Michigan United Conversation Clubs
Linn Duling, Michigan Department of Natural Resources
Karen East, Michigan Legislative Services Bureau
John Emerson, National Fisheries Institute
Van Esser, Council of Great Lakes Governors
Carlos Fetterolf, Jr., Great Lakes Fishery Commission
Delano Graff, Pennsylvania Fisheries Commission
Jim Grant, Great Lakes & Environmental Assessment Section, Michigan DNR
David Gray, Minnesota Department of Health
John Hartig, Ph.D., Great Lakes Regional Office, International Joint Commission
Rebecca Head, Washtenaw County Department of Public Works
David Herbst, National Wildlife Federation
John Hesse, Michigan Department of Public Health
Harold E.B. Humphrey, Michigan Department of Public Health
Carol Jones, Ph.D., School of Natural Resources, University of Michigan
Benjamin Johnson, Michigan Department of Public Health
Barbara Knuth, Ph.D., Department of Natural Resources, Cornell Univ.
Herb Krauch, Indiana Wildlife Federation
Robert Kreiger, Ph.D., Delta Environmental Consultants, Inc.
Thomas E. Lauer, Indiana Divison of Fish and Wildlife
Lee Liebenstein, Wisconsin Department of Natural Resources
George Loechl, American Fishing Tackle Manufacturer's Association

Jim Martin, Lake St. Clair Walleye Association
Steven Moyer, National Wildlife Federation
David Newhouse, National Audubon Society
Christine H. Pennisi, Illinois-Indiana Sea Grant Extension Program
Charles Pistis, Michigan Sea Grant Program
Norville Prosser, Sport Fishing Institute
Richard Reuss, Great Lakes Sport Fishing Council
David Rolland Sager, Environmental Contaminants Branch, Resource Protection Division, Texas Parks and Wildlife Department
John Schwartz, Michigan Sea Grant Program
Wendy Silverman, School of Natural Resources, University of Michigan
John Skrypek, Ecological Services Section, Minnesota DNR
Dan Thomas, Great Lakes Sport Fishing Council
Lisa Williams, Pesticide Research Center, Michigan State University
Asa Wright, Fisheries Division, Michigan Department of Natural Resources

* The individuals who reviewed and commented on drafts of the Technical Support Document or the Project booklet:

Henry Anderson, M.D., Wisconsin Division of Health
David Bayliss, Human Health Assessment Group, Office of Health & Environmental Assessment, U.S. EPA
C.D. Besadny, Wisconsin Department of Natural Resources
Randy Bruins, Environmental Criteria & Assessment Office, U.S. EPA
Sally Churchill, Michigan Attorney General
Milt Clark, Ph.D., U.S. EPA Region V
James Cogliano, Human Health Assessment Group, Office of Health & Environmental Assessment, U.S. EPA
James Colquhoun, New York Department of Environmental Conservation
Cameron Davis, Lake Michigan Federation
William Davis, Wisconsin's Environmental Decade
David DeVault, U.S. EPA Great Lakes National Program Office
Art Dittmar
Michael Dourson, Ph.D., Environmental Criteria & Assessment Office, U.S. EPA
Linn Duling, Michigan Department of Natural Resources
Van Esser, Council of Great Lakes Governors

William Farland, Ph.D., Office of Health & Environmental Assessment, U.S. EPA
L.J. Fischer, Ph.D., Michigan State University
Joel Fisher, Ph.D., International Joint Commission
Robert Ginsberg, Ph.D.
David Gray, Minnesota Public Health Department
John Hartig, Ph.D., Great Lakes Regional Office, International Joint Commission
Charli Hiremath, Human Health Assessment Group, Office of Health & Environmental Assessment, U.S. EPA
Delbert Hoggard, Indiana Wildlife Federation
Barbara Knuth, Ph.D., Department of Natural Resources, Cornell University
Robert Kreiger, Ph.D., Delta Environmental Consultants, Inc.
James P. Ludwig, Ph.D., Ecological Research Services, Inc.
Debdas Muckerjee, Ph.D., Environmental Criteria & Assessment Office, U.S. EPA
Susan Mudd, Citizens For A Better Environment
Cindy Sonich-Mullin, Environmental Criteria & Assessment Office, U.S. EPA
Susan Braen Norton, Exposure Assessment Group, Office of Health and Environmental Assessment, U.S. EPA
Andy Pelt, Michigan Steelhead and Salmon Fishermen's Association
George Loechl, American Fishing Tackle Manufacturers Association
Michigan Departments of Public Health, Natural Resources & Agriculture (joint comments)
Steven Moyer, National Wildlife Federation
David Newhouse, National Audubon Society
Christine Pennisi, Illinois-Indiana Sea Grant Program
Peter Preuss, Ph.D., Office of Research & Development, U.S. EPA
Warren (Pete) Redmon, U.S. EPA Region V
Rudolph Rosen, Ph.D., National Wildlife Federation
David R. Sage, Ph.D., Texas Parks & Wildlife Department
Wendy Silverman
Art Talsma, South Dakota Department of Fish, Game & Parks
Dan Thomas, Great Lakes Sport Fishing Council
Thomas L. Washington, Michigan United Conservation Clubs
Howard Zar, U.S. EPA Region V

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Note: Each chapter includes a separate, detailed table of contents.

INTRODUCTION

The National Wildlife Federation's Lake Michigan Sport Fish Consumption Advisory Project was a two-year study of the human health risks that result from eating Lake Michigan sport fish. The Project technical staff included a full-time toxicologist, an environmental biologist, and graduate students in public health and natural resources. This Technical Support Document describes in detail the methodology and conclusions of the Project. Included as an appendix is a copy of the booklet, Lake Michigan Sport Fish: Should You Eat Your Catch?, which was designed to communicate our conclusions to the sport fish-eating public. NWF has also produced a 15 minute video describing the Project's conclusions, which is available for $10.00 from NWF's Great Lakes Natural Resource Center, 802 Monroe, Ann Arbor, Michigan, 48103.

This Project has been the subject of intense scrutiny and criticism from many organizations and individuals interested in the Lake Michigan sport fishery. On occasion, our critics have not distinguished between the merits of our technical evaluation and the method we chose to communicate our results to Lake Michigan sport anglers and their families.

This Technical Support Document addresses only the methods we employed to evaluate and quantify (where possible) the risks of eating Lake Michigan sport fish. We hope that this publication will encourage reasoned discussion about the proper methodology for evaluating the human health risks of eating contaminated sport fish. In that regard, it is important to understand that our quantitative risk assessment method is essentially the same as that used by the U.S. EPA, the Michigan Department of Natural Resources, and the Wisconsin Department of Natural Resources to authorize the continued dumping of toxic chemicals into the Great Lakes and the resulting contamination of sport fish.

A copy of the Project booklet is included in the appendix so that readers can evaluate for themselves how we translated our quantitative health assessments into advice for the lay public. As anyone involved in risk communication knows, many people want only a simple yes-or-no answer to the question: Can I eat my catch? But risk (or safety) is a continuum and there are no simple answers.

We believe, along with noted risk communication expert Baruch Fischoff, that "it is better to make people face the complexity of risk issues than to seek to satisfy their desire

for simple solutions. Keeping complexity alive is a continual challenge for those hoping to conduct intelligent debates about risk." We kept the complexity alive in our booklet by presenting quantitatively the results of our health assessments at different risk levels. We did so in a comparative context to make the point that risk is a relative concept and that anglers' choices about which fish and how much to eat drastically affect the risks levels. We included the appropriate caveats about the uncertainties and assumptions of our methodology.

Time will tell whether we succeeded in improving sport anglers' understanding of risk assessment in general or of the problem of Lake Michigan pollution specifically. In the meantime, NWF's Great Lakes office will continue to encourage state agencies to give anglers the most current and accurate information about the risks of eating their catch and to press state and federal agencies to stop toxic pollution at its source. Our vision for the Great Lakes is a sport fishery for which consumption advisories are no longer necessary.

September, 1989

Mark Van Putten, Director
Great Lakes Natural Resource Center
National Wildlife Federation

CHAPTER 1

SUMMARY OF QUANTITATIVE HEALTH ASSESSMENTS FOR PCBs, DDT, DIELDRIN, AND CHLORDANE

Great Lakes Natural Resource Center
National Wildlife Federation
802 Monroe
Ann Arbor, MI 48104

INTRODUCTION

The discovery of contaminated sport fish in the Great Lakes in the early 1970s prompted the health agencies in the Great Lakes States and in Canada to advise that individuals reduce or eliminate consumption of the most contaminated fish. Consumption advisories for sport fish were triggered by mercury and by certain halogenated organic compounds such as polychlorinated biphenyls (PCBs), DDT and its metabolites DDD and DDE, dieldrin, and chlordane. Concentrations of these compounds are especially high in the tissues of large, predatory species such as lake trout and salmon. Tissue concentrations of these compounds can range as high as 100,000 times concentrations in surrounding water.

All of the Great Lakes States issue consumption advisories for Great Lakes sport fish. Most of the states use the Food and Drug Administration action levels for chlorinated organic toxicants and for mercury to determine when consumption advisories are necessary and how stringent advisories should be. Minnesota is the only state in the Great Lakes basin that does not rely on FDA action levels to trigger consumption advice. Rather, Minnesota uses a risk-based procedure to trigger consumption advice for Great Lakes sport fish. Consumption advisories are issued by all states on a species and size class specific basis (for more information on the procedures used to develop consumption advice for Great Lakes sport fish, see Foran and VanderPloeg, 1989).

As a result of consumption of contaminated fish, high levels of PCBs and DDT have occurred and been measured in the blood serum and breast milk of

some Great Lakes residents. In fact, the National Research Council and the Royal Society of Canada concluded in 1985 that the consumption of Great Lakes fish and food grown in the region is the largest pathway of human exposure to halogenated pollutants.

In May of 1986, the Governors of the eight Great Lakes States (Michigan, Minnesota, Wisconsin, Illinois, Indiana, Ohio, Pennsylvania, and New York) signed the Great Lakes Toxic Substance Control Agreement. One of the provisions in the Agreement calls for the development of uniform fish consumption advisories for each of the Great Lakes. Although the Agreement does not call for development of risk-based consumption advisories, the U.S. EPA and other researchers have proposed that the use of quantitative risk assessment procedures to develop consumption advice more adequately protects the health of individuals who consume Great Lakes sport fish.

In this report we describe a risk-based procedure, and the results of that procedure, that were used by National Wildlife Federation (NWF) scientists to develop a brochure describing the potential health risks from eating contaminated sport fish from Lake Michigan. This procedure was applied to four contaminants that occur commonly in sport fish from Lake Michigan: PCBs, DDT and its metabolites, dieldrin, and chlordane. The procedure involved the evaluation of the combined effects of the four chemicals as a mixture. This procedure should be used to replace the current practice of triggering advisories by the use of chemical-specific FDA action levels, and can be applied to sport fish in the other Great Lakes and U.S. waters.

This report summarizes the hazard assessments, dose-response assessments, exposure estimates and risk characterizations for DDT, dieldrin, PCB and chlordane. The report also presents some risk management considerations and risk communication tools which contributed to the development of the brochure for concerned sport fish anglers and consumers. The process of combining quantitative health assessments with risk management considerations corresponds to the framework used by the U.S. EPA for regulator decision-making and is appropriate for use in the development of consumption advisories for Great Lakes sport fish.

A complete description of the project methods and results is available in a technical document from: Lake Michigan Fish Advisory Project, 802 Monroe, Ann Arbor, MI 48104. The technical document contains full, quantitative health assessments and risk characterizations for PCB, dieldrin, DDT and chlordane as well as the assessment methodology. This two volume, 500 page document will be available by August, 1989 at a cost of $13.00 for duplication and mailing.

Finally, this report contains detailed recommendations to reduce the uncertainty surrounding the amount of toxics in the fish and to use the risk-based process described in the development of advisories as well as the control of the sources of toxic pollution. Briefly, the recommendations are:

* State agencies should collect adequate sample sizes of <u>all</u> important sport fish species.

* State agencies should issue consumption advice based on the average tissue concentration in all fish of a species sampled except when statistical analysis allows the consideration of specific size classes.

* State agencies should expand their Lake Michigan monitoring programs to include all important contaminants found in fish and should conduct baseline testing of fish tissues to identify all contaminants in Great Lakes sport fish.

* The Lake Michigan states should develop one document that describes the quality assurance/quality control (QA/QC) programs used for fish collection and analysis, and a report on the outcome of the Lake Michigan QA/QC program should be published annually.

* The state agencies in the Great Lakes Basin, as well as in other parts of the United States, should adopt a risk-based procedure to develop consumption advice for contaminated sport fish that assesses exposure to combinations of toxics.

* The state agencies should conduct health risk assessments for metals and other contaminants that occur at low but detectable concentratons in sport fish, and consumption advice should be developed when people may be exposed to unacceptable risks.

* The Great Lakes states should support and fund long-term epidemiological research of the health status of sport fish eaters, an acknowleged highly exposed population.

* The state and federal agencies should develop pollution controls and clean-up plans based on the health risks of consuming Great Lakes sport fish. A four-point plan for doing so is described in the report, "Promises to Keep," proposed by NWF and other organizations.

METHODS

HAZARD ASSESSMENT

Hazard assessments involved the review and evaluation of published scientific studies of health effects in laboratory animals and in human populations exposed to PCBs, DDT, dieldrin, or chlordane. The goal at this phase of the risk assessment was to assess the amount and quality of the data, in other words, the weight-of-evidence for the existence of a potential hazard to people exposed to one of the chemicals. Criteria for assessing the quality of the data were developed as part of the NWF fish consumption advisory study and are presented in the full version of the technical report. Well-conducted studies contribute the greatest weight-of-evidence that a hazard may exist in humans exposed exposed at low levels.

A weight-of-evidence scheme provides a framework to support a conclusion that a chemical may be a systemic toxicant in humans. The number and types of studies that have been conducted for individual chemicals varies tremendously. Well-conducted epidemiologic studies in highly exposed populations provide a greater degree of certainty that an effect will be elicited in humans exposed to low doses of a toxicant than do well-conducted animal studies. A chemical that has produced a toxic response in more than

one laboratory animal species and in both sexes is considered to be more likely to produce a similar response in humans than when data are available for only one species or strain, or only one sex. On the other hand, when the quality of a study is compromised by a small sample size, questionable practices, poor reporting of data, or problems in study design, the degree of certainty of impacts in humans is decreased.

Weight-Of-Evidence For Systemic Toxicity

A weight-of-evidence scheme used to evaluate the amount and degree of evidence for or against the capability of a substance to cause reproductive or developmental toxicity in humans is presented in the full version of the technical report. A formal weight-of-evidence scheme was not used for other non-cancer effects because none have been developed. However, reports of all types of health effects caused by these four chemicals were evaluated for their applicability to environmentally exposed human populations.

Weight-Of-Evidence For Carcinogenicity

The hazard assessment supporting the dose-response and risk characterizations for cancer for the four compounds monitored in Lake Michigan sport fish was based on the U.S. EPA Guidelines for Cancer Risk Assessment and the recommended criteria for evaluating epidemiologic studies and animal bioassay data presented by the Office of Science and Technology Policy.

DOSE-RESPONSE ASSESSMENT

The dose-response assessment involved an evaluation of how the incidence or severity of disease in an exposed animal or human population increases with the level of exposure. For systemic effects, an attempt was made to identify a dose level that did not result in significant adverse

effects in the exposed animal or human population. For cancer, the potency of the chemical was evaluated.

Systemic Toxicity

The relative hazard of PCBs, dieldrin, chlordane, and DDT were determined in this study by the calculation of a reference dose (RfD). A chemical-specific reference dose was calculated according to a procedure recommended by the U.S. EPA. Because data from epidemiologic studies was not available, the RfD for each chemical was based on a no-observed-adverse-effect-level (NOAEL) identified from animal studies. A reference dose estimates the dose of a substance expected to produce no toxicity in sensitive human populations. It is assumed that noncarcinogenic toxic responses are not induced in individuals below a certain threshold dose level that is specific to a particular chemical.

The reference dose (RfD) for each chemical was derived from a no-observed-adverse-effect-level (NOAEL) in animals by applying uncertainty factors (UF) reflecting the quality and type of data used to predict toxicity. Uncertainty factors were used to account for the variation in sensitivity within the human population, the differences between humans and non-human animal species, and limitations in the quality of the data used. A modifying factor (MF) was also used when uncertainties still existed with respect to the data base. The NOAEL was defined as the highest experimental dose of a chemical at which no statistically or biologically significant increase in frequency or severity of an adverse effect in exposed individuals was observed when compared with individuals in an appropriate control group. The reference dose was calculated by dividing the NOAEL by the UFs and MF and is expressed in milligrams per kilogram body weight per day (mg/kg/day). When a NOAEL was

not determined, an additional uncertainty factor was applied to a lowest-observed-adverse-effect-level (LOAEL) selected from an appropriate study.

$$RfD = NOAEL/(UFs * MF)$$

$$RfD = LOAEL/(UFs * MF)$$

The product of the uncertainty factor(s) and modifying factor results in a margin of exposure (MOE) between the reference dose for humans and the NOAEL or LOAEL in animals. The MOE is increased as the level of certainty that the NOAEL is correct decreases. The U.S. EPA considers the reference dose to be "an estimate (with uncertainty spanning perhaps 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."

The U.S. EPA recommends that when exposure to a chemical occurs through more than one source, the reference dose should be multiplied by a factor which represents the fraction contributed by the source of concern. The NWF Project estimated the chemical contribution from sources other than sport fish and subtracted that contribution from the overall reference dose for each chemical.

Carcinogenicity

The U.S. National Research Council defines dose response assessment as the description of the relationship between the dose of an agent and the probability of induction of a carcinogenic effect. A dose response assessment for cancer involves three distinct steps, (1) selection of the appropriate

data, (2) extrapolation from the response observed at high doses to the expected exposure in the human population at risk, and (3) adjusting the dose to reflect species differences if the selected data are from a laboratory experiment.

Studies investigating the induction of cancer in humans by PCB, dieldrin, DDT and chlordane were preferred for use in the NWF fish consumption advisory project. However, epidemiological studies that quantified both exposure and cancer incidence were not available for any of the chemicals. Therefore, studies using laboratory animals were selected for the NWF project based on the science-policy decisions promulgated by the U.S. EPA. These are outlined in Table 1.

EXPOSURE ASSESSMENT

Tissue concentration data for PCB (total), dieldrin, chlordane (total), and DDT (total) were gathered for individual sport fish species and size classes of individual species from each of the states surrounding Lake Michigan. Data were gathered for the years 1985, 1986 and 1987. However, only data for 1985 and 1986 were used to develop quantitative health impact assessments. Data for 1987 were not used since the states surrounding Lake Michigan will not incorporate 1987 data into state fish consumption advisories until next year (1990) and since all 1987 data are not yet available from the states. Thus, we chose to use only 1985 and 1986 data to allow comparison of the NWF model advisory with the individual Lake Michigan state advisories. Tissue concentrations of PCB, dieldrin, chlordane and DDT were used in the analysis as these are the only contaminants with a number of measurements adequate to allow appropriate statistical analysis. Tissue concentration data for walleye and perch did not include measurements for Green Bay, Wisconsin

because these species are not wide ranging in the Lake, and a representative average concentration for the species in Lake Michigan was desired.

Fish tissue data were entered and stored on a mainframe computer system. All data were analyzed for distribution and to determine relationships between length and contaminant concentration. Statistical comparisons of individual contaminant concentrations between 1985 and 1986 were made for each species. Only two species (walleye and chinook) had statistically different contaminant concentrations between 1985 and 1986 (PCB). For both of these species, contaminant concentrations in 1986 were higher than in 1985. However, due to the relatively small sample sizes for these and all other species sampled in 1985 and 1986, the arithmetic average of 1985 and 1986 contaminant concentrations was used to develop quantitative health impact assessments.

Not all species exhibited significant relationships between length and contaminant concentration. Where there was not a statistically significant relationship between length and contaminant concentration, the average, minimum and maximum contaminant concentrations were determined for all sizes (combined) of that species. Where a statistically significant relationship between length and contaminant concentration existed, the average, minimum and maximum contaminant concentrations were determined for four size classes: 0 - 10 inches (0 - 254 mm), 10 - 20 inches (254 - 508 mm), 20 - 30 inches (508 - 762 mm) and >30 inches (>762 mm). The average contaminant concentrations for 1985 and 1986 are reported for individual species (and size classes of species where appropriate) in Table 2.

RISK CHARACTERIZATION

The risk characterization phase combines the estimates of potency for systemic toxicity and cancer (the RfD and potency factor) with the exposure data (fish tissue concentrations and meal frequencies) to arrive at an estimate of the degree of risk of developing disease associated with various fish consumption levels.

Systemic Toxicity

An estimate of the hazard of adverse health effects other than cancer was obtained through the calculation of a hazard index. The hazard index is described in the U.S. EPA guidelines for the Health Risk Assessment of Chemical Mixtures. An estimate of environmental exposure (mg/kg/day) using the average fish tissue concentration, an assumed 1/2-pound meal size, and various fish consumption rates is divided by a reference dose (mg/kg/day) to arrive at a hazard index;

$$HI = \text{Exposure Dose}/RfD.$$

We have followed the recommendation of the U.S. EPA for estimating the combined hazard of multiple contaminant exposures when data on interactions are not available. Chemical-specific hazard indices based on the same health endpoint were added to predict the total hazard of fish consumption for each species.

The equation as presented in the U.S. EPA guidelines is:

$$HI = E_1/RfD_1 + E_2/RfD_2 + \ldots + E_i/RfD_i$$

where: E_i = exposure level to the i_{th} toxicant and, RfD_i = the reference dose for the i^{th} toxicant.

The hazard index is considered to be an interim hazard index only because many toxicants have been identified in sport fish in the Great Lakes but adequate tissue concentration data are available for only a few. Therefore, the actual total hazard of these fish is not known, but is likely to be higher than the interim hazard index.

Cancer

Chemical-specific upper-bound potency factors (q^*) were calculated using the Global 82 linearized multistage model. The dose levels that would result in a lifetime upper-bound risk of 10^{-3} to 10^{-7} (one-in-one-thousand to one-in-ten-million) were then calculated. These dose levels were presented in mg/kg/day and number of meals per time period. The evaluation includes the use of average tissue concentrations for six sport fish species, a meal size of 1/2-pound, and a range of estimates for fish consumption.

The overall upper-bound risk for each species was estimated consistent with The U.S. EPA Guidelines for Cancer Risk Assessment. The risk estimates were calculated based on the linear relationship,;

Risk = Dose * Potency.

The guidelines advise that if interactive effects between chemicals cannot be precisely determined, an assumption of additivity should be used to predict the upper-bound cancer risk of eating fish containing several chemicals. Therefore, individual risks were added to determine an overall upper-bound cancer risk.

RESULTS

HAZARD ASSESSMENT

Pharmacokinetics

In general, humans and experimental animals absorb, metabolize, store, and eliminate PCBs, DDT/DDE, dieldrin, and chlordane in a similar manner. There are identifiable differences between certain species, for example, between rodents, dogs, and monkeys, between rats and mice, or between experimental animals and humans. The available literature on these chemicals is not sufficient to conclude that one particular animal species, specifically, rats or mice, are a better model for the prediction of effects in humans.

Two findings are particularly important. First, humans do not metabolize these compounds easily, and they are stored in the body's tissues. Hence, as contaminated fish consumption continues, the chemicals build up in the tissues and are available to exert their toxic effects in greater amount. Second, when a woman becomes pregnant, the PCBs, DDT/DDE, dieldrin, and chlordane that have been stored in her tissues are readily transferred across the placenta to the developing fetus. Therefore, the children of exposed mothers are especially susceptible to these compounds.

Health Effects

PCBs, DDT/DDE, dieldrin, and chlordane may be developmental toxicants in environmentally exposed people. Subtle abnormalities have been reported in the children of women exposed to PCBs and DDT. A study conducted in Michigan found subtle physical and developmental abnormalities in the children of women who were regular consumers of Lake Michigan sport fish prior to and during their pregnancies compared to a nonexposed group. The effects observed included lower birth weights, smaller head circumferences, and less developed motor and cognitive skills. The differences were associated with the mothers' fish consumption and with the level of PCBs measured in umbilical cord blood. Similar developmental problems were reported in the children of Taiwanese and Japanese mothers, who used cooking oil contaminated with PCBs, and the children of women in North Carolina with environmental exposure to PCBs and DDT. While no one study by itself proves that PCBs cause reproductive problems in people, the evidence as a whole suggests that there is cause for concern.

Several types of cancer have been associated with occupational exposure to PCBs, and, although the results are too limited to conclude that a causal relationship exists, they are suggestive of an effect. Several limited epidemiologic studies have indicated a possible association of pesticide exposure with cancer.

The major targets for subchronic and chronic toxicity in mammals are the liver, immune system, endocrine system, reproductive system, the unborn fetus, and nursing offspring. Liver toxicity, reproductive/developmental toxicity, and carcinogenesis appear to be the most sensitive toxicological

endpoints that are common to PCBs, DDT/DDE, dieldrin, and chlordane exposure. PCBs, DDT/DDE, dieldrin, and chlordane have produced evidence of carcinogenicity in mice and/or rats in chronic bioassays. The positive results have been determined by the U.S. EPA to be sufficient to place the compounds into the weight-of-evidence classification of B2, probable human carcinogens.

DOSE-RESPONSE ASSESSMENT

Reference Dose (RfD)

The liver has been identified in the literature as the most sensitive target organ for PCBs, DDT/DDE, dieldrin, and chlordane toxicity. Table 3 presents the four Reference Doses (RfDs) for noncarcinogenic toxicity, and the NOAEL or LOAEL, uncertainty factors (UF), and modifying factor (MF) upon which they are based. The NOAEL or LOAEL for all four of the compounds was divided by an UF of 10 to account for the potential variability in sensitivity within humans and by 10 to account for the potential difference in sensitivity between humans and rats.

A LOAEL was also identified for developmental toxicity in rhesus monkeys exposed to PCBs. The literature concerning DDT, dieldrin, or chlordane was not sufficient to allow the identification of a LOAEL or NOAEL for reproductive or developmental effects. A reference dose was calculated for PCBs based on developmental effects and is shown in Table 3. A reference dose for developmental toxicity was not calculated for the other compounds.

Potency Factor for Carcinogens

Table 4 presents the carcinogenic potency factors for PCBs, DDT/DDE, dieldrin, and chlordane. For this report, upper-bound cancer risks were calculated based on the potency factor for the most sensitive species.

RISK CHARACTERIZATION

The risk characterization for possible cancer effects in humans is presented in three ways. Table 5 presents the combined 95% upper-bound cancer risks associated with four meal frequencies for the Lake Michigan sport fish species evaluated by NWF. The four meal frequencies used for this report -- one meal per year, one meal per month, one meal per week, and three meals per week -- span a wide range of fish consumption habits characteristic of certain segments of the Lake Michigan sport fishing population. Table 6 presents the number of sport fish meals per year for each species and size class associated with five upper-bound cancer risk levels, 1-in-1,000, 1-in-10,000, 1-in-100,000, 1-in-1,000,000, and 1-in-10,000,000. Table 7 presents the number of sport fish meals that, if eaten over a 70 year lifetime, may be associated with the same five risk levels. A meal size of 0.5 pounds was used for the estimates.

The cancer risk projections estimate the upper bound probability of developing cancer from a lifetime of consuming Lake Michigan sport fish containing the same concentrations of PCBs, DDT/DDE, dieldrin and chlordane that were measured in 1985 and 1986. The cancer risk numbers are presented in scientific notation and should be interpreted to be the number of extra cases of cancer expected from sport fish consumption in a population of a specific size. The population sizes can be 100 (10^2), 1000 (10^3), 10,000 (10^4), 100,000 (10^5), 1,000,000 (10^6) and so on.

The risk characterization for health problems other than cancer is shown in Tables 8 and 9. Table 8 presents the combined hazard indices associated with four meal frequencies for the Lake Michigan sport fish species evaluated by NWF. Table 9 presents the number of sport fish meals per year for the same species associated with a hazard index equal to one, based on liver toxicity for all four chemicals and based on developmental toxicity for PCBs.

The hazard indices reflect the degree to which sport fish consumption, based on a specific meal frequency, exceeds the level of consumption that would be expected to result in negligible toxicity. Again, these estimates assume that the fish are consumed for a lifetime with a constant fish tissue concentration. When the combined chemical exposure for a species (number of meals per year) is associated with a hazard index equal to one or less, the risk of disease is probably quite low. But as consumption increases, the margin between the animal NOAEL and the human RfD becomes smaller, and the possibility of disease developing in the sport fish consumer increases.

Note that the meal frequencies based on liver toxicity in Table 9 are for the combined effects of PCBs, DDT, dieldrin, and chlordane while the meal frequencies based on developmental toxicity are for the effects of PCBs only. Even so, these data suggest that a smaller number of meals should be eaten to protect against developmental toxicity than to protect against liver toxicity. Although between one to five 1/2-pound meals of the smaller size classes and species may be eaten each year without concern for developmental toxicity in offspring based on PCBs concentrations, the added effect of DDT,

dieldrin, chlordane, and other developmental toxicants found in the fish cannot be quantified. Moreover, reproductive effects have been reported in human populations exposed to PCBs. The authors of the Michigan study reported that the incidence of effects in the infants of mothers who had regularly eaten sport fish from Lake Michigan increased with the amount of fish eaten. Several potential confounding factors, including cigarette smoking and alcohol consumption, were accounted for in the researchers' analysis of the data. It is therefore prudent to conclude that women who intend to have children and children not yet sexually mature should avoid consuming the fish species examined in this study.

Table 10 combines the conclusions drawn from the risk characterization for cancer and other potential health effects in humans consuming the contaminated sport fish species. The number of meals eaten in a 70 year lifetime associated with an excess cancer risk of 1-in-10,000, 1-in-100,000, and 1-in-1,000,000 is presented for each species and size class. In addition, the maximum number of meals eaten in any one year that would not be expected to result in liver toxicity is shown. This table is included in the brochure on consuming these contaminated Lake Michigan sport fish species. This table provides a large amount of information to the concerned sport fish eater and should facilitate informed decisions about consumption of these fish species in light of their potential health effects.

The hazard assessments and risk characterizations were used in combination with risk management and risk communication considerations to develop fish consumption recommendations for Lake Michigan sport fish species. The cancer risk projections and hazard indices are intended to assist sport

fish consumers to make decisions about protecting themselves from exposure to toxic contaminants.

Table 11 describes various cancer risk levels that have triggered regulatory action in the past and those that are part of the U.S. FDA's and EPA's current regulatory framework. These levels may assist in the interpretation of the upper-bound cancer risk levels found in this investigation of Lake Michigan sport fish consumption. Generally, regulators take action to reduce excess cancer risks in a population when they are found to be between 1-in-1,000,000 to 1-in-10,000 or above. Programs designed to prevent exposure to carcinogens in food regulate above an excess risk level of 1-in-1,000,000. The risk level selected to trigger action depends on the statutory mandate for a particular program, which may include requirements for balancing the costs and benefits of actions.

The presentation of comparative cancer risks is a valuable tool for interpreting the significance of the data on risk for different consumption rates of the Lake Michigan fish species evaluated by NWF. Table 12 presents cancer risks associated with three meal frequencies for the Lake Michigan sport fish species compared to sport fish from two other U.S. locations, Quincy Bay, Massachusetts and Puget Sound, Washington. The cancer risks for Lake Michigan sport fish are also compared to cancer risks associated with consuming the same amounts of commercial protein sources, red meat, cod, haddock, canned tuna, and chicken. The risk estimates are for the same four chemicals that were evaluated in the Lake Michigan fish. The data for the commercial sources were obtained from the U.S. Food and Drug Administration's Total Diet Survey for the years 1982-1986. This information is depicted graphically in the brochure for consumers of Lake Michigan sport fish.

The FDA survey reports the levels of contaminants in cooked foods while the data for the sport fish represent raw fillets. Nevertheless, these risks can still be compared because studies of the effects of cooking fish containing these chemicals have not shown consistent reductions in toxicant levels in all species, ages, and size classes. Moreover, even if cooking reduced the toxics in sport fish by 50%, the cancer risks from these fish would still be significantly greater than those for the commercial fish shown on this chart.

RECOMMENDATIONS

We offer the following recommendations based on our analysis of contaminant concentration data in Lake Michigan sport fish and based on our assessment of the health risks posed by exposure to these contaminants through consumption of contaminated Lake Michigan sport fish.

1. Inadequate data exist to properly (statistically) assess the extent of contamination in several species and size classes of Lake Michigan sport fish. Because of data inadequacies, we were not able to determine representative concentrations of contaminants in some sport fish species nor were we able to develop consumption advice for these species. Therefore, we recommend that state resource and health agencies collect adequate sample sizes of the following fish species to determine the types and concentrations of contaminants: Whitefish, steelhead trout/rainbow trout, northern pike, pink salmon, and smallmouth bass. Although we did develop advice for walleye and yellow perch, monitoring data for these species are only marginally adequate and we recommend that larger sample sizes of these species be collected. Further, the smaller size classes of many species are not sampled adequately,

and we recommend increased sampling of smaller sizes of all salmonids as well as the fish species cited above.

2. Once adequate sample sizes of Lake Michigan sport fish species are collected, we recommend that states conduct a thorough analysis of the relationship between fish length and contaminant concentrations. Size class specific advice should be issued only where a positive relationship exists between length and contaminant concentration. Further, where consumption advice is issued for specific size classes of fish species, different advice for different size classes should be issued only where there are statistically significant differences in contaminant concentrations between species.

3. Most of the states surrounding Lake Michigan conduct tissue analysis only for PCBs in sport fish. We recommend 1) that the states expand their Lake Michigan monitoring programs to include chemicals other than PCB. At the very least, expanded programs should include analysis of DDT (and its metabolites), chlordane, and dieldrin. Other toxicants including lead and other metals, toxaphene, mirex, polynuclear aromatic hydrocarbons (PAHs), lindane, heptachlor and heptachlor epoxide, chlorinated styrenes, hexachlorobenzene, and other chlorinated compounds (including dioxins and furans) should be analyzed in Lake Michigan sport fish, and 2) that states develop a program to analyze and identify all contaminants in Great Lakes sport fish. Further, states should develop a process to identify new contaminants that would be included in the fish monitoring program.

4. Quality assurance/quality control programs in the Lake Michigan states are not well documented. Only Michigan has developed a document that describes QA/QC procedures for its Great Lakes fish monitoring program. No

comprehensive summary of QA/QC information exists in individual states or for the Lake Michigan monitoring program as a whole. Therefore, we recommend that the Lake Michigan states develop one document that describes the tissue collection and analysis procedures used for Lake Michigan sport fish and the QA/QC procedures used in the monitoring program. Further, we recommend that a yearly report on the outcome of the Lake Michigan QA/QC program be published by the Lake Michigan states and made available to all interested persons.

5. We strongly recommend that the Lake Michigan states, as well as the rest of the states in the Great Lakes basin, utilize a risk-based procedure to develop consumption advice for Great Lakes sport fish. This procedure should recognize both risk assessment and risk management issues, assess these issues in a broad, open public format, incorporate them into consumption advice presented to the public, and present them openly and clearly in all communications. Further, consumption advice should be based on a thorough assessment of the health risks associated with concurrent exposure to combinations of contaminants. Where data are not available that address the risks of concurrent exposure to combinations of contaminants, the assumption should be used that risks are additive and consumption advice based on the assumption.

6. Thorough health assessments for metals and other contaminants that occur at low but detectable concentrations in sport fish (including toxicants cited in Recommendation #3) should be conducted and consumption advice developed for fish contaminated with these materials where health assessments indicate that potential impacts on human health may occur through consuming contaminated sport fish.

7. We strongly recommend that long-term, epidemiological research be funded and conducted to determine the types and extent of impacts of consuming contaminated Great Lakes sport fish on humans in the Great Lakes basin.

8. The state and federal agencies should develop pollution controls and clean-up plans based on the health risks of consuming Great Lakes sport fish. A four-point plan for doing so is described in the report, "Promises to Keep," proposed by NWF and other organizations.

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Table 1.

ASSUMPTIONS AND SCIENCE POLICY DECISIONS USED IN THE DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

SELECTION OF CANCER INCIDENCE DATA

Well-conducted epidemiologic studies preferred if quantified data on exposure and cancer incidence are available. If not available, data based on carcinogenic responses in laboratory animals responding in a manner similar to humans will be used.

Use total number of animals with one or more tumor type or site with incidence that is statistically increased.

Benign tumors of the same histogenic origin will be combined with malignant tumors. The contribution of benign tumors to the total risk estimate will be indicated.

Greater emphasis will be placed on results in the most sensitive species, strain and sex.

Incidence data will reflect the same route and pattern of exposure as the human environmental exposure, otherwise adjustments will be made to account for the difference in delivered dose expected.

DOSE RESPONSE MODEL

An assumption of no threshold is made.

An assumption of low dose linearity is made.

The linearized multistage model is the mathematical model of choice. 95% upper bound risk estimates will be presented.

An assumption of lifetime exposure (70 years) at a constant dose rate will be used.

An assumption of additivity will be used to incorporate background tumor incidence.

A scaling factor based on body surface area will be used to extrapolate from the test species to humans. The percent reduction in risk that would be observed if a body weight scaling factor were used will be stated since the actual appropriate interspecies dose equivalency is as yet undetermined.

If the test animal absorbs only a fraction of the administered dose, or if evidence suggests that humans absorb more or less of the chemical than does the test animal, and the administered dose would be otherwise used, this dose will be adjusted accordingly.

TABLE 2. Average tissue concentrations of PCB, dieldrin, total DDT and total chlordane in lake trout, chinook salmon, coho salmon, brown trout, yellow perch, and walleye pike expressed by size class (where at least one contaminant concentration is significantly different between size classes). Groupings evaluated with a Duncan's multiple range test. Averages within species (between size classes) are not significantly different if connected by the same letter. Size classes: 1 - 0 to 10 inches; 2 - 10 to 20 inches; 3 - 20 to 30 inches; 4 - greater than 30 inches.

SPECIES	SIZE CLASS	CONTAMINANT	AVERAGE TISSUE CONCENTRATION (mg/kg)	N	GROUPING
Lake Trout	4	PCB	8.30	25	A
	3	PCB	3.40	116	B
	2	PCB	0.92	33	C
Lake Trout	4	Chlordane	0.78	2	A
	3	Chlordane	0.42	35	A
	2	Chlordane	0.22	8	A
Lake Trout	4	Dieldrin	0.37	2	A
	3	Dieldrin	0.18	35	B
	2	Dieldrin	0.17	8	B
Lake Trout	3	DDT	1.88	26	A
	2	DDT	0.23	1	A
Chinook	4	PCB	2.43	58	A
	3	PCB	1.17	48	B
	2	PCB	0.39	74	C
Chinook	4	Chlordane	0.16	8	A
	3	Chlordane	0.04	4	B
	2	Chlordane	0.0	8	B
Chinook	4	Dieldrin	0.21	8	A
	3	Dieldrin	0.04	4	A
	2	Dieldrin	0.01	8	A
Chinook	4	DDT	1.78	6	A
	3	DDT	0.29	3	A
Brown Trout	all	PCB	1.76	127	-
	all	Chlordane	0.13	45	-
	all	Dieldrin	0.10	45	-
	all	DDT	0.43	31	-
Coho	all	PCB	0.56	56	-
	all	Chlordane	0.07	28	-
	all	Dieldrin	0.03	28	-
	all	DDT	0.19	25	-

TABLE 2. Continued.

SPECIES	SIZE CLASS	CONTAMINANT	AVERAGE TISSUE CONCENTRATION (mg/kg)	N	GROUPING
Perch	all	PCB	0.19	16	-
	all	Chlordane	0.01	7	-
	all	Dieldrin	0.02	7	-
	all	DDT	0.19	10	-
Walleye	all	PCB	0.67	15	-
	all	Chlordane	0.03	9	-
	all	Dieldrin	0.02	9	-
	all	DDT	0.11	9	-

Table 3. Parameters Used to Calculate the Reference Dose (RfD) for Systemic Effects in Humans Exposed to PCBs, DDT/DDE, Dieldrin, and Chlordane.

Reference	Target Organ	Dose mg/kg/day	End-point	Study Length weeks	UF[2]	MF[3]	Reference Dose mg/kg/day Total	Reference Dose mg/kg/day Fish
PCBs								
Bruckner et al., 1974	Liver	0.05	LOAEL[1]	5	10H 10A 10L	1	5E-05	4.5E-05
DDT/DDE								
Laug et al., 1950	Liver	0.05	NOAEL[1]	6	10H 10A	1	5E-04	4.6E-04
DIELDRIN								
Fitzhugh et al., 1964	Liver	0.03	LOAEL[1]	24	10H 10A 10L	1	3E-05	1.2E-05
CHLORDANE								
RIASBT, 1983	Liver	0.06	NOAEL[1]	130	10H 10A	0.7	4.2E-04	4.2E-04

[1] LOAEL = Lowest-Adverse-Effect-Level; NOAEL = No-Adverse-Effect-Level

[2] UF = Uncertainty factor. Uncertainty factors are used to calculate the reference dose. The no-adverse-effect-level (NOAEL) is divided by factors of 10 to account for variation in sensitivity among the members of the human population, 10H; uncertainty in extrapolating from animal data to humans, 10A; and uncertainty in extrapolating from a LOAEL instead of a NOAEL, 10L. See Table 5, Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment, and Risk Characterization for details.

[3] MF= Modifying factor. An additional uncertainty factor, greater than zero and less than or equal to 10, to further adjust for scientific uncertainties of the study.

Table 4. Species-Specific Potency Factors Used to Derive Upper Bound Cancer Risk Projections for Lake Michigan Sport Fish Consumption.

Chemical	Potency Factor (per mg/kg/day)	Comments
PCBs	7.6	Rats, Norback and Weltman, 1985
DDT/DDE	0.4	Mice, Turusov et al., 1973
DIELDRIN	16.0	Mice, Geometric mean of 13 data sets
CHLORDANE	1.3	Mice, Geometric mean of four data sets

Table 5.
Additive 95% Upperbound Cancer Risk From Four Compounds Found in Selected Lake Michigan Sport Fish Species.

Species/ Size Class	Meal Frequency			
	One/Year	One/Month	One/Week	Three/Week
Lake Trout				
10-20 inches	9E-05	1E-03	5E-03	1E-02
20-30 inches	3E-04	3E-03	1E-02	4E-02
30 inches or more	6E-04	7E-03	3E-02	1E-01
Brown Trout (All Sizes)	1E-04	2E-03	7E-03	2E-02
Chinook				
10-30 inches	9E-05	1E-03	5E-03	1E-02
30 inches or more	1E-04	1E-03	6E-03	2E-02
Walleye (All Sizes)	5E-05	6E-04	3E-03	8E-03
Coho (All Sizes)	4E-05	5E-04	2E-03	7E-03
Perch (All Sizes)	2E-05	2E-04	8E-04	3E-03

*Cancer risk projections involve an assumption of lifetime exposure with 1/2-pound meals and average fish tissue concentrations.

Table 6.
Meal Frequency (Meals Per Year) For Selected Lake Michigan Sport Fish Species Associated With Five 95% Upper-Bound Cancer Risk Levels For Four Compounds.

Species/ Size Class	Cancer Risk 1E-03	1E-04	1E-05	1E-06	1E-07
Lake Trout					
10-20 inches	10.97	1.10	0.11	0.01	0.00
20-30 inches	3.70	0.37	0.04	0.00	0.00
30 inches or more	1.60	0.16	0.02	0.00	0.00
Brown Trout					
(All Sizes)	7.32	0.73	0.07	0.01	0.00
Chinook					
10-30 inches	11.57	1.16	0.12	0.01	0.00
30 inches or more	4.93	0.49	0.05	0.00	0.00
Walleye (All sizes)	20.46	2.05	0.20	0.02	0.00
Coho (All sizes)	22.93	2.29	0.23	0.02	0.00
Perch (All Sizes)	60.54	6.05	0.61	0.06	0.01

*These meal frequencies involve an assumption of lifetime exposure with 1/2-pound meals and average fish tissue concentrations.

Table 7.
Number of Meals Over A 70 Year Lifetime Associated With Five Upper-Bound (95%) Cancer Risk Levels for Four Compounds Found in Selected Lake Michigan Sport Fish Species.

Species/ Size Class	Cancer Risk 1E-03	1E-04	1E-05	1E-06	1E-07
Lake Trout					
10-20 inches	767.75	76.77	7.68	0.77	0.08
20-30 inches	258.79	25.88	2.59	0.26	0.03
30 inches or more	111.88	11.19	1.12	0.11	0.01
Brown Trout					
(All Sizes)	512.27	51.23	5.12	0.51	0.05
Chinook					
10-30 inches	810.02	81.00	8.10	0.81	0.08
30 inches or more	345.10	34.51	3.45	0.35	0.03
Walleye (All sizes)	1431.89	143.50	14.32	1.40	0.00
Coho (All sizes)	1604.77	160.48	16.05	1.60	0.16
Perch (All Sizes)	4237.64	423.76	42.38	4.24	0.42

*These meal frequencies involve an assumption of lifetime exposure with 1/2-pound meals and average fish tissue concentrations.

Table 8.
Additive Hazard Index From Four Compounds Found in Selected Lake Michigan Sport Fish Species.

Species/ Size Class	Meal Frequency			
	One/Year	One/Month	One/Week	Three/Week
Lake Trout				
10-20 inches	0.2	2.3	9.8	29.4
20-30 inches	0.3	4.0	17.5	52.5
30 inches or more	0.7	8.5	36.8	110.4
Brown Trout (All Sizes)	0.2	2.1	9.0	26.9
Chinook				
10-30 inches	0.1	1.2	5.0	15.1
30 inches or more	0.3	3.7	16.4	49.1
Coho (All Sizes)	0.1	0.7	2.9	8.6
Walleye (All Sizes)	0.0	0.6	2.5	7.5
Perch (All Sizes)	0.0	0.3	1.3	4.0

*Hazard indices involve an assumption of lifetime exposure with 1/2-pound meals and average fish tissue concentrations.

Table 9.
Meal Frequencies (Meals Per Year) Associated With A Hazard Index Of One For Liver Toxicity (PCBs, DDT, Dieldrin, and Chlordane) And For Developmental Toxicity (PCBs only).

Species/Size Class	Combined Liver Toxicity	Developmental PCBs only
Lake Trout		
10-20 inches	5.31	1.10
20-30 inches	2.86	0.29
30 inches or more	1.41	0.12
Brown Trout		
(All Sizes)	5.80	0.57
Chinook		
10-30 inches	10.64	0.86
30 inches or more	3.18	0.42
Coho (All Sizes)	14.64	1.22
Walleye (All Sizes)	19.97	1.51
Perch (All Sizes)	35.47	5.31

*These meal frequencies involve an assumption of lifetime exposure with 1/2-pound meals and average fish tissue concentrations.

TABLE 10:

NUMBER OF LAKE MICHIGAN SPORT FISH MEALS THAT YOU CAN EAT DURING YOUR LIFETIME ASSOCIATED WITH A 1-IN-10,000 1-IN-100,000, OR 1-IN-ONE-MILLION LIFETIME RISK OF CANCER (1/2-pound meal size, skin-on fillets)

SPECIES/ SIZE CLASS	CANCER RISK		
	1-IN-10,000	1-IN-100,000	1-IN-1,000,000
Lake Trout (30" or more)	11 meals in your lifetime (but not more than 1 meal in any year).	1 meal in your lifetime.	No meals in your lifetime.
Lake Trout (20 - 30") Chinook Salmon (30" or more)	30 meals in your lifetime (but not more than 3 meals in any one year).	3 meals in your lifetime.	No meals in your lifetime.
Brown Trout (Any Size) Lake Trout (10 - 20") Chinook Salmon (10 - 30 ")	70 meals in your lifetime (but not more than 7 meals in any one year).	7 meals in your lifetime.	One meal in your lifetime.
Coho Salmon (Any Size) Walleye (Any Size)	150 meals in your lifetime (but not more than 17 meals in any one year).	15 meals in your lifetime.	One meal in your lifetime.
Yellow Perch (Any Size)	420 meals in your lifetime (but not more than 35 meals in any one year).	42 meals in your lifetime (but not more than 35 meals in any one year).	4 meals in your lifetime.

The maximum number of meals for any one year is shown to protect against the development of health problems other than cancer, like liver damage. The possibility of these other health problems becomes greater if the fish are eaten more frequently.

WOMEN WHO INTEND TO HAVE CHILDREN, PREGNANT WOMEN, NURSING MOTHERS, AND CHILDREN (15 YEARS AND YOUNGER) SHOULD NOT EAT ANY OF THESE LAKE MICHIGAN SPORT FISH SPECIES.

Table 11. Cancer risk levels for various federal agency actions.

RISK LEVEL	REGULATORY CONTEXT	REFERENCE
4×10^{-3}	*de manifestis*[1] level for small populations based on 132 regulatory decisions, 126 made since 1980[23]	Travis et al., 1987
3×10^{-4}	*de manifestis* level for large populations based on 132 regulatory decisions, 126 made since 1980[23]	Travis et al., 1987
1×10^{-4}	*de minimis*[4] level for small populations based on 132 regulatory decisions, 126 made since 1980[23]	Travis et al., 1987
1×10^{-6}	*de minimis* level for large populations based on 132 regulatory decisions, 126 made since 1980[23]	Travis et al., 1987
1×10^{-6}	*de minimis* level for food additives, Delaney Clause, Food, Drug and Cosmetic Act	U.S. FDA
1×10^{-6}	*de minimis* level for National Contingency Plan, Superfund cleanup remedies	Env. Rptr., BNA, Crnt. Dvlpmts., 1275, 1988.
1×10^{-6}	*de minimis* level for carcinogens, U.S. EPA Cancer Risk Assessment Guidelines	Fed. Reg. v51, #185 24 Sept. 1986

1. *de manifestis* - "risk of obvious or evident concern" - all risks at or above this level generated a regulatory action to reduce the risk.

2. Regulatory decisions made by U.S. FDA, U.S. EPA, Consumer product Safety Commission, and Occupational Safety and Health Administration.

3. Risks between the *de manifestis* and *de minimis* levels may or may not have generated a regulatory action. Regulatory decisions made in this risk region were generally governed by cost: Substances with risk reduction costs of less than $2 million per life saved were regulated; substances that cost more were not regulated.

4. *de minimis* - "acceptable level of risk that is below regulatory concern" - all risks below this level did not generate a regulatory action to reduce the risk.

Table 12. Comparative Cancer Risks for Lake Michigan Fish and Other Foods.

Species/Food Type	Meal Frequency		
	1/Week	1/Month	1/Year
Lake Trout (>30")	3E-02	7E-03	6E-04
Lake Trout (20-30")	1E-02	3E-03	3E-04
Brown Trout (20-30")	9E-03	2E-03	2E-04
Lake Trout (10-20")	5E-03	1E-03	9E-05
Brown Trout (10-20")	5E-03	1E-03	1E-04
Chinook Salmon (All Sizes)	5E-03	1E-03	1E-04
Coho Salmon (20-30")	2E-03	5E-04	4E-05
Flounder (Quincy Bay)	8E-04	2E-04	2E-05
Pacific Cod (Puget Sound)	8E-04	2E-04	2E-05
Perch (All Sizes)	8E-04	2E-04	2E-05
Lobster (Quincy Bay)	8E-04	2E-04	2E-05
Chinook Salmon (Puget Sound)	3E-04	6E-05	5E-06
Red Meat	1E-05	<1E-06	<1E-06
Cod/Haddock	5E-06	<1E-07	<1E-07
Chicken	5E-06	<1E-07	<1E-07
Canned Tuna	1E-06	<1E-07	<1E-07

*Cancer risks for the Puget Sound fish are for PCBs only. Cancer risks for the Quincy Bay fish are for PCBs, DDT, and chlordane.

CHAPTER 2

METHODS AND SCIENTIFIC RATIONALE FOR HAZARD ASSESSMENT, DOSE RESPONSE ASSESSMENT, AND RISK CHARACTERIZATION

TABLE OF CONTENTS

INTRODUCTION

This document describes the methods and rationale for the chemical-specific hazard assessments, dose-response assessments, and risk characterizations conducted for PCBs, DDT/DDE, dieldrin and chlordane. In general, guidelines developed by the U.S. EPA for evaluating and characterizing the health risks from carcinogenic compounds and other systemic toxicants were adopted. Other resources were also used to develop assessment criteria as appropriate and are cited in this document.

HAZARD ASSESSMENT

Hazard assessments involved the review and evaluation of published scientific studies of health effects in laboratory animals and in human populations exposed to PCBs, DDT, dieldrin, or chlordane. The goal at this phase of the risk assessment was to assess the amount and quality of the data, in other words, the weight-of-evidence for the existence of a potential hazard to people exposed to one of the chemicals. Criteria for assessing the quality of the data were developed as part of the NWF fish consumption advisory study. Well-conducted studies contribute the greatest weight-of-evidence that a hazard may exist in humans exposed exposed at low levels.

A weight-of-evidence scheme provides a framework to support a conclusion that a chemical may be a systemic toxicant in humans. The number and types of studies that have been conducted for individual chemicals varies tremendously. Well-conducted epidemiologic studies in highly exposed populations provide a greater degree of certainty that an effect will be

elicited in humans exposed to low doses of a toxicant than do well-conducted animal studies. A chemical that has produced a toxic response in more than one laboratory animal species and in both sexes is considered to be more likely to produce a similar response in humans than when data are available for only one species or strain, or only one sex. On the other hand, when the quality of a study is compromised by a small sample size, questionable practices, poor reporting of data, or problems in study design, the degree of certainty of impacts in humans is decreased.

Weight-Of-Evidence For Systemic Toxicity

A weight-of-evidence scheme used to evaluate the amount and degree of evidence for capability of a substance to cause reproductive or developmental toxicity in humans is presented in this section. A formal weight-of-evidence scheme was not used for other non-cancer effects because none have been developed. However, reports of all types of health effects caused by these four chemicals were evaluated for their applicability to environmentally exposed human populations.

Weight-Of-Evidence For Carcinogenicity

The hazard assessment supporting the dose-response and risk characterizations for cancer for the four compounds monitored in Lake Michigan sport fish was based on the U.S. EPA Guidelines for Cancer Risk Assessment and the recommended criteria for evaluating epidemiologic studies and animal bioassay data presented by the Office of Science and Technology Policy.

WEIGHT OF EVIDENCE FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Assessing Data Quality

The following section discusses various principles and criteria which were used to evaluate studies of reproductive and developmental effects in humans and experimental animals exposed to the chemicals covered by the NWF Sport Fish Consumption Advisory Project. While other types of responses to chronic exposures are not specifically discussed, many of the same concerns apply generally.

Studies of Reproductive and Developmental Effects in Humans

Human studies include epidemiologic studies and case reports of adverse effects in individuals. While all human data provide valuable information on potential hazard, only those studies which are able to provide estimates of the degree of exposure in relation to observed effects are adequate for a quantitative risk assessment. Studies in humans cannot be tightly controlled, therefore criteria for the determination of data quality cannot be as specific as for experiments in animals.

The Intra-agency Regulatory Liaison Group (IRLG) (1981) developed guidelines for the documentation of epidemiologic studies. These were used to evaluate the adequacy of human studies for use in weight-of-evidence and quantitative risk evaluations. The guidelines identify elements which should be discussed in any documentation of an epidemiological study including background and objectives, study design, study and comparison subjects, data collection procedures, analysis, and supporting documentation.

The IRLG Workshop on Reproductive Toxicity Risk Assessment (1986) identified some major issues to be considered in the design of studies or their analysis.

The issues apply generally to epidemiologic research, however there are special considerations which must be given careful attention in the study of reproductive outcomes. These are (1) the definition and ascertainment of the reproductive outcomes of interest, (2) the interpretation of dose-response relationships, (3) the definition, identification, and quantification of exposures, and (4) the application of appropriate statistical techniques to determine if an association between exposure and outcome occurs more often than would be expected by chance, and to determine the strength of that association. Each of the issues are discussed briefly.

1. Reproductive Outcomes

A broad spectrum of outcomes are associated with parental exposure to reproductive toxicants (Sever and Hessol, 1984). Relevant outcomes measured in human populations are;

* altered fertility,
* single gene defects,
* chromosomal abnormalities,
* spontaneous abortions,
* congenital malformations,
* intrauterine growth retardation,
* altered sex ratio,
* perinatal deaths,
* developmental disabilities,
* behavioral disorders, and
* malignancies.

A combination of inter-related outcomes may contribute to the total adverse effect elicited by a toxicant including fetal loss, subfertility, and malformations in live births (Selevan and Lemasters, 1987).

Various sources of data on reproductive and teratogenic risk (vital statistics, hospital records, questionnaires) are subject to bias and information gaps (IRLG, 1986). Complete ascertainment of embryonic and fetal deaths and subfertile individuals in a population sample is very difficult. Therefore, many studies collect data only on live births or recognized pregnancies and consider only one endpoint. The entire range of outcomes produced by an agent cannot be learned from these studies. Epidemiologic studies are able to collect the following types of information; semen evaluations, indirect measures of fertility/infertility, and reproductive history studies of some pregnancy outcomes (e.g., fetal loss, birth weight, sex ratio, congential malformations, postnatal function, and neonatal growth and survival) (U.S. EPA, 1988b).

2. Dose Response

An increase in the incidence of a toxic response as the dose level increases is important evidence that the agent is responsible for the effects observed. However, a traditional dose-response curve may not be observed for some effects. This is because different dose levels of a toxicant may cause different responses so that measurements of only one type of effect might show a decreasing incidence when the dose reaches a certain level. The timing of exposure may also affect the type of response observed and its dose-response behavior. Studies which do not show an increasing dose-response curve at all doses are not necessarily negative studies and should be examined carefully. Selevan and Lemasters (1987) concluded that "because of these potentially

inverse dose-response relationships with increasing exposure, the most sensitive assessment of effects of exposure on reproduction would examine several outcomes at several exposure levels, possibly combining adverse outcomes into some sort of adverse pregnancy index."

3. Exposure

The exposure period should correspond to the critical period for the reproductive outcomes that are studied (Sever and Hessol, 1984). The ability to do this may be limited by a poor ascertainment of exposure among study and comparison groups (IRLG, 1986). The confirmation of exposure estimates through an additional source or by direct measurement increases the validity of the study results. The use of an internal or local comparsion group is also essential.

4. Statistical Analysis

Sample size should be large enough to minimize the probabilities of finding a false positive (Type I) or false negative (Type II) association. When Type II error is minimized, the power of the study is increased which strengthens conclusions regarding the exposure-disease relationships (Sever and Hessol, 1984).

According to the IRLG (1986), "a negative or inconclusive study result does not demonstrate the absence of a hazard or the safety of an agent, unless the study was of sufficient statistical power and adequately designed to have been able to detect a difference had one existed." The statistical power of epidemiological studies is often limited by a small number of exposed individuals, poorly defined exposures, or a small number of observed outcomes.

Successive pregnancies by the same mother are not independant events and should be adjusted for in the analysis (U.S. EPA, 1986, 1988a and b). The use of nonindependent observations overestimates the true size of the population at risk and artificially increases the significance level.

Studies with a low probability of biased data should carry more weight (U.S. EPA, 1986, 1988a and b). Potential sources of selection and information bias and their effect on the study conclusions should be evaluated and discussed. Other risk factors, effect modifiers, and potential confounders should be controlled for in the study design and/or the analysis. These factors may vary depending on the outcomes studied.

Studies of Reproductive and Development Effects in Animals

The U.S. Food and Drug Administration and the U.S. Environmental Protection Agency have developed testing guidelines for investigations of reproductive and developmental toxicology in laboratory animals (U.S. FDA, 1970; U.S. EPA, 1982; U.S. EPA, 1985). In addition, guidelines have been developed for the purpose of evaluating published studies concerning evidence of reproductive and developmental toxicity in humans and nonhuman mammals (IRLG, 1981; IRLG, 1986; U.S. EPA, 1986; U.S. EPA, 1988a; U.S. EPA, 1988b). The study and interpretation of such evidence for the purpose of establishing the potential hazard to occupational populations and the general public has been discussed by serval authors including Collins (1978), Manson et al. (1982), Dixon and Hall (1982), Sever and Hessol (1984), Manson (1987), and Nisbet and Karch (1983).

The FDA states in its guidelines for the testing of drugs, food additives, and pesticides that multigeneration reproduction studies are appropriate for compounds likely to concentrate in the body with long-term exposure (Manson et al., 1982). This is the type of test required for evaluating food additives. During earlier years, a three generation bioassay was considered acceptable. Laboratory animals were exposed to the chemical of concern in the diet or drinking water beginning at weaning of the parental generation through the entire duration of the study. More recently, FDA guidelines have called for a minimum of a two generation reproductive study design, with a teratology phase.

The three generation test protocol is as follows. After 60 days of treatment or at age 100-120 days of age, parental (P1) males and females are mated to produce the F1a generation. Within 24 hours after birth, pups are weighed, sexed and examined. The litters are culled to ten animals or less on day 4 using a random procedure. Separate weights for male and female offspring and survival are recorded at birth, and at days 4, 7, 14, and 21 days of age. The pups in the first litters are weaned at 21 days, sacrificed and autopsied.

One to two weeks postweaning, a second mating of the P1 animals is accomplished to produce the F1b generation. The same measurements are taken as for the F1a offspring. At weaning, a sufficient number of offspring are selected to produce the F2 generations. The procedure is repeated again to produce the F3 generations. The F3 offspring are sacrificed at weaning, and at least 10 animals are preserved for histologic examination.

The following indices of reproductive capability and developmental health are calculated.

Fertility Index:	(Number of pregnancies/number of matings)100
Gestation Index:	Number of live fetuses per litter, or number of live-born per total number born
Sex Ratio:	Percentage of each sex against the total number of offspring calculated at birth, and 4, 7, 14, and 21 days of age.
Viability Index:	(Number of pups alive at 4 days/number of pups born alive)100
Weaning Index:	(Number of pups alive at 21 days/number of pups maintained at 4 days)100
Growth Index:	Average weights of male and female offspring at birth and 4, 7, 14, and 21 days of age.

The U.S. FDA specifies that a complete necropsy and histopathology should be conducted on a group of ten F1 males and 25 F1 females per dose level that were parents of the F2 generation as well as on five male and female F1 and F2 weanlings per dose level.

The EPA guidelines for registering pesticides contain a few significant variations of the FDA protocol for multigenerational tests (U.S. EPA, 1982). The test suggested by the EPA is a two generation study involving the production of only one litter per generation. The agency reasons that no toxic effects have been known to first appear in the third generation.

Dosing of the parental animals (P1) should begin at about 8 weeks of age (U.S. EPA, 1982) and continue through the weaning of the F1 animals. Treatment of the F1 generation should begin at weaning and continue to the time of weaning the F2 offspring three weeks after delivery (U.S. EPA, 1982). The F2

generation is exposed to the test chemical only *in utero* and via nursing. The U.S. EPA (1982) recommends that the perinatal animal be dosed for at least 8 weeks prior to the mating period. The duration of observation recommended by the U.S. EPA is at least 28 weeks from dosing of P1 animals to sacrifice of F2 offspring at weaning.

The U.S. EPA states that gross necropsy should be done on all animals including those who died during the experiment. Histopathology should be conducted on the vagina, uterus, ovaries, testes, epididymus, seminal vesicles, prostate and target organs for all high dose and control P1 and F1 animals selected for mating. Only those organs showing pathology should be examined in animals of the other dose groups.

A teratology test may also be conducted if structural malformations are of concern and should follow procedures established for drug testing by the U.S. FDA (Collins, 1978) or U.S. EPA guidelines (1982). The study design of such a test attempts to increase the sensitivity of the study to detect such abnormalities by using a different pattern of exposure. The compound is administered orally throughout gestation or only during organogenesis (day 6-15 for rats and mice, 6-14 for hamsters, or 6-18 for rabbits). All the pregnant females are sacrificed approximately 24 hours before the expected delivery. The data to be evaluated include number of live and dead fetuses, corpora lutea, and early and late resorptions. Live fetusus are examined and weighed; the one-third of them are preserved to study the visceral organs, and two-thirds are cleared and stained for skeletal examination.

Other developmental outcomes in addition to those traditionally measured may be more sensitive indicators of toxicity in offspring. It has been

recommended that the evaluation of neurobehavioral toxicity be incorporated into standard protocols for reproductive and developmental toxicity (Collins, 1978; Nisbet and Karch, 1983). Development of the human and animal brain occurs well into the postnatal period. Toxicants may kill neurons or disrupt the neurochemical development of brain circuits disrupting normal functioning. These effects can be associated with abnormalities in the behavior of offspring including disturbances in arousal (depression or hyperactivity), learning disabilities, impaired motor function and mental retardation.

Nisbet and Karch (1983) concluded that behavioral indices of toxicity to the developing nervous system may be most sensitive screens for developmental hazard to the brain. Some drugs determined to be nonteratogenic in traditional screens produce behavioral impairments, and doses below the teratogenic dose of some compounds will elicit behavioral alterations. Therefore, behavioral screening methods have proved sensitive to a wide range of toxicants including drugs, alcohol, pesticides and heavy metals. Although interest in this field of toxicology has been evident for some time, standard testing procedures have not been established for behavioral screening assays (U.S. EPA, 1986).

The interpretation of reproductive and developmental toxicity studies is not straight forward because of the diversity of possible responses and the relationship of various responses to toxicant exposure. Several critical factors outlined by Nisbet and Karch (1983) were the (1) species selection, (2) dosage, (3) route and pattern of exposure, (4) the number of animals, (5) end points, (6) dose-response relationships, (7) statistical analysis, and (8) maternal toxicity. These are discussed widely in the scientific literature.

The following discussion summarizes the principles and/or criteria which were used by the NWF project to judge the validity of a study's results.

1. Species Selection

Nisbet and Karch stress that it is important to consider the ability of the animal to mimic the expected concentration and persistence of toxic metabolites at the target tissue in humans. The concordance of response between humans and animal species has been investigated and is discussed in the section of weight-of-evidence in this report. Various species and strains are not consistent in their sensitivity to different reproductive or developmental toxicants. Basic species differences can occur in the mother, the placenta, or in the embryo (IRLG, 1986). In general, the basic processes of gamete development and transport, fertilization, and implantation, and neuroendocrine control are similar between humans and laboratory animals (IRLG, 1986).

2. Dosage

Both the U.S. FDA and U.S. EPA guidelines recommend the use of three dose levels plus a control group. The high dose should be a multiple of human exposure or one-tenth of the LD50. The low dose should be expected to be nontoxic to the dams and their offspring.

The high dose used should produce some maternal or adult toxicity. Toxicity would be indicated by marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity. A maximum of 10% mortality in this dose group is specified. The interpretation of developmental effects observed only in the highest dose group is made complicated because developmental toxicity may be caused secondarily to maternal toxicity, and the dose-response

pattern induced by the chemical in the embryo/fetus would be difficult to discern (Manson, 1987).

The lowest dose selected should demonstrate a no observed effect level (NOEL) for adult and offspring effects. At least one intermediate dose level should be included. A concurrent control group treated with the vehicle used for administration of the test chemical should also be included.

Animals should be randomly allocated to dose groups. The evaluation of a positive control group as well as an untreated control group has been recommended by Nisbet and Karch (1983).

3. Route and Pattern of Exposure

The U.S. EPA (1988a) suggests that the route of exposure should be considered with respect to available pharmacokinetic data. The pattern and duration of exposure directly affects the reproductive outcomes observed (U.S. EPA, 1986). The critical periods for implantation, early embryogensis, and organogenesis differ, and there are species differences as well (Nisbet and Karch, 1983). Effects on sperm in males depend on the stages of spermatogenesis during which exposure occured (U.S. EPA, 1988). Exposure at different stages of male and female reproductive development can also result in different outcomes. Acute versus subchronic dosing at a specific dose rate may produce different effects due to bioaccumulation or inductive effects on enzyme systems. Therefore, the absence of an effect may reflect inappropriate timing of observations relative to the exposure pattern experienced by the animals. In general, the route of exposure should be based on the expected human exposure (U.S. EPA, 1986; Nisbet and Karch, 1983).

4. Number of Animals per Dose Group

Enough animals should be used in a study to provide statistically meaningful data. Factors which bear on the selection of appropriate number are cost, required sensitivity of the experiment, toxicologic effect to be measured, reproducibility of the effect and incidence of spontaneous occurrences of the effects (Nisbet and Karch, 1983). The determination of appropriate sample size requires knowledge of the analytic methods to be used, the magnitude of a meaningful effect, the level of Type I and Type II errors desired, and the background rate of the endpoint of interest. Continuous endpoints (e.e., birth weight) can be evaluated with more powerful statistical techniques than categorical endpoints (e.g., presence or absence of a congential malformation) (IRLG, 1986). Therefore birth weight is a more sensitive endpoint than malformation for a given sample size. IRLG (1986) emphasized that the use of sample size calculations is preferable to reliance on arbitrary sample size requirements.

The U.S. FDA and U.S. EPA guidelines specify that enough animals per dose should be used to obtain at least 20 pregnant females per dose level per generation (20 for rats, mice, and hamsters; 12 for rabbits). At least ten males per dose level are also required by the U.S. FDA, however the U.S. EPA specifies 20 males.

Nelson and Holson (1978) demonstrated that indices of background fertility mearsured in five strains of mice were quite variable: The percent pregnant out of mated animals was 5% for C3H/he mice, 6% for BALB/c mice and 14.8% for CD1 mice indicating that depending on the strain used, different sample sizes are required to obtain a predesignated number of pregnant animals. Sample size calculations resulted in quite different estimates of the number of

animals needed to observe a 5% and 10% difference in the incidence of a continuous and categorical variable between treated and control animals. These estimates are reproduced in Table 1 for illustrative purposes.

Table 1. Calculated Number of Litters (N)[a] to Detect Designated Changes in Fetal Weight and Embryolrthality in Mice and Rats

	Number Litters Needed to Show		Number Litters Needed to Show	
	5% Change in Fetal Weight	10% Change in Fetal Weight	5% Change in Embryolethality	10% Change in Embryolethality
Mice				
A/J	84	22	1176	324
C57BL/6	198	50	992	288
CD1	84	22	805	235
Rats				
CD[b]	62	16	858	248
OM[c]	44	12	723	216

[a] Number of Litters/group
[b] Charles River, Wilmington, MA
[c] Osborne-Mendel, Charles River, Wilmington, MA

Source: Nelson and Holson, 1978

The U.S. EPA noted that using 20 rodents per dose group, an increased incidence of malformations can be detected in the range of 5 to 12 times control levels (U.S. EPA, 1986). An increase in the *in utero* death rate can be detected that is 3 to 6 times the rate in controls, and a decrease in fetal weights can be observed that is 0.15 to 0.25 times control values.

5. Endpoints

A variety of functional, morphological, and biochemical reproductive parameters are vulnerable to toxic effects during the liftime of an animal (Dixon and Hall, 1982). According to Nisbet and Karch (1983), "an ideal assessment of reproductive toxicity should be conducted so that it reveals effects on each of the mechanisms leading to fetal abnormality, fetal loss, or damage of offspring in later life."

Potentially affected stages of reproduction include:

* Damage to parental gametes resulting in sterility of abnormal development of the fertilized egg or embryo
* Interference with normal uterine development and the nutrition of the conceptus
* Damage to the embryo or inhibition of embryogenesis
* Toxic effects on the fetus, fetal membrane (yolk sac and amnion), or placenta
* Inhibition of maternal metabolism causing secondary effects on the fetus
* Inhibition of uterine growth
* Adverse effects on parturition
* Adverse effects on lactation or weaning
* Latent effects on the progeny, manifested in later life (e.g., impaired development, infertility, or cancer)

Unfortunately the interpretation of some reproductive endpoints is complicated by a high degree of variability among control values and a less than clear association with altered function. Further, several parameters are measured only indirectly or are inaccurate (Schwetz et al., 1980). Schwetz et al. concluded that if adequate numbers of animals are used, good information can be obtained only for fertility, fetal and neonatal growth and survival, and post-weaning growth and maturity.

An integrated evaluation of all endpoints studied must be done to make determinations of potential maternal and developmental toxicity in humans (U.S. EPA, 1986). This is also true for evaluations of male and female reproductive toxicity. Such studies should include information on a variety of end points that reflect the full spectrum of potential reproductive responses (U.S. EPA, 1988a and b). This would include measures that are

sensitive enough to detect low-dose effects (e.g., properly performed histopathological evaluations) as well as measures of reproductive function (e.g., fertility).

6. Dose-Response Relationships

Evidence for a dose-response relationship is an important criterion in the assessment of developmental toxicity (U.S. EPA, 1986). However, the response patterns of some developmental endpoints require careful interpretation. As was discussed previously, the timing and pattern of exposure as well as the dose level can influence the incidence of the endpoints observed.

Dose-response patterns of prenatal developmental toxicity can be clearly identified only at dose levels that do not cause overt maternal toxicity (Manson, 1987). Embryo/fetal lethality, growth retardation, and dysmorphogenesis may have individual dose-response curves that may overlap and have different slopes. The action of some agents (e.g., cytotoxicity) may cause all three effects reflecting differing degrees of response to the same primary insult. Other toxicants may cause a qualitative difference in response leading to either embryolethality or to malformations alone. Therefore, in some cases, the incidence of affected implants (nonlive and malformed conceptuses combined) may reflect a better dose-response relationship than does the incidence of nonlive or malformed offspring taken individually (U.S. EPA, 1986). The endpoint observed in tests of reproductive toxicity may also vary as the exposure level increases (e.g., effects on spermatogenesis) and evaluation of dose-response should take this into account (U.S. EPA, 1988a and b).

7. Statistical Treatment of the Data

The U.S. EPA (1986, 1988a and b) has determined that since offspring response is dependent upon the environment of the litter, the litter should be used as the experimental unit for statistical analysis. The mother is the individual treated during studies of developmental toxicity. The power of a test is limited by the numbers of litters. Nisbet and Karch (1983) and IRLG (1981) discuss which entity is the appropriate experimental unit, the litter or individual fetus. Although the parents are the treated unit, all fetuses in a litter do not respond indentically, and the degree of response within a litter can provide as much or more information about toxicity than a categorical indication of response. On the other hand, fetuses in a litter are not independent. Counting every fetus may artificially inflate the sample size and overstate the sensitivity of the test. Various approaches have been proposed to account for litter effects and the impact of litter size on response parameters. In tests of male reproductive toxicity, the male is the experimental unit to be considered. The EPA guidelines on developmental toxicity specify that analytical procedures used and their results should be clearly indicated in the presentation of data along with an indication of the variance in each end point. The EPA guidelines (U.S. EPA, 1986) also specify that "any risk assessment should present the detection sensitivity for the study design used and for the end point(s) evaluated." The power of a study to detect a true effect is limited by the sample size used, the background incidence of the end point observed, the variability in the incidence of the end point, and the analysis method.

Biological plausibility is also important because the investigation of multiple endpoints may give rise to the chance occurrence of a few statistically significant findings. In contrast, the power of a test may not

be great enough to find signigicant changes in certain endpoints which are nonetheless biologically important. The internal consistency of results increases the weight-of-evidence for predicting a human hazard (IRLG, 1981).

8. Maternal Toxicity

One purpose in evaluating the developmental toxicity of a chemical is to determine whether it is a greater hazard to the conceptus than it is to the preganat female or adult male. It is important to assess the relative severity of effects caused in the conceptus and adults at a particular dose level in addition to the incidence of effects (IRLG, 1986). The adult may experience a mild, reversible effect while the lesion in the embryo or fetus may be irreversible.

It has been found that reduction in fetal body weight, increased resorptions and, rarely, fetal deaths can occur in association with maternal toxicity. Some variations and major malformations may also be produced by toxicity in the mother (Manson, 1987). According to the EPA guidelines (U.S. EPA, 1986), "current information is inadequate to assume that developmental effects at maternally toxic doses result only from the maternal toxicity; rather, when the lowest observed effect level is the same for the adult and developing organisms, it may simply indicate that both are sensitive to that dose level. Moreover, the maternal effects may be reversible while effects on the offspring may be permanent.".

Weight of Evidence Scheme for Predicting Reproductive and Developmental Toxicity in Humans Used for the NWF Sport Fish Consumption Advisory Project

A weight-of-evidence scheme used to evaluate the amount and degree of evidence for the capability of a substance to cause reproductive or developmental toxicity in humans is presented in Table 3. It is essentially the same as the weight-of-evidence classification proposed by the U.S. EPA (1988b) for male reproductive risk except that it applies to both sexes and to both developmental and reproductive toxicity.

It is important to note that this weight-of-evidence scheme does not include a ranking of chemicals by the severity of response or a weighting for potency. It also gives no relative information to judge compounds for which there is no or inadequate data to draw conclusions as to potential human toxicity.

The adequacy of studies of reproductive and developmental outcomes in humans were evaluated according to the IRLG Guidelines for Documentation of Epidemiologic Studies (1981), and the U.S. EPA Guidelines for the Health Assessment of Suspect Developmental Toxicants (1986), Proposed Guidelines for Assessing Female Reproductive Risk (1988a), and Proposed Guidelines for Assessing Male Reproductive Risk (1988b). Studies of reproductive and developmental toxicity in laboratory animals were evaluated in light of the previous discussion on study protocols and the interpretation of study results. Specific decision rules for the selection or rejection of studies are not possible because the examination of different reproductive and developmental endpoints requires various study designs. In addition, the reporting of results, primarily in older studies, is variable.

Table 3. Weight of Evidence Classification for Reproductive and Developmental Toxicity.

Known Positive Reproductive or Developmental Toxin	A convincing body of evidence exists that an agent causes an adverse reproductive or developmental effect in humans indicated by an adequate epidemiological study.
Probable Positive	A convincing body of evidence exists that an agent causes an adverse reproductive or developmental effect in nonhuman mammals with or without evidence in humans.
Probable Positive Developmental	Two adequate animal studies in two species, strains, or a replicate test reporting developmental toxicity, or one adequate animal study plus positive, but inconclusive, epidemiologic evidence.
Probable Positive Reproductive I	At least one adequate animal study reporting reproductive toxicity, plus at least one epidemiologic study finding a statistical association between reproductive deficits and exposure to an agent although bias, confounders, and other risk factors have not been completely explained.
Probable Positive Reproductive II	At least one adequate animal study reporting reproductive toxicity
Possible Positive	Evidence from human or other mammalian studies shows statistically significant adverse effects, but the quality of the studies is questionable.
Known Negative	A convincing body of evidence exists that an agent does not cause an adverse effect in humans. Indicated by more than two studies measuring a wide array of endpoints.
Probable Negative	A convincing body of evidence exists that an agent does not cause an adverse effect in nonhuman mammals. Indicated by more than two studies measuring a wide array of endpoints.
Possible Negative	Studies with acceptable quality produce no adverse effects, but important aspects of the male reproductive system have not been evaluated.

No Data or Inadequate Data	No data are available, or Negative data are available from studies for which the confidence in quality is questionable, or Results are available for which the predictive value of the test system or endpoint has not been established, or Studies with acceptable quality produce inconsistent and conflicting results such that the possibility of adverse effect cannot be discounted, or Other data exist from which biologically meaningful adverse effects are plausibly indicated.

Factors Affecting the Weight of Evidence Classification

1. Relevance of Effects in Animals to Humans

Although the data are limited, there is a scientific basis for extrapolating from animals to humans on the basis of reproductive and developmental toxicity (U.S. EPA, 1986). Comparisons of human and animal data show that for a limited number of agents that are positive in humans there was almost always concordance of effects between humans and at least one non-human species tested (Schardein et al., 1983; Nisbet and Karch, 1983). This section presents evidence that supports the use of animal data in a weight-of-evidence system to predict effects in humans. The following discussion also illustrates the difficulty in concluding that data from only one adequate study in animals is sufficient to predict adverse effects in humans.

An evaluation of the concordance of results in animal models and humans led Schardein et al., (1985) to the conclusion that rats and mice provided the highest level of predictability for known human teratogens. These two species predicted a positive response for 70% of compounds known to be teratogens in

humans. Rabbits had a 75% predictability of human effects for teratogenic and other developmental and reproductive responses combined. Background malformation rates are more consistent and are significantly lower in rats and rabbits than in mice (Schardein et al., 1985). Mice exhibit considerable intraspecies variability in response.

Studies using rats and mice did not predict the teratogenic potential of thalidomide, a drug which caused tragic consequences in the infants of women who used it. However, rabbits exhibited a teratogenic response to thalidomide, and nine nonhuman primate species developed limb defects in offspring upon administration of the drug. Therefore, no one species can be expected to be predictive for humans for every compound tested.

A 1980 U.S. FDA literature review identified 38 compounds for which birth defects are reported in humans associated with their intake during pregnancy (Schardein, 1983). One hundred sixty-five compounds for which human teratologic effects were not reported were also identified. The responses of various animal species to exposure to these chemicals were compared and the analysis is presented in Table 4.

At least one test animal species responded positively to 37 of the 38 "human teratogens". The exception, an otological defect in humans, would not normally be found in test animals at the time of caesarean section. Eighty percent of the known or suspected human teratogens tested positive in multiple nonhuman species. Eighty-five percent and eighty percent of the compounds tested positive in mice and rats, respectively. The rabbit tested positive for 60% of the human teratogens, while the hamster and monkeys showed a teratogenic response to 45% and 30% of the compounds, respectively.

Therefore, rats and mice appear to predict teratogenicity in humans more frequently than the rabbit, hamster, and monkey as demonstrated by this set of substances. Further, the data suggest that a substance with unknown human teratogenic potential would be expected to cause a teratogenic response in at least one animal species if the material was a human teratogen.

Table 4. Comparison of teratogenicity in humans and animals.

38 Known or Suspected Human Teratogens		165 Chemicals With No Evidence of Human Teratogenicity	
Species	Percent of Chemicals Testing Positive in Each Species (Sensitivity)	Species	Percent of Chemicals Testing Negative in Each Species (Specificity)
Mouse	85%	Mouse	35%
Rat	80%	Rat	50%
Rabbit	60%	Rabbit	70%
Hamster	45%	Hamster	35%
Monkey	30%	Monkey	80%
Two or more species	80%	Two or more species	50%
Any one species	97%	All species	28%

Source: Schardein, 1983.

The frequency with which mice and rats "correctly" predict a negative response in humans appears to be lower than the frequency with these species "correctly" predict a positive response in humans. Table 4 indicates that of the 165 tested chemicals considered to be nonteratogenic in humans, 35% and 50% were not teratogenic in the mouse and rat, respectively. Tests of the monkey and rabbit resulted in a negative response for 80% and 70% of these substances. It should be noted that the monkey's low response may be due to the small sample size used in toxicity tests and therefore may not be "true"

negative response (Brown and Fabro, 1983). Fifty percent of the compounds tested negative in two or more species but only 28% of the compounds "correctly" tested negative in all species tested.

The problems that arise in attempts to compare human and animal data bases for concordance in response have been discussed in the literature and should be considered when interpreting the conclusions of the U.S. FDA literature review. The methods used to collect the data lead to differences in the completeness with which specific outcomes are detected in humans and laboratory animals (Brown and Fabro, 1983). Animal data are obtained in controlled experiments where pregnant females are given relatively high doses throughout organogenesis and the fetuses are then thoroughly examined. Human data are obtained through case studies and epidemiologic investigations where people have generally been exposed to low doses for an unknown duration of time at an unknown frequency. In addition, early spontaneous abortions often aren't detected or reported in humans which causes evaluations of specificity (the concordance of negativity) to be particularly problematic. It is possible that some chemicals with no evidence of teratogenicity in humans are responsible for adverse effects that could not be detected in the epidemiologic study. Manson (1987) suggested three possible explanations for the low specificity observed; no studies or inadequate studies have been conducted in humans with the animal teratogen; the animal studies have yielded false-positive results because of test conditions and interpretation; or the true risk for adverse pregnancy outcome is underestimated in human studies that only measure outcomes from the time of birth onward.

Positive animal teratology studies can be interpreted to be suggestive of the potential human response (Schardein et al., 1985). The IRLG (1986) concluded

that testing for developmental toxicity in animal models will identify most chemicals that are potentially hazardous to human development. Manson (1987) stressed that any manifestation of exposure-related developmental toxicity in animal studies can be indicative of a variety of responses in humans. Conversely, lack of an effect in a particular organ in animals does not predict the absence of an effect in that organ in humans (Schardein et al., 1985).

The conclusion that reproductive effects observed in animals are relevant to exposed humans is based on assumptions regarding the correlation of reproductive processes. Nisbet and Karch (1983) found that for ten chemicals, similar types of adverse effects on reproductive capability were caused in both humans and animals. According to the IRLG Workshop (1986), "the basic processes of gamete development and transport, fertilization, and implantation are similar, as is overall neuroendocrine control." The IRLG Workshop concluded, "in the absence of specific data to the contrary, adverse effects in experimental animals should be presumed to indicate a potential risk to human reproduction.

2. Number of Adequate Animal Studies

Both the "known positive" and "probable positive" weight-of-evidence categories rely on the determination that a convincing body of evidence exists to indicate a hazard to humans. The number of adequate animal studies that should be required to conclude that the evidence is sufficient is particularly problematic. The U.S. EPA provides some guidance on the amount of data necessary to conclude that "a convincing body of evidence" exists for male reproductive risk. The U.S. EPA (1988b) states,

> For example, positive results from a *single* study based on a *single* end point may be sufficient to place an agent into a known positive or probable positive category. However, negative results from an adequate array of end points, and from more than one study, may be necessary to place an agent into one of the equivalent negative categories.

The agency also determines that,

> While a single study of high quality could be sufficient to achieve a relatively high level of confidence, replication increases the confidence that may be placed in such results.

While the U.S. EPA would allow a minimum of one adequately conducted animal study to place a chemical in the probable positive classification for male reproductive risk, it is acknowledged that replication increases the confidence in the prediction of adverse effects in humans. Other classification schemes have placed greater weight on evidence of toxicity demonstrated in two or more species, strains, or a replicated study.

A system developed by Brown et al. (1986) involves five weight-of-evidence categories; confirmed evidence, substantial evidence, suggestive evidence, insufficient evidence, and no data. At least two positive animal tests are required to place a substance into the substantial evidence category for developmental toxicity. At least one positive animal test and some positive (although not conclusive) human evidence is required to place a compound into this category for reproductive toxicity. The substantial and suggestive categories are subdivided into groups based on the severity of the effect induced. Other weight-of-evidence schemes also give greater weight to two or more positive animal studies (Toxic Substances Control Act Interagency Testing Committee Scoring System and the Michigan Critical Materials Register).

The degree of sensitivity across species to reproductive and developmental toxicants is not consistent, and no one animal species is considered to be most appropriate for predicting effects in humans (Schardein et al., 1985). Interspecies variability may be attributed to differences in absorption, metabolism, excretion, diet, size, developmental patterns, intercurrent disease processes, placental transfer, etc. (Schardein et al., 1985).

The confidence in animal to human extrapolations increases as the number of species tested increases. The U.S. EPA, under the auspices of the TSCA and FIFRA, recommend that two species be tested for developmental and reproductive toxicity. Under TSCA, the rat, mouse, rabbit, or hamster are acceptable for developmental toxicity, while the rat is the preferred species for reproductive toxicity. Under FIFRA, the rat and rabbit are preferred for developmental toxicity, and the rat or mouse is acceptable for reproductive toxicity. The U.S. FDA recommends that two of the following species be used to test for teratogenicity; mouse, rat, rabbit, or others.

3. Sensitivity of End Points

Developmental toxicity end points observed in experimental animals do not and should not be expected to mimic those measured in humans exposed to the same toxicant (IRLG, 1986). According to U.S. EPA guidelines (1988b), "any statistically significant deviation from baseline levels for an *"in vivo"* effect warrants closer examination. To determine whether such a deviation constitutes an *"adverse"* effect requires an understanding of its role within a complex system and the determination of whether a *"true effect"* has been observed."

The U.S. EPA guidelines propose that,

> The greatest weight for male reproductive hazard identification should be placed on effects on sexual behavior, fertility and/or pregnancy outcomes, or other end points that are directly related to reproductive function such as sperm measures, reproductive histopathology, reproductive organ weight(s), and reproductive endocrinology. Agents producing effects on those end points can be assigned to known positive (human data) or probable positive (test species data) categories if study quality was adequate.

The agency also states that,

> Less confidence should be placed in results from other measures such as "*in vitro*" tests, data from nonmammalian species or structure-activity relationship evaluation, but positive results may trigger follow-up studies to determine the likelihood and extent to which function might be affected. Positive results from these types of studies would be assigned to the possible positive category, while negative results would be assigned to the inadequate data category.

In the female, more subtle effects on the reproductive system may be induced at dose levels lower than those that result in infertility or produce other overt effects (U.S. EPA, 1988a). These more sensitive endpoints include alterations in structural or functional competence of the ovaries, hypothalamus, and pituitary, or alterations in feedback mechanisms occurring during critical periods of reproductive development as well as during other periods of reproductive life. Alterations in the onset of puberty, alterations in the reproductive cycle, oocyte toxicity, and premature reproductive senescence are endpoints suggesting toxicity to the female reproductive system. Weight and histologic changes in ovaries, uterus, and pituitary gland are other potentially more sensitive endpoints, however they must be carefully evaluated (U.S. EPA, 1988a).

4. Negative Studies

The Brown et al. (1986) methodology for assessing reproductive and developmental toxicity specifies that nonpositive results are not utilized to counterbalance positive results. The authors reason that non-positive data reflect specific testing and exposure conditions and cannot be used to provide assurance of safety, or to prove non-effect. Therefore, the system focuses on evaluating observed effects rather than establishing non-effects. Counterbalancing negative effects with positive effects can be avoided by the requirement that data indicating probable or possible positive effects must not exist for a chemical to be placed in any of the negative categories as was done in the system described in this technical document.

Criteria for the Weight of Evidence Classification System Used for the NWF Sport Fish Consumption Advisory Project

Because laboratory animals demonstrate species and strain variability in response to reproductive and developmental toxicants, and no species is more predictive of the human response than any other in every instance, one positive response in a well conducted animal study was sufficient to establish a probable hazard of reproductive toxicity in humans. However, a positive response in two or more species, strains, or replicated experiments was given a greater weight-of-evidence. In the absence of human data, at least two well conducted animal studies were required to establish a probable developmental hazard to humans. Two positive studies decrease the probability that a false-positive result has occurred. The breadth of the available toxicity information for a substance were thoroughly investigated and clearly presented.

WEIGHT OF EVIDENCE FOR CARCINOGENICITY

The following section discusses the evaluation criteria used for the NWF Sport Fish Consumption Advisory Project to determine the adequacy of published studies to predict a potential cancer hazard in humans. Next, the weight of evidence scheme used to identify a substance as a human carcinogen is described.

The hazard assessments supporting the dose-response and risk characterizations for cancer for the compounds monitored in Lake Michigan sport fish rely on the weight-of-evidence scheme developed by the U.S. EPA and the recommended criteria for evaluating epidemiologic studies and animal bioassay data presented in the OSTP review.

The Evaluation of Evidence Concerning Carcinogenicity

Studies of Carcinogenicity in Humans

Epidemiological studies have established an association between cancer and exposure to tobacco products and alcohol, ultraviolet and ionizing radiation, certain occupational and medicinal chemicals, dietary factors, and some infectious agents (OSTP, 1985). Studies on human populations provide the most relevant evidence for the protection of public health from toxic exposures. However, epidemiologic studies have several limitations which must be accounted for by investigators in order to establish a causal relationship between an exposure to a substance and cancer. If exposure is low or rare, or if the excess risk is small compared to the baseline incidence rate, a large number of human subjects are required to detect a statistical relationship. The long

latency period for the development of cancer complicates the detection of a relationship and subjects must be followed for an adequate period of time. Often exposure cannot be measured directly and subjects may be classified by exposure incorrectly in either a random or biased manner. The implication of a specific material when the causes of a cancer are multifactorial is difficult and all factors which are associated with both the disease and the exposure variable must be controlled for by study design or in the data analysis. Unfortunately, it may not be possible to adjust for all confounding factors, particularly if some risk factors are unknown.

The OSTP report makes the following statement regarding determining causality from epidemiologic studies.

> In interpreting epidemiological findings, one is guided by the magnitude of the risk estimates, their statistical significance (likelihood of being due to chance), and the rigor of the study design to avoid various kinds of bias, including those related to selection, confounding, classification, and measurement. A determination of causality in epidemiology is bolstered by dose-response relationships, the consistency and reproducibility of results, the strength and specificity of the association, its biological plausibility, and other considerations.

There are three types of human studies which provide evidence of cancer, case reports, descriptive studies, and analytical studies (OSTP, 1985). Case reports of individual cancer patients who were exposed to the agent can alert health professionals to a possible connection. Descriptive epidemiologic studies identify the distribution or patterns of disease in populations. This type of study can stimulate etiological hypotheses for investigation by analytical studies, or can support the findings of analytical studies, but fall

short of establishing causal relationships by themselves. Analytical studies, which include case-control and cohort studies, obtain data on disease occurrence and potential risk factors for specific individuals. Cohort studies can directly estimate the risk of disease associated with a given exposure and provide the best evidence for a causal relationship, while case-control studies do so indirectly.

Some methodological guidelines were offered by the Office of Science and Technology Policy for study designs.

> The study groups should be sufficiently large, and the time intervals between initial exposure and tumor onset sufficiently long, to identify the lowest excess risk considered important to detect. Reliable and valid estimates of exposure should be sought, with quantitiative measurements to permit dose-response evaluations. Studies should be designed in a manner that minimizes potential sources of bias, and permits detection and control of confouding variables.

Analytical studies using large sample size and controlling for effect modifiers and confounding factors that do not detect a hazard can be useful. However, studies that follow individuals for much less than 30 years after the initial exposure cannot provide evidence for a lack of carcinogenicity (IARC, 1987). The absence of an association cannot be estabalished by a negative study, however, likely upper and lower bounds on the estimates of risk can be made relative to the specific conditions of that study. The OSTP recommended that the statistical power of negative studies should be routinely considered when such studies are reviewed.

The NWF sport Fish Consumption Advisory Project used the criteria discussed by the OSTP (1985) to evaluate the adequacy of the epidemiologic evidence on

cancer in human populations exposed to the chemicals monitored in Lake Michigan sport fish. The NWF Project also evaluated the animal studies pertaining to these chemicals according to the criteria recommended by the OSTP (1985). The criteria for animal studies are discussed in the next section.

Studies of Carcinogenicity in Animals

IARC notes that of the 44 agents for which IARC has determined there is sufficient or limited evidence of carcinogenicity to humans, the 37 substances that have been tested adequately in animals produce cancer in at least one species (IARC, 1987). Therefore, IARC has concluded that

> Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, nevertheless in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans.

The following summary delineates the OSTP recommendations for proper study design in chronic animal studies which identify mammalian carcinogens (OSTP, 1985). Several issues relative to design, conduct and reporting should be considered in the interpretation of cancer bioassays. Specifically, there are important considerations with respect to (1) animal species and strain, (2) animal care and diet, (3) test and control groups, (4) dose levels, and frequency and route of exposure, (5) study duration, and (6) pathological techniques.

1. Species and Strain of Animal

The ideal species and strain predicts human response accurately. Unfortunately, a prior knowledge of the ideal animal for testing a particular substance is generally not available. The species should be practical for use under experimental conditions yet similar to humans in metabolism and pathologic responses. The OSTP (1985) reported that the mouse, rat, and hamster are felt by many researchers to meet these conditions. Although inbred strains do not have the genetic variability characteristic of human populations, they are recommended because a large amount of data has been collected on organ specific cancer rates in certain inbred strains. The use of two species reduces the possibility of a false negative result. Therefore consistency in response is emphasized in the U.S. EPA weight-of-evidence classification for human carcinogens.

2. Animal Care and Diet

The outcome of a study can be influenced by housing conditions, intercurrent disease, drug therapy, impurities in diet, air, water, and bedding, and animal care facilities. Therefore, stringent control of evironmental conditions and proper animal care techniques are mandatory. The selection and allocation of animals between control and treatment groups should be random and there should be no bias in the treatment of dose groups with regard to diet, husbandry, necropsy and pathology. The basal diet should be analyzed periodically for nutrients and unintentional contaminants.

3. Test and Control Groups

A sufficient number of animals should be used so that enough animals are available at the end of the study for statistical analysis. At least fifty

animals of each sex are recommended for each dose group. At least one concurrent control group, identical in every respect to the exposed groups except for exposure to the test substance, should be used.

4. Dose Levels, Frequency and Route of Exposure

At least three dose levels in addition to the concurrent control group are recommended for cancer detection and risk assessment. The highest dose should cause signs of minimal toxicity without significantly altering the animal's normal life span due to effects other than carcinogenicity. This is called the maximally tolerated dose (MTD) and is used to provide the best probability of detecting a neoplastic response. The MTD has been defined as the highest dose that causes no more than a 10% decrease in weight gain in a subchronic study. The lower dose levels provide information on the shape of the dose-response curve.

The use of the MTD introduces complexity into the interpretation of bioassays. The MTD increases the sensitivity of the test to detect a carcinogenic response. However, high doses may produce altered physiologic conditions which can qualitatively affect the induction of malignant tumors. Normal physiology, homeostasis and detoxification or repair mechanisms may be overwhelmed and cancer induced or promoted when otherwise it would not have been. In contrast, cancer incidence could be decreased if metabolic activation is overwhelmed. Therefore, it is very important that dose be selected on the basis of pharmacokinetics and metabolic data as well as the result of subchronic toxicity studies.

Route of exposure should be similar to the anticipated human exposure, although if the substance is absorbed, distributed and metabolized, the response is considered to be meaningful. Knowledge of metabolism and pharmacokinetics is important for interpreting potential differences.

5. Duration of Study

Animals should be exposed for the majority of their normal life spans. Treatment should begin as soon as possible after weaning and continue for 18 months for mice and hamsters and 24 months for rats. Longer time is advisable if cumulative mortality at the time of sacrifice is less than 10%.

6. Pathology

According to the OSTP,

> Most experts agree that the incidence of total tumors at all organ sites is not a very useful expression of cancer incidence, nor is the calculation of the incidence of total benign or total malignant tumors. Most useful appears to be the number of histologically unique tumors at specific organ sites.

The OSTP (1985) stated that many pathologists believe that benign tumors should be combined with malignant tumors that occur in the same tissue at the same organ site because they represent a stage in the progression to malignancy.

Weight of Evidence Scheme for Predicting Carcinogenic Potential in Humans Used for the NWF Sport Fish Consumption Advisory Project

The U.S. EPA classification is adapted from a system developed by the International Agency for Research on Cancer and was described in the 1986

Guidlines for Carcinogen Risk Assessment (U.S. EPA, 1986). The NWF Sport Fish Consumption Advisory Project used the U.S. EPA weight-of-evidence classifications for summarizing the level of certainty regarding the carcinogenicity of a chemical in humans.

The U.S. EPA weight-of-evidence system was used as follows. Evidence from human and animal studies are analyzed separately and a determination is made as to whether the data are sufficient, limited, or inadequate, or if there are no data or no evidence of carcinogenicity. These categories are defined as follows.

Human Evidence

Sufficient: Data which indicates that there is a causal relationship between the agent and human cancer.

Limited: Data which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded.

Inadequate: Data which indicates that one of two conditions prevail: (a) there are few pertinent data, or (b) the available studies, while showing evidence of association, did not exclude chance, bias, or confounding and therefore a causal interpretation is not credible.

No Data: Data are not available.

No Evidence: Data which indicates that no association was found between exposure and an increased risk of cancer in well-designed and well-conducted independent analytical epidemiologic studies.

Animal Evidence

Sufficient: Data which indicates that there was an increased incidence of malignant tumors or combined malignant and benign tumors: (a) in multiple species or strains; or (b) in multiple experiments; or (c) to an unusual degree in a single experiment with regard to high incidence, unusual site or type of tumor, or early age at onset.

Limited: Data which suggest a carcinogenic effect but the evidence is limited because: (a) the studies involve a single species, strain, or experiment and do not meet criteria for sufficient evidence; (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting; or (c) an increase in the incidence of benign tumor only.

Inadequate: Data which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

No Data: Data are not available.

No Evidence: Data which indicates that there is no increased incidence of neoplasms in at least two well-designed and well-conducted animal studies in different species.

The human and animal data are then combined into a classification of the overall weight-of-evidence for human carcinogenicity in the following manner.

Group A-Human Carcinogen
There is "sufficient" evidence from epidemiologic studies to support a causal association between exposure to the agents and cancer.

Group B-Probable Human Carcinogen
"Limited" human epidemiologic evidence and/or "sufficient" evidence from animal studies is available. There are two subgroups in this category which divide evidence according to the degree of certainty.

> *Group B1:* "Limited" epidemiologic evidence and "sufficient" evidence from animal studies.
> *Group B2:* "Inadequate" or "no data" from epidemiologic studies but "sufficient" evidence from animal studies.

Group C-Possible Human Carcinogen
"Limited" evidence from animal studies and "no data" from epidemiologic studies.

Group D-Not Classifiable as to Human Carcinogenicity
"Inadequate" human and animal evidence or "no data" are available.

Group E-Evidence of Non-Carcinogenicity for Humans
"No evidence" in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

Additional supporting evidence may cause the overall weight-of-evidence designation to be modified. This may include structure-activity relationships, short-term test findings, results of appropriate physiological, biochemical, and toxicological observations, and comparative metabolism and pharmacokinetic studies.

The criteria for categorizing human and animal studies involve judgments regarding the quality of the studies, the type of response and the strength of the association, the number of well conducted studies for which data is available, and the consistency of results if data on more than one study is available. The evaluation of study results and data quality were discussed in depth by the Office of Science and Technology Policy (1985). The guidelines for chemical carcinogen risk assessment developed by the U.S. EPA (1986) and the California Department of Health Services (1985) are consistent with the principles elaborated in the OSTP report.

DOSE RESPONSE ASSESSMENT

DOSE-RESPONSE ASSESSMENT FOR CHRONIC TOXICITY

The NWF Sport Fish Consumption Advisory Project assessed the potency of the chemicals selected for review by the calculation of a reference dose (RfD). A reference dose estimates the dose of a substance that would be expected to produce no systemic toxicity or reproductive/developmental toxicity in sensitive human populations. A chemical specific reference dose was developed according to a procedure recommended by the U.S. EPA (1988) which is described in this section. The selection of adequate data for the calculation of a reference dose is also discussed. It is assumed that the mechanism of action of noncarcinogenic toxic responses involves a threshold for dose below which the toxic response is not induced in an individual. However, some theoretical exceptions have been proposed. The time of fertilization may be one such exception where the risk of cell death to the one-cell embryo might be nonthreshold in nature (Brent, 1987). A discussion of proposals to

incorporate the experimentally observed dose-response relationship into the calculation of an acceptable human dose is also included in this section.

Selection Of Data

Adequate studies on reproductive and developmental toxicity were selected according to the principles and criteria discussed in the weight-of-evidence section. Studies of chronic systemic toxicity were evaluated using the U.S. EPA Pesticide Assessment Guidelines (1982).

The U.S. EPA (1988) provides some guidance on the selection of critical studies and end points to use in the derivation of a reference dose for a chemical. When information on the level of the exposure associated with a particular end point in humans is available, then epidemiologic studies can be used as the basis of a quantitative dose-response assessment. When this information is not available, then the animal model that is most biologically relevant to humans should be selected. If there is no animal model that is more relevant than the others, then the most sensitive species should be used. The most sensitive species is defined as the species showing a toxic effect at the lowest administered dose. There is some evidence which suggests, at least for teratogens, that humans may be more sensitive than laboratory animals to toxic exposures (Springer, 1987; Nisbet and Karch, 1983). The critical end point to be used in the dose-response assessment is the response that occurs at the highest no-observed-adverse-effect-level (NOAEL) for a particular species, or a lowest-observed-adverse-effect-level (LOAEL) when a NOAEL has not been determined. We have adopted these data selection criteria for the calculation of chemical specific reference doses for the NWF Project.

Calculation of a Reference Dose

The guidelines in Table 5 are recommended by the U.S. EPA (1988) for determining what uncertainty factors and modifying factor should be used to calculate the reference dose. The guidelines were adapted from Dourson and Stara (1983) who presented the scientific evidence supporting the specific values to be used. Zielhuis and van der Kreek (1979) also discuss rationale behind traditional safety factors. These guidelines were adopted by the NWF Sport Fish Consumption Advisory Project.

A reference dose (RfD) is derived from the no-observed-adverse-effect-level (NOAEL) by applying uncertainty factors (UF) which reflect the quality and type of data used to predict the human toxic potential. Uncertainty factors are used to account for the variation in sensitivity within the human population, the differences between humans and animal species, and limitations in the quality of the data used. A modifying factor (MF) may also be used which reflects a professional judgement of the remaining uncertainties with respect to the data base pertaining to the chemical. The NOAEL is defined as the highest experimental dose of a chemical at which there is no statistically or biologically significant increase in frequency or severity of an adverse effect in individuals in an exposed group when compared with individuals in an appropriate control group. The reference dose is developed by dividing the NOAEL by the UFs and MF and is expressed in milligrams per kilogram body weight per day (mg/kg/day). When a NOAEL has not been determined, an additional uncertainty factor is applied to a LOAEL selected from an appropriate study.

The product of the uncertainty factor(s) and modifying factor is the margin of exposure (MOE). The MOE is increased as the level of certainty that the NOAEL is correct decreases. The U.S. EPA considers the reference dose to be "an estimate (with uncertainty spanning perhaps 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, 1988)."

Table 5. Guidelines for the Use of Uncertainty Factors in Deriving Reference Doses and Modifying Factors (U.S. EPA, 1988).

Standard Uncertainty Factors (UFs)

Use a 10-fold factor when extrapolating from valid experimental results in studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population and is referenced as "10H".

Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty involved in extrapolating from animal data to humans and is referenced as "10A".

Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty involved in extrapolating from less than chronic NOAELs to chronic NOAELs and is referenced as "10S".

Use an additional 10-fold factor when deriving an RfD from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty involved in extrapolating from LOAELs to NOAELs and is referenced as "10L".

Modifying Factor (MF)

Use professional judgment to determine the MF, which is an additional uncertainty factor that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above; e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is one.

The U.S. EPA (1988) recommends that when exposure to a chemical occurs through more than one source, the reference dose should be multiplied by a factor which represents the fraction contributed by the source of concern. The NWF Project developed a procedure to account for multiple exposure sources. A reference dose for sport fish consumption, exclusive of other routes of exposure, was

calculated for each compound. The derivation of these reference doses is described in each chemical-specific dose-response section.

Discussion

The reference dose (safety factor approach) has been criticized as an unsophisticated method for evaluating potential human risk (Gaylor, 1983; Kaplan et al., 1987). The NOAEL is a statistical phenomenon that depends heavily on the sample sizes used in an experimental assay. Studies that use a smaller number of animals per dose level may result in a higher NOAEL; therefore, an understanding of the confidence limits surrounding estimates of no effect is important (Gaylor, 1983). Gaylor (1983) has proposed the use of mathematical dose-response models that provide a good fit of the experimental data to derive an upper confidence limit for the effective dose that results in an increased response of 1% in the test animals (ED_{01}). A safety factor is then applied that will reduce the risk of disease in the animal population to a desired level. The premise of this approach is that the size of a safety factor should reflect the dose-response relationship. The author stresses that to arrive at an "acceptable" dose for human exposure, additional adjustment for interspecies differences, the nature and extent of human exposure, and possible risk/benefit considerations must be evaluated.

Kaplan et al. (1987) used a simple model to evaluate the potential proportion of the human population demonstrating a response threshold below a calculated acceptable daily intake (ADI). It was found that the size of the ratio, $NOEL/u_A$ (the no effect level divided by the true (unknown) animal threshold) will have a large effect on the proportion of the human population with

thresholds below the ADI. In addition, the slope of the animal dose-response curve is important in affecting the probability of obtaining a large NOEL/u_A ratio. As the dose-response slope becomes less steep, the probability of obtaining a large ratio becomes increased. The authors concluded that if the slope of the experimental dose-response curve is very flat or if there is evidence that the animal threshold is well below the lowest experimental dose considered, a safety factor of 100 may not be adequate.

Efforts are currently underway at the U.S. EPA to develop extrapolation models to derive the reference dose for noncarcinogenic effects (U.S. EPA, 1988).

DOSE RESPONSE ASSESSMENT FOR CARCINOGENICITY

The dose-response assessments for each chemical were conducted in accordance with the U.S. EPA Guidelines for Carcinogen Risk Assessment (1986). The National Research Council defines dose-response assessment as the description of the relationship between the dose of an agent and the probability of induction of a carcinogenic effect (U.S. EPA, 1986). A dose-response assessment involves three distinct steps, (1) selection of the appropriate data, (2) extrapolation from the response observed at high doses to the expected exposure in the human population at risk, and (3) adjusting the dose to reflect species differences if the selected data are from a laboratory experiment. Each of these steps will be detailed in this section.

The prediction of cancer incidence by a specific chemical exposure has as its goal the prevention of cancer. The latency of cancer and its irreversibility require that a protective stance be taken, and this involves making decisions

in the face of large unknowns. A risk assessor must make science policy decisions and rely on various assumptions to bridge the uncertainties inherent in all of the steps of a dose-response assessment. The procedures and assumptions used for the NWF Project to accomodate the uncertainties in the risk assessment are found in Table 6 at the end of this section.

The selection of data from epidemiological or laboratory animal experiments for use in a dose reponse assessment implies that it will appropriately predict the experience of the human population at risk. Epidemiological studies must be properly conducted, show a statistically significant relationship between exposure to the chemical of interest and a carcinogenic response, quantify levels of exposure, and explore the impact of potential confounders and effect modifiers. Animal studies must also be designed to establish that the observed response is due to exposure to the chemical of interest and must have statistically significant results. Variability in sensitivity can occur between species, strains, sex, and age. Data selection is based on the entire weight of evidence that indicates that the substance is probably carcinogenic to humans. A study's statistical variability, and the degree to which its design deviates from the relevant human experience, as well as the accuracy and precision of exposure estimates all insert uncertainties into the final estimate of carcinogenic risk to the exposed human population.

Epidemiologic and animal studies, because of economic and physical constraints, are able to detect an increase in cancer incidence due to a chemical exposure of 10% or greater (OSTP, 1985). Therefore risk assessors must extrapolate from the higher doses which cause this degree of cancer incidence to determine the

dose region that will result in a negligible risk. The shape of the dose-response curve in the low dose region is not known. A mathematical model must be selected that describes the dose-response curve at low doses based on assumptions of the mechanism of cancer and on a desire to determine the entire range of cancer risks that might be expected in the human population at risk.

Finally, the unit of dose can affect the size of the estimate of risk. Unfortunately, it is not known what unit of dose is equivalent across species, and this will insert a large degree of uncertainty into the accuracy of the risk estimate. The physiological and biochemical similarities and differences across species have been widely discussed but the quantitative relationships have not been elucidated well. Therefore, a difference of opinion exists among scientists regarding what interspecies scaling factor is appropriate when extrapolating to the human experience.

Some uncertainties can be quantified but others can only be treated in a qualitative manner. For example, chemical interactions involving synergism, potentiation, or antagonism potentially have a great impact on the actual risk to human beings who are exposed to many substances at once and over their lifetimes, but data are usually lacking on chemical mixtures. The final risk estimates must be presented in the context of what is known about the impact of the uncertainties inherent in the risk assessment process.

Selection of the Cancer Incidence Data

Selection of cancer incidence data is the first step in a dose-response assessment. The data used to conduct a dose-response assessment should come

from a properly conducted study using a species similar to humans in biochemical and pathologic response, and a route of exposure that is relevant to the expected human exposure. Therefore the U.S. EPA and other governental guidelines advise that data from well conducted epidemiologic studies should be chosen, if available, followed by data from an animal species that responds to the subtance in a manner similar to humans (U.S. EPA, 1986; U.S. OSTP, 1985; CDHS, 1985). More often than not, epidemiologic studies with well quantified exposure estimates are not available for a particular substance. The dose-response assessments conducted by the NWF project followed this general policy.

The following science policy-decisions, recommended by the U.S. EPA, were used in the extrapolation process in situations where more than one relevant data set of good quality in animals was available. These issues are also carefully discussed in guidelines developed by the California Department of Health Services (CDHS, 1985).

1. The data should be presented for each tumor site with statistically increased incidence. When a species has developed a statistically increased incidence of tumors of more than one type or at multiple sites, an estimate of total cancer incidence is preferred. The estimate of total risk should be based on a count of the total number of animals with one or more tumor type or site. Total cancer risk is important because animals and humans do not always develop tumors at the same site (Cothern and Marcus, 1984).

2. Benign tumors of the same histogenic origin should be combined with malignant tumors if they would be expected to progress to malignancy. The

contributions of benign tumors to the total risk estimate should be indicated. In addition to the potential to progress to malignancy, the CDHS reasons that the induction of benign tumors in the experimental animals reflects the biological activity of the carcinogen. Other species may manifest this activity in the form of malignant tumors (CDHS, 1985).

3. Greater emphasis should be placed on results in the most sensitive species, strain and sex. However, the range of risk estimates from all relevant studies should also be presented. The CDHS (1985) emphasizes that differences in sensitivity should be evaluated in light of whether they reflect true biological differences, or limitations in the studies. The decision to use the most sensitive species, strain and sex is, in part, an effort to account for the wide variability in human sensitivity. Risk estimates based on other relevant data sets may also allow the consideration of studies that may be of better quality.

4. Ideally, the incidence data should reflect the same route and pattern of exposure as the human enviromental exposure. If they do not, adjustments should be made to account for the difference in delivered dose expected and they should be clearly documented.

The Mathematical Dose-Response Models

The second step in a dose-response assessment is the extrapolation of cancer incidence data using mathematical models from the experimental dose range to the low doses commonly encountered in the human population. Several dose-response models have been used or proposed by different investigators to derive risk estimates in the region of the dose-response curve where experimental data are nonexistent. The models include tolerance distribution models, mechanistic models, and physiologically-based pharmacokinetic models.

Since the mechanisms of cancer are not precisely known, choice of the appropiate model should depend on what is known about the activity of the substance under study including pharmacokinetics and target organ dose (OSTP, 1985). When information of this nature is limited, the OSTP (1985) recommends that models incorporating low dose linearity be used to estimate low dose cancer risks when the available data are compatible with this procedure. The linearized multistage model, generally recommended by the U.S. EPA (1986), was used to calculate potency factors for each chemical-specific dose-response assessment.

This section discusses the mathematical extrapolation models, some characteristics of carcinogenesis that suggest possible mechanisms for its induction, and two assumptions that are used for the NWF Project; a no dose threshold for response and low dose linearity. Finally, the extrapolation model selected for the NWF Project and the type of data that will be used with the model are discussed.

A. A Description of Some Mathematical Models

The following discussion of mathematical models is based on reviews by the Food Safety Council (1980), Kreski and Van Ryzin (1981), the California Department of Health Services (1985), and Downs (1985).

1. Tolerance distribution models

Tolerance distribution models are based on the assumption that each individual in a population has a threshold dose level. The tolerance distribution

describes the distribution of individual threshold values in a population such that the probability of a response is a function of the dose of a toxicant; P = f(D). These models vary according to the function that relates the probability of response to the dose level. Three commonly discussed tolerance distribution functions are the probit, logit, and extreme value (Weibull) models.

The probit model assumes that the tolerances have a lognormal distribution. The proportion of individuals responding to dose D is P(D), and is described as;

$$P(D) = \phi(a+B\log D)$$

where ø is the standard normal distribution, and a and B are the intercept and slope at dose, D. A typical plot of this dose-response curve is given by an S-shaped curve. The curve becomes linear when P is transformed into probits. As dose decreases, the response, P, approaches zero very rapidly. The Mantel-Bryan extrapolation method uses the probit function. The model does not fit experimental data well, however, and the arbitrarily shallow slope of the dose-response curve as the dose approaches zero causes the estimate of risk at low doses to be much lower than that estimated by other nonthreshold models.

The logit model, based on the logistic distribution, also has an S-shaped dose-response curve, symmetric about the 50% response point. It's equation is;

$$P(D) = 1/[1+\exp\{-(a+B\log D)\}]$$

This model approaches zero response more slowly than the probit curve and leads to a higher estimate of risk at low doses than the probit model.

Both the probit and logit models have been used to evaluate acute toxicity, but there is no theoretical reason why either should adequately represent the cancer response of a population (Hoel, 1980). Moreover, the two models cannot be differentiated statistically. Hoel (1980) and others have stressed the need to use carefully understood assumptions regarding mechanism of action in order to meaningfully restrict the class of models.

The extreme value model (Weibull) is described by the equation;

$$P(D) = [1 - \exp(-BD^m)]$$

where BD^m is the mean of a Poisson distribution.

This model can also be related to both time and dose by the expression:

$$P(t,D) = 1 - \exp-[g(D)t^k]$$

where g(D) is a function of dose. The model is adaptive in shape. The graph of the dose-response curve at low doses is convex upward, linear, or convex downward accordingly as the shape parameter, m, is less than, equal to, or greater than unity.

2. Mechanistic models

Mechanistic models rely on simplistic assumptions of the mechanism of action of carcinogens. These models include the one-hit, gamma multi-hit, multi-stage.

The one hit model assumes that only one event or hit is required to transform a normal cell. This model has a dose-response curve defined by

$$P(D) = 1 - \exp(-BD)$$

where BD is the expected number of hits at dose level D. The concept of a hit encompasses a variety of possible elementary biochemical events leading to carcinogenesis. The shape of the dose-response curve is determined by only one parameter and is linear in the low-dose region. Thus, it often fails to provide a satisfactory fit to dose-response data in the observable range. The one-hit model is a special case of the Weibull, multi-stage, and multi-hit models.

The gamma multi-hit model assumes that at least k hits are required to produce a response and the probability of a hit is proportional to dose. The equation is shown as:

$$P(D) = 1 - \sum_{j=0}^{k-1} (BD)^j \exp(-BD)/j!$$

When the model is generalized to any positive number, k, the dose-response curve assumes a gamma distribution of tolerances with the shape parameter, k.

The additional parameter, k, allows a better description of the data in the experimental range. This model is adaptive in shape; in the low dose region, the equation is linear for k=1, concave for k<1 and convex for k>1. The model provides a blend of the probit model at high dose levels and the logit at low doses. It will fit a wider variety of data sets than the multi-stage model in part because the constant k is estimated from the data.

Some practical problems arise from the use of this model (CDHS, 1985). A background rate may be generated even where the data do not indicate one and may discount a possible significant increase in tumors at low doses. If the number of hits is less than one, the model can yield unrealistically low estimates of "virtually safe dose" because the slopes can become extremely steep at low doses. In addition, the model has no clear biological basis if k is not an integer, or becomes extremely large. Finally, a reliable procedure for calculating low dose confidence limits for the gamma multihit model has yet to be derived.

The multistage model assumes that several distinct, heritable changes are necessary to transform a normal cell into a malignant cell which can then progress to cancer. The response is shown to be a polynomial function of dose with nonnegative coefficients.

$$P(D) = 1 - \exp - \sum_{i=0}^{k} (B_i D^i),$$

where $B_1>0$ and k equals the number of stages required to observe cancer. A carcinogen is assumed to be able to increase any of the event rates, and each transition (i) is dependent on two constants, a_i and b_i. The model is fitted using maximum likelihood theory, and the coefficients and k are estabished by the best fit to the data. The number of stages, k, can also be assumed to be no more than the number of dose levels, and this is the procedure used by the U.S. EPA (Anderson et al., 1983). A linear term is used in the estimation of the upper confidence limits for risk at low doses which makes this model equivalent to the one-hit model in the low dose region. One criticism of this model is that the number of stages in the model is arbitrary when based on the number of dose groups. It also does not describe well functions that are initially flat, then curve rapidly upward. Use of the maximum likelihood estimate (MLE) as a "best estimate" for risk is also problematic because it is very sensitive to small changes in the data for incidence and dose.

A time-to-tumor model used by the U.S. EPA is an extension of the multistage model which incorporates age-adjusted incidence. The equation is:

$$P(d,t) = 1 - \exp - (q_0 + q_1 d + q_2 d^2 + q_3 d^3 t^k)$$

where q_0, q_1, q_2, q_3, and k are nonnegative parameters estimated from the data. It is called the multistage Weibull model and allows an estimation of the probability of cancer by a fixed age in the absence of competing risks. Information must be available as to whether the tumors of interest were fatal or incidental. A problem in assessing time-to-tumors is the difficulty in determining the precise time of appearance of the tumor (CDHS, 1985).

3. Physiologically-based pharmacokinetic models

Physiologically-based pharmacokinetic models (PB-PK) incorporate what is known about the kinetics of the behavior of the chemical in the body (rates of activation, detoxification, clearance and distribution) and its carcinogenic activity (mutation, cell proliferation etc.) to derive risk estimates. These models use species specific information to estimate the amount of parent material and metabolites distributed to various physiological compartments in the body, their rate of distribution, and rate of disappearance. Three types of information are used to describe the behavior of the chemical. Partition coefficients express the relative solubility of the substance in blood and tissues. Physiologic constants describe tissue and organ volumes and blood flow. Biochemical constants define the rate coefficients for biotransformation pathways. Thus, PB-PK models are useful to account for species differences, the kinetics of metabolism, and mechanisms of uptake (Krewski et al., 1987).

Krewski et al. (1987) discussed the application of pharmacokinetic data to estimate the effective dose delivered to the target organ. More accurate estimates of risk can be obtained in the mathematical extrapolation models using delivered dose instead of the administered dose. Nonsaturable vs saturable kinetics of activation and detoxification, and the time pattern of dosing has an effect on the size of the delivered dose. Sielken (N.D.) also presented an Individualized Response Model which models the probability of response as a function of time and the biologically effective dose (BED). The BED itself is a function of individual susceptibility, background dose, and the administered dose.

One model currently being evaluated by the U.S. EPA is the two-stage Moolgavkar, Venzon, and Knudson model (Moolgavkar, 1986; Moolgavkar and Knudson, 1981; Moolgavkar, 1983, Thorsland, 1987). This model describes the development of a malignant cell as a function of the rate of transition of a normal stem cell to an initiated cell (u_1), the rate of growth of normal and initiated cells (a_1,a_2), the rate of differentiation or death of normal and initiated cells (B_1,B_2), and the rate of the transition from initiated cell to malignant cell (u_2). The estimates of risk may be quite variable in the low dose region however, when different methods of estimating the rate of cell proliferation are used.

4. Use of the Dose-Response Models by Government Agencies

The U.S. EPA uses the linearized multistage procedure in the absence of evidence supporting adoption of an alternate model for a particular substance

(U.S. EPA, 1986). A "plausible upper limit" to the risk is presented and the lower limit is generally stated to be as low as zero. Elizabeth Anderson and the U.S. EPA's Carcinogen Assessment Group state that the "recognition that the lower bound may approach zero or be indistinguishable from zero stems from the uncertainties associated with mechanisms of carcinogenesis, including the possibility of detoxification and repair mechanisms, metabolic pathways, and the role of the agent in the cancer process (Anderson et al., 1983)." The guidelines state that it is not possible to calculate a best estimate of the risk because of the wide range of uncertainty bounded by the upper and lower confidence limits. The 1986 guidelines suggest that a risk estimate using the linearized multistage model might be compared with estimates derived from other models where appropriate.

The Food Safety Council (1980) and Krewski and Van Ryzin (1981) compared the behavior of various mathematical models using bioassay data on several chemicals. Generally most of the models will fit the experimental data relatively well. Estimates of the dose that will result in a risk in the range of 10^{-6} to 10^{-8} derived by the different models are quite divergent however. Krewski and Van Ryzin found that when the background rate is greater than about 1%, the empirical results obtained using the probit, logit, and extreme value models suggest that the assumption of additive background provides risk estimates close to those based on linear extrapolation for all the models.

The Food Safety Council (1980) recommended that the "best estimate" of risk from a variety of models be used to evaluate risk in the low dose region. The California Department of Health Services stated in its Guidelines for Chemical

Carcinogen Risk Assessments and Their Scientific Rationale (1985) that "an appropiate procedure may be to present a range of risk estimates with an explanation of the reliability of the data that support estimates at different points on the range." Both the maximum likelihood estimate and the upper confidence value are calculated by the CDHS.

B. Characteristics of Carcinogenesis

Certain fundamental characteristics of carcinogenesis have bearing on conclusions concerning the identification of carcinogens and their low dose behavior (CDHS, 1985).

1. Carcinogenesis involves heritable changes in a cell.

2. A carcinogenic transformation in a cell is a multistage process.

3. Some of the stages may be reversible under some circumstances, while others appear to be permanent alterations.

4. The development of cancer involves a latent period of variable duration for each stage.

5. The initial change in a cell is believed to be an alteration in the genetic material contained in the DNA molecule. Later stages may involve changes that enhance the survival and growth of the transformed cell line.

6. Exogenous factors (viruses, chemical carcinogens, cofactors) affect critical changes at each stage. Combinations of exogenous factors may interact in an additive, inhibitory, or multiplicative manner.

7. Endogenous factors (oncogenes, hormones) also influence the development of cancer.

Certain critical issues concerning mechanism of action impinge on the selection of an appropriate dose-response model. These include the possibility that the

development of cancer may involve a threshold, and evidence for low dose linearity.

C. The Assumption of No Threshold

There are several arguments for the operating hypothesis that there is no threshold dose for the induction of cancer. The initial target for carcinogenicity is at the molecular level involving DNA or other macromolecules. The occurrence of events in a single cell is expected to lead to cancer (CDHS, 1985). The California Department of Health Services, in their Guidelines for Chemical Carcinogenesis Risk Assessments and Their Scientific Rationale, points out that, even in the presence of internal defense systems, there is a finite probability that a few molecules of a carcinogen will be able to evade various protective systems within the body (including detoxifying enzymes) and produce the initiating event.

Another argument for the use of a "no threshold" assumption is that even if individuals have thresholds, the establishment of a population threshold would be even more difficult due to the genetic heterogeneity and physiologic variability that exists in the human population. The relevant threshold would be that of the most sensitive individual which may approach zero (CDHS, 1985). In addition, the existence of a background incidence of cancer in humans suggests that any possible population threshold for some mechanisms of action has been exceeded. Since it is not known which mechanisms of action are currently contributing to background cancer incidence, any additional exposure can be assumed to have increased risk.

Two mechanisms of action for which thresholds have been proposed are the induction of malignant cells through cytotoxicity and other epigenetic mechanisms." Cytotoxicity is an epigenetic mechanism which hypothetically increases the number of altered cells by causing cell death and the stimulation of cellular regeneration. The rate of cell replication overwhelms the DNR repair capabilities of the cell, and therefore cells initiated either by the cytotoxic agent or another agent are allowed to persist. The California Department of Health Services was unable to find evidence in the literature of a decreased efficiency of DNA repair in the presence of cytotoxins. In addition, no data are available from bioassays with sufficient statistical power at low doses of a cytotoxic agent to verify a negative carcinogenic response at dose levels associated with no cell death (CDHS, 1985).

The California Department of Health Services asserts that since epigenetic carcinogens are defined operationally by a negative response in short term mutagenesis assays and DNA binding assays, it is important that the frequency of false negatives be low. False negatives can arise in these tests if the active form of the substance is not generated or because of the limited sensitivity of certain short term tests. Therefore, it is difficult to conclusively indentify an epigenetic agent. Indeed, the International Agency for Research on Cancer (IARC) has stated that, "there is as yet insufficient information to implement a classification of agents according to their mechanism of action (IARC, 1987).

D. The Assumption of Low Dose Linearity

The assumption of low dose linearity is a more protective model of cancer risk than a dose-response curve with the same intercept but a concave upwards shape

(CDHS, 1985). All nonthreshold models will result in low dose linearity if it is assumed that at least one of the agents responsible for background incidence has the same mechanism of action as the test substance. The response is therefore additive. It is also possible for some agents to interact antagonistically or synergistically.

The treatment of background incidence in a dose-response model has a large effect on the value of the risk estimate. If independence is assumed, low dose linearity does not necessarily follow, and a much greater variability in the risk estimated by different mathematical models will be observed (Krewski and Van Ryzin, 1981). The assumption that a substance will have a common mechanism with at least one background agent seems reasonable. Even if a substance was deactivated through a unique pathway, the inactivation would have to be instantaneous and complete for additivity not to be operative. For these reasons, a policy of additivity has been adopted by the California Department of Health Services for the purposes of cancer risk estimation (CDHS, 1985). Hoel (1980) demonstrated that low dose linearity will prevail unless background incidence is 100% independent. The author also states that extrapolation from an animal study with no background incidence to humans which exhibit spontaneous background cancer incidence will again involve a linear framework.

Data from mutagenesis studies support a linear relationship at low doses, and a limited amount of epidemiological evidence has been cited that is consistent (Anderson et al., 1983). Studies determined to have adequate data include evaluations of radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, and liver cancer induced by

aflatoxin in the diet. However, it has been pointed out that errors in the reporting of dose can also lead to an apparent low dose linearity in epidemiological studies (Food Safety Council, 1980).

Krewski et al. (1987) state that the Armitage-Doll multistage model and the two-stage birth-death-mutation model of Moolgavkar and Knudson (1981) lead to low dose linearity when the transition intensity functions between stages are linear functions of dose. Pharmacokinetic models will show low dose linearity in cases where all kinetic processes are linear at low doses if the probability of tumor occurrence is proportional to the dose delivered to the target tissue in the low dose region. Krewski et al. demonstrate that if both the activation and detoxification pathways are nonsaturable processes, then the delivered dose is a linear function of the administered dose, and the probability of tumor induction is also a linear function of the administered dose. However, the delivered dose is not a linear function of the administered dose when activation or detoxification is saturable.

E. Choice of Model for the NWF Sport Fish Consumption Advisory Project

It is recognized that a physiologically-based pharmacokinetic model is the preferable basis for dose-response assessment since knowledge of the behavior of the test material in the body and physiologic differences between species can be incorporated. However, more often than not, the detailed biological data required for the use of these models is not available. In addition, the variability of the risk estimates in the low dose region when different methods of calculating the effective dose for certain organ specific models has not been fully characterized. Finally, more detailed knowledge of the specific

mechanism of action is required than is currently available for most substances.

In light of the above limitations, the linearized multistage model was the mathematical model of choice for dose-response assessments as recommended by the U.S. EPA (1986), OSTP (1985), and CDHS (1985). This model incorporates two assumptions regarding the mechanism of action for carcinogenisis; no dose threshold for response and low-dose linearity. Specifically, the Global 82 computer program developed by Dr. Kenneth Crump (Crump and Howe, 1985) has been used to estimate upper-bound cancer risks at specified dose levels and the lower-bound "virtually safe dose" at specific low levels of risk.

As was stated in the discussion of study selection, the results of epidemiologic studies were used if incidence and exposure could be properly quantified. In the absence of human data, studies using laboratory animals were used. The available data concerning chemical absorption, metabolism, distribution, and excretion were evaluated to determine the relevancy of the animal species and strain to predict human hazard. An estimate of the delivered dose was not possible. Therefore, the administered dose, was used to derive upper-bound risk estimates in the Global 82 model.

When the data were compatible, upper-bound risk estimates were calculated and presented using the Weibull and gamma multihit models to evaluate the uncertainty caused by the choice of models. Th computer program, Risk81, developed by Dr. Daniel Krewski of Health and Welfare Canada, was used. The assumption of additivity was used to incorporate background tumor incidence.

Interspecies Extrapolation

The third, and final, step in a dose-response assessment is interspecies extrapolation. Several adjustments need to be made when converting the dose associated with a specific risk in an experimental animal to humans. Differences in size, physiology, exposure, pharmacokinetics, and susceptibility must be accounted through the use of scaling factors (CDHS, 1985).

A. Scaling Factors

Humans differ from experimental animals in size, lifespan, surface area, metabolic rate, and food consumption in addition to other physiologic processes. Examples of scaling factors are mg/kg body weight/day, ppm in the diet, $mg/m^2/day$, and mg/kg body weight/lifetime (U.S. EPA, 1986). If the dose expressed in a certain dose rate is the same for rats and humans, then the dose expressed in other dose rates will be quite different. For example, for the same dose expressed in mg/kg/day, the relative risk for humans compared to rats would increase from 1 for mg/kg/day to 6 for ppm in diet to 14 for $mg/m^2/day$ to 40 for mg/kg/lifetime (CDHS, 1985).

There is no consensus as to the most appropriate scaling factor for carcinogenesis. The Food and Drug Administration scales dose according to mg/kg body weight/day or $mg/m^2/day$ depending on whether the active carcinogen is the parent compound or a metabolite. It is reasoned that if the active carcinogen is the parent compound, then metabolic rate, which is equivalent on a surface area basis, does not become a factor. Therefore, body weight is the correct scaling factor. The U.S. EPA extrapolates between species using the

dose scale, mg/m^2 body surface area per day when there are no data indicating that this is inappropriate (U.S. EPA, 1986). This is based on observations that many physiological rates including ventilation, basal metabolic, and clearance rates scale in proportion to a fractional power of body weight among animal species. Therefore, even if the carcinogenic agent is the parent compound, its activity is related to its time in the body, which in turn is related to clearance time, and hence to a dose per surface area relationship. The surface area relationship also holds for the acute effects of anticancer agents (U.S. EPA, 1988).

Evaluations of dose scaling factors between animals and humans for carcinogens have presented evidence supporting factors ranging from mg/kg/day, and mg/kg/lifetime. The California Department of Health Services concluded that "in the absence of decisive empirical evidence, it seems best to use an intermediate measure of dose such as $mg/m^2/day$, recognizing that uncertainties in this scaling assumption may lead to differences up to a factor of 14 when data on mice are used, and up to a factor of 6 when data on rats are used. The Minnesota Department of Health selected a cancer induction potency estimate for TCDD derived by the U.S. Centers for Disease Control in part because it used a body weight scaling factor (Kreiger, 1985). This was felt to be appropriate because of the correlation of body weight with target organ weight. The Food Safety Council (1980) asserted their preference for the use of body weight as a scaling factor stating that the ratio of absolute dose by weight to absolute surface area in man is out of proportion to that in other animals. The ratios were one in the mouse, 1.42 in the rat, 2.74 in the rabbit, 3.12 in the monkey, and 9.8 in humans. The use of ppm in diet was criticized because changes in

food consumption by rodents in the postweaning stage is not accounted for. Surface area was challenged because procedures for estimating surface area do not represent true skin area.

Brown et al. (1988) conducted a survey of the literature on the validity of interspecies risk extrapolations and found approximately 25 quantitative analyses for either radiation or chemical hazards. The conclusions of the studies varied widely. Brown et al. reached the following conclusions regarding the current state of knowledge concerning interspecies extrapolation.

1. Meaningful comparisons are limited by the paucity of quantitative potency estimates for carcinogenicity in humans. The lack of data is compounded by great uncertainty in human exposure estimates.

2. The number of thorough risk comparisons is insufficient to allow judgment of the overall validity and value of quantitative extrapolations.

3. Simple scaling rules may be useful for narrow classes of agents.

4. More complex relationships may be required for more confident extrapolations including consideration of exposure duration, dose rate, life span, latency, pharmacokinetics, and baseline cancer rate.

B. Other Differences

Absorption differences between species will lead to variation in the dose delivered to the tissues when the same dose is administered. It is generally assumed that humans absorb at least as much material as the experimental animal (CDHS, 1985). If systemic distribution is demonstrated, differences in the

route of exposure is not a major consideration unless a high local concentration of the material is generated. Metabolic and pharmacokinetic differences should be investigated to increase confidence in the interspecies extrapolation.

Humans may be more susceptible than inbred animal populations to carcinogens due to additional factors such as genetic variability and exposure to multiple active materials. The limited empirical evidence suggests that humans are approximately as sensitive as the most sensitive animal species. Therefore, additional adjustments are not carried out (CDHS, 1985).

C. Factors Used to Derive the NWF Sport Fish Consumption Advisory Project Risk Estimates

A scaling factor based on body surface area was used to extrapolate from the test species to humans. The percent reduction in risk that would be observed if a body weight scaling factor were was stated since the actual appropriate interspecies dose equivalency is as yet undetermined.

If the test animal absorbs only a fraction of the administered dose, or if evidence suggests that humans absorb more or less of the chemical than does the test animal, and the administered dose would otherwise be used, this dose will be adjusted accordingly.

Table 6.
ASSUMPTIONS AND SCIENCE POLICY DECISIONS USED IN THE DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

SELECTION OF CANCER INCIDENCE DATA

Well-conducted epidemiologic studies preferred if quantified data on exposure and cancer incidence are available. If not available, data based on carcinogenic responses in laboratory animals responding in a manner similar to humans will be used.

Use total number of animals with one or more tumor type or site with incidence that is statistically increased.

Benign tumors of the same histogenic origin will be combined with malignant tumors. The contribution of benign tumors to the total risk estimate will be indicated.

Greater emphasis will be placed on results in the most sensitive species, strain and sex.

Incidence data will reflect the same route and pattern of exposure as the human environmental exposure, otherwise adjustments will be made to account for the difference in delivered dose expected.

DOSE RESPONSE MODEL

An assumption of no threshold is made.

An assumption of low dose linearity is made.

The linearized multistage model is the mathematical model of choice. 95% upper bound risk estimates will be presented.

An assumption of lifetime exposure at a constant dose rate will be used.

Upperbound risk estimates will be calculated and presented using the Weibull and gamma multihit models to evaluate the uncertainty caused by the choice of model.

An assumption of additivity will be used to incorporate background tumor incidence.

A scaling factor based on body surface area will be used to extrapolate from the test species to humans. The percent reduction in risk that would be observed if a body weight scaling facotr were used will be stated since the actual appropriate interspecies dose equivalency is as yet undetermined.

If the test animal absorbs only a fraction of the administered dose, or if evidence suggests that humans absorb more or less of the chemical than does the test animal, and the administered dose would be otherwise used, this dose will be adjusted accordingly.

THE TREATMENT OF UNCERTAINTY

Exposure Assumptions
The deviation of exposure assumptions from the exposure pattern experienced in the studies used in the dose-response assessments will be discussed.
Choice of Model
When appropriate, a potency value will be calculated using the Weibull and gamma multihit models using the Risk 81 computer program (Krewski and Van Rysin, 1981) and the range in values will be compared to the potency value from the linear multistage model.
Choice of Data
When more than one data set of good quality is available, potency values will be calculated using the alternative data and the range of values will be compared to the data set of choice.
Interpretation of Selected Data
When tumor incidence has been combined for tumor type or different sites, the contribution of the different types or sites will be quantified.
Scaling Factor
The decrease in risk if body weight were used to extrapolate between the animal data and human predictions will be specified.
Interactions
The interactive effect of chemical mixtures will be discussed and if possible examined quantitatively.

The overall upper bound risk for each species will be estimated. As a general rule, if it is not possible to precisely determine interactive effects, an assumption of additivity will be used to predict the upper bound cancer risk of eating fish containing several chemicals. The risks will be added to determine an overall upper bound risk.

RISK CHARACTERIZATION

Systemic Toxicity

An estimate of the hazard of adverse health effects other than cancer was obtained through the calculation of a hazard index for each chemical. The hazard index is described in the U.S. EPA guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986b). An estimate of environmental exposure (mg/kg/day) using a range of fish consumption estimates was divided by a reference dose (mg/kg/day) to arrive at a chemical-specific hazard index; HI = Exposure Dose/RfD.

As the HI approaches unity, concern for the potential hazard of the substance increases. The reference dose is considered to have a range of uncertainty of an order of magnitude in either direction. The hazard index should be viewed in light of that range of uncertainty.

A similar risk characterization procedure is discussed by the U.S. EPA (1988) in its guidelines for deriving the reference dose. The margin of exposure (MOE) between the expected environmental exposure dose and the animal study's NOAEL is compared to the product of the uncertainty factor and the modifying factor selected to derive the reference dose. If the MOE is less than the UF x MF, then the need for regulatory concern is carefully examined.

Areas of uncertainty were discussed qualitatively. The data upon which the hazard index is based were described and interpreted in light of the study limitations and sensitivity, and the type of effect observed.

An estimate of the overall hazard due to the consumption of multiple chemicals was derived for each species. The U.S. EPA guidelines state, "when little or no quantitative information is available on the potential interaction among the components, additive models are recommended for systemic toxicants." The guidelines go on to note that "several studies have demonstrated that dose additive models often predict reasonably well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds." This assumption is plausible only when the components in a mixture cause the same response by the same mode of action.

We followed the recommendation of the U.S. EPA for estimating the combined hazard of multiple contaminant exposures when data on interactions was not available. Chemical-specific hazard indices based on the same health endpoint were added to predict the total hazard of fish consumption for each species.

The equation as presented in the U.S. EPA guidelines is:

$$HI = E_1/AL_1 + E_2/AL_2 + \ldots + E_i/AL_i$$

where: E_1 = exposure level to the i^{th} toxicant and,

AL_i = maximum acceptable level for the i^{th} toxicant.

The hazard index is considered to be an interim hazard index only because many toxicants have been identified in sport fish in the Great Lakes, but adequate tissue concentration data is available for only a few. Therefore, the actual total hazard of these fish is not known.

Cancer

The following procedure was used for the NWF Fish Consumption Advisory Project to characterize the risk associated with consuming contaminated sport fish

caught in Lake Michigan. Chemical-specific upper-bound risk estimates were calculated using the Global 82 linearized multistage model (Crump and Howe, 1985). Risk estimates were presented for technical purposes as the excess upper-bound individual lifetime risk assuming a lifetime exposure at a constant dose rate. This is the number of excess cancer cases expected (one significant figure) per standard population size (million, ten thousand etc.).

The dose that would result in a lifetime upper-bound risk of 10^{-4} to 10^{-6} was also calculated using the Global 82 computer program. These dose levelsa were presented in mg/kg/day and number of meals per time period.

The evaluation included the use of tissue concentrations for several sport fish species and size classes, and a range of estimates for fish consumption and meal size. The variance of risk experienced by males and females, and different age groups was evaluated quantitatively and qualitatively as appropriate. An assumption of dose equivalency between species based on surface area was used.

Uncertainty was qualitatively and quantitatively described for the following areas.

Exposure Assumptions

The deviation of exposure assumptions from the exposure pattern experienced in the studies used in the dose-response assessments was discussed.

Choice of Model

When appropriate, a potency value was calculated using the Weibull and gamma multihit models using the Risk 81 computer program (Krewski and Van Rysin,

1981) and the range in values was compared to the potency value from the linear multistage model.

Choice of Data

When more than one data set of good quality was available, potency values were calculated using the alternative data and the range of values was compared to the data set of choice.

Interpretation of Selected Data

When tumor incidence was combined for tumor type or different sites, the contribution of the different types or sites were quantified.

Scaling Factor

The difference in risk estimates if body weight were used to extrapolate between the animal data and human predictions was specified.

The overall upper bound risk for each species was estimated. As a general rule, if it was not possible to precisely determine interactive effects, an assumption of additivity was used to predict the upper-bound cancer risk of eating fish containing several chemicals. The risks were added to determine an overall upper-bound risk for the consumption of multiple chemicals.

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CHAPTER 3

EXPOSURE ASSESSMENT

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GENERAL PROCEDURES

Contaminant concentration data were gathered for individual sport fish species and size classes of individual species from each of the states surrounding Lake Michigan (Indiana, Wisconsin, Illinois and Michigan). Data were collected for the years 1985, 1986 and 1987. However, only data for 1985 and 1986 were used to develop quantitative health impact assessments. Data for 1987 were not used since some states surrounding Lake Michigan will not incorporate 1987 data into state fish consumption advisories until 1990 and since all 1987 data are not yet available from all Lake Michigan states. Thus, we chose to use only 1985 and 1986 data to facilitate comparison of the NWF model advisory with the individual Lake Michigan state advisories.

Sport fish were collected by states surrounding Lake Michigan from several sites in the lake during 1985, 1986 and 1987 (Table 1). Upon collection, each state followed uniform monitoring procedures for contaminants listed in Table 2. Monitoring and Quality Assurance/Quality Control procedures for Great Lakes sport fish are described in the State of Michigan's procedural statement - Sport Caught Fish Consumption Advisories: Philosophy, Procedures, and Process (1986). All contaminant data were collected from skin-on fillets and are reported as mg/kg (ppm) wet weight.

Seventeen fish species were collected from Lake Michigan or from mouths of its tributaries as part of the Lake Michigan fish monitoring program (Table 3). Concentration in edible tissue for all contaminants reported in Table 2 and all species reported in Table 3 were entered and stored both by state and by combining individual state data into one, grand file on the University of Michigan's computer system (Michigan Terminal Service - MTS). All computer operations were conducted on the University of Michigan's IBM 3090 mainframe computer.

Many data points (contaminant concentrations of PCB, DDT, dieldrin, or chlordane in individual samples) were reported as below the analytical limit of detection (LOD). Where contaminant concentrations were reported as less than the LOD, the data point was entered onto the computer and included in our statistical analysis as zero (0.00). The data point was entered onto the computer as a missing value and not included in statistical analysis where contaminant concentrations were not reported for a sample. The use of zero for contaminant concentrations reported as less than the LOD likely underestimates the actual contaminant concentration in a species or size class of species.

Only a portion of the fish sampled by each state, and a portion of the contaminants detected in their tissues were selected for analysis in the NWF study. We chose to use only those fish (Walleye, Lake Trout, Coho Salmon, Brown Trout, Chinook Salmon and Perch) and those contaminants (Total PCB, Total Chlordane, Dieldrin, and Total DDT) for which sample sizes were large enough to allow statistical analysis of tissue contaminant concentrations, fat (lipid) content of individual species, and fish length and weight. Generally, we required data for each contaminant from analysis of at least 20 individuals of each species combined from 1985 and 1986 sampling. Only those contaminants and fish noted in Tables 2 and 3 had sample sizes large enough to meet this requirement. Therefore, only these contaminants and fish were addressed in the health assessment portion of the study and in the NWF model consumption advisory.

STATISTICAL ANALYSIS

After choosing contaminants and species that met the minimum data base requirements, we performed regression analyses to determine the relationship between fish length and contaminant concentration, lipid content

and contaminant concentration, and weight and contaminant concentration. Statistical distributions of contaminant concentrations, length, weight and lipid content were determined prior to regression analysis. We used SAS (1982) procedures to examine statistical distributions and to perform regressions. SAS calculates two statistics to test for normality, the Shapiro-Wilk statistic (W) when sample sizes are less than 51, and the Kolomogorov D statistic when sample sizes are greater than 50. Both test the null hypothesis that input data values are a random sample from a normal distribution.

Not all variables were normally distributed (Table 4). Length was the variable most often non-normally distributed in the species evaluated for this study. The non-random nature of sampling conducted by state resource agencies likely results in the problem of non-normality. Some evidence suggests that larger fish contain higher concentrations of contaminants. Therefore, sampling by state agencies is intentionally biased toward larger size classes of fish.

The problem of non-normality alone may prohibit performing regression analyses using parametric statistics to examine the relationship between length and contaminant concentration. However, the general linear model used to perform regression analysis (SAS, 1982) is relatively robust and insensitive to data that are non-normally distributed. More important statistical requirements are those for linearity between independent (length, weight or lipid content) and dependent variables (contaminant concentrations) and the requirement that variables be statistically independent or uncorrelated (orthogonal).

We plotted the relationships between length and contaminant concentrations (PCB, dieldrin, DDT, and chlordane) for each of the species examined in this report. We did not examine the relationships between weight

and contaminant concentration or between lipid content and contaminant concentration. Length/contaminant concentration was the only relationship examined since this is the only useful relationship for developing a fish consumption advisory. Ideally, some predictor of contaminant concentration should be developed, assuming that not all individuals of a species carry identical contaminant concentrations, since contaminant concentration cannot be measured readily by sport anglers. Where a predictor is available, is reliable, and is easily measured, that predictor can be used in place of contaminant concentration to determine the extent and nature of consumption advice based on contaminant concentration. Fish length is a simple and accurately measured variable; thus, where it predicts contaminant concentration, it will be a useful variable by which to issue consumption advice.

The relationship between length and contaminant concentration was not linear for all species and contaminants nor was there a significant relationship between length and contaminant concentration for all species and contaminants (Table 5). Where a non-linear relationship exited between length and contaminant concentration, log/log transformation of variables was performed and statistical analysis rerun. Significant relationships were not observed for length of yellow perch and walleye and their corresponding tissue concentrations of PCB, dieldrin, DDT or chlordane (Table 5). Thus, consumption advice for these species was not based on size class.

Where a statistically significant relationship between length and contaminant concentration existed (F-test, $P < 0.05$) for a species, consumption advice was determined based on average contaminant concentrations for each of three size classes of that species: 10 - 20 inches, 20 - 30 inches, and greater than 30 inches. These size classes were chosen for their ease of measurement in the field. Comparison of mean contaminant

concentrations between size classes was conducted after consumption advice was determined based on size classes. Final consumption advice was issued on a size-class specific basis only where statistically significant differences existed for PCB concentrations between size classes (ANOVA, Duncan's multiple range test, $P < 0.05$).

The relationship between length and contaminant concentration was statistically significant for at least one contaminant for lake trout, chinook salmon, coho salmon and brown trout (Table 5). However, a significant relationship existed between length and each of the four contaminants only in lake trout. We chose to issue size-class specific advice for lake trout and chinook salmon in our model advisory based on the significant relationship between PCB and length in these species and based on the differences in PCB concentrations between size classes (Table 6). PCB is the most important of the four contaminants in these fish due to its high concentration in tissues and its potency.

We did not issue size-class specific advice in our advisory for contaminants in coho salmon since only one size class of this species was sampled by the Lake Michigan states during 1985 and 1986. Size-class specific advice was not issued for brown trout since contaminant concentrations were not significantly different between size classes. Average contaminant concentrations for all species addressed in this project, calculated by combining monitoring data from 1985 and 1986, are shown in Table 6 by size class where appropriate.

RECOMMENDATIONS

We offer the following recommendations based on our analysis of contaminant concentration data in Lake Michigan sport fish and based on our assessment of the health risks posed by exposure to these contaminants through consumption of contaminated Lake Michigan sport fish.

1. Inadequate data exist to properly (statistically) assess the extent of contamination in several species and size classes of Lake Michigan sport fish. Because of data inadequacies, we were not able to determine representative concentrations of contaminants in some sport fish species nor were we able to develop consumption advice for these species. Therefore, we recommend that state resource and health agencies collect adequate sample sizes of the following fish species to determine the types and concentrations of contaminants in these species: Whitefish, steelhead trout/rainbow trout, northern pike, pink salmon, and smallmouth bass. Although we did develop advice for walleye and yellow perch, monitoring data for these species are only marginally adequate and we recommend that larger sample sizes of these species be collected. Further, the smaller size classes of many species are not sampled adequately and we recommend increased sampling of smaller sizes of all salmonids as well as the fish species cited above.

2. Once adequate sample sizes of Lake Michigan sport fish species are collected, we recommend that states conduct a thorough analysis of the relationship between fish length and contaminant concentrations. Size class specific advice should be issued only where a positive relationship exists between length and contaminant concentration. Further, where consumption advice is issued for specific size classes of fish species, different advice

for different size classes should be issued only where there are statistically significant differences in contaminant concentrations between species.

3. Most of the states surrounding Lake Michigan conduct tissue analysis only for PCBs in sport fish. We recommend that the states expand their Lake Michigan monitoring programs to include chemicals other than PCB. At the very least, expanded programs should include analysis of DDT (and its metabolites), chlordane (including nonachlor), dieldrin and mercury. Other toxicants including lead and other metals, toxaphene, mirex, polynuclear aromatic hydrocarbons (PAHs), lindane, heptachlor and heptachlor epoxide, chlorinated styrenes, hexachloro-benzene and other chlorinated compounds (including dioxins and furans) should be analyzed in Lake Michigan sport fish.

4. Quality assurance/quality control programs in the Lake Michigan states are not well documented. Only Michigan has developed a document that describes QA/QC procedures for its Great Lakes fish monitoring program. No comprehensive summary of QA/QC information exists in individual states or for the Lake Michigan monitoring program as a whole. Therefore, we recommend that the Lake Michigan states develop one document that describes the tissue collection and analysis procedures used for Lake Michigan sport fish and the QA/QC procedures used in the monitoring program. Further, we recommend that a yearly report on the outcome of the Lake Michigan QA/QC program be published by the Lake Michigan states and made available to all interested persons.

5. We strongly recommend that the Lake Michigan states, as well as the rest of the states in the Great Lakes basin, utilize a risk-based procedure to develop consumption advice for Great Lakes sport fish. This procedure should recognize both risk assessment and risk management issues, assess these issues

in a broad, open public format, incorporate them into consumption advice presented to the public, and present them openly and clearly in all communications. Further, consumption advice should be based on a thorough assessment of the health risks associated with concurrent exposure to combinations of contaminants. Where data are not available that address the risks of concurrent exposure to combinations of contaminants, the assumption should be used that risks are additive and consumption advice based on that assumption.

6. Thorough health assessments for metals and other contaminants that occur at low but detectable concentrations in sport fish (including toxicants cited in Recommendation #3 above) should be conducted. Consumption advice should be developed for fish contaminated with these materials where health assessments indicate that potential impacts on human health may occur through consuming contaminated sport fish.

7. We strongly recommend that long-term, epidemiological research be funded and conducted to determine the types and extent of impacts of consuming contaminated Great Lakes sport fish on humans in the Great Lakes basin.

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TABLE 1. Sites of 1985 and 1986 fish collections as part of the Lake Michigan fish consumption advisory monitoring program.

WISCONSIN	MICHIGAN	ILLINOIS	INDIANA
Door County*	Charlevoix	Cook County*	Lake Michigan
Kewanee R. mouth	South Haven	Waukegan harbor	
Manitowoc County*	Manistique River	Zion (offshore)	
Milwaukee County*	St. Joseph River	Cook County*	
Ozaukee county	Grand Haven		
Green Bay	Platte River Weir		
Oconto River	Thompson Creek Weir		
Pensaukee County	Little Manistee Weir		
Kenosha County			
Kewanee County*			
Racine			
Sheboygan County*			
Sturgeon Bay			

* Indicates several sampling locations at this site

TABLE 2. Contaminants in fish tissue monitored by states surrounding Lake Michigan. Contaminants marked with an * are those addressed in NWF health assessments.

CONTAMINANT	
Cadmium	Cis-nonachlor
Chromium	Trans-nonachlor
Copper	Total nonachlor
Lead	Aldrin
Nickel	Dieldrin*
Zinc	DDE
Mercury	DDD
Aroclor 1248	DDT
Aroclor 1254	Total DDT*
Aroclor 1260	Hexachlorobenzene
Total PCB*	Heptachlorepoxide
Total Chlordane*	Lindane
Alpha-chlordane	Toxaphene
Gamma-chlordane	Octachloro-styrene
Oxychlordane	

TABLE 3. Fish species monitored by states as part of the Lake Michigan fish consumption advisory monitoring program. Fish marked with an * are species addressed in NWF health assessments.

SPECIES	
Walleye*	Brook Trout
Rainbow Trout	Largemouth Bass
Lake Trout*	Whitefish
Coho Salmon*	Rock Bass
Chinook Salmon*	Smallmouth Bass
Yellow Perch*	Steelhead
Northern Pike	White Sucker
Brown Trout*	Black Crappie
Pink Salmon	

TABLE 4. Independent and dependent variables with non-normal distributions by fish species.

	NON-NORMALLY DISTRIBUTED VARIABLES	
SPECIES	DEPENDENT	INDEPENDENT
Walleye	Chlordane Concentration	Length
Lake Trout	---	Weight
Coho Salmon	---	Length, Weight
Perch	---	Length, Weight
Brown Trout	Dieldrin, Chlordane Conc.	Length, Lipid Content
Whitefish	Dieldrin, DDT Conc.	Length

TABLE 5. Nature of the relationship between fish length and contaminant concentration. NL - Nonlinear, L - Linear, NO - no observed relationship. R^2 (in parentheses) based on log/log transformation for nonlinear data and on non-transformed data for linear relationships. Where R^2 is reported, null hypothesis that slope of regression line is equal to zero is rejected ($P < 0.05$). NS - null hypothesis not rejected ($P > 0.05$).

SPECIES	Length and PCB (R^2)	Length and DIELDRIN (R^2)	Length and DDT (R^2)	Length and CHLORDANE (R^2)
Lake Trout	NL (0.68)	L (0.26)	L (0.53)	L (0.25)
Chinook	L (0.48)	L (NS)	NL (NS)	L (0.38)
Coho	L (0.30)	L (NS)	L (NS)	L (NS)
Brown Trout	NL (0.16)	L (0.34)	L (0.37)	L (NS)
Yellow Perch	NO (NS)	NO (NS)	NO (NS)	NO (NS)
Walleye	NL (NS)	NO (NS)	NO (NS)	NO (NS)

TABLE 6. Average tissue concentrations of PCB, dieldrin, total DDT and total chlordane in lake trout, chinook salmon, coho salmon, brown trout, yellow perch, and walleye pike expressed by size class (where at least one contaminant concentration is significantly different between size classes). Groupings evaluated with a Duncan's multiple range test. Averages within species (between size classes) are not significantly different if connected by the same letter. Size classes: 1 - 0 to 10 inches; 2 - 10 to 20 inches; 3 - 20 to 30 inches; 4 - greater than 30 inches.

SPECIES	SIZE CLASS	CONTAMINANT	AVERAGE TISSUE CONCENTRATION (mg/kg)	N	GROUPIN
Lake Trout	4	PCB	8.30	25	A
	3	PCB	3.40	116	B
	2	PCB	0.92	33	C
Lake Trout	4	Chlordane	0.78	2	A
	3	Chlordane	0.42	35	A
	2	Chlordane	0.22	8	A
Lake Trout	4	Dieldrin	0.37	2	A
	3	Dieldrin	0.18	35	B
	2	Dieldrin	0.17	8	B
Lake Trout	3	DDT	1.88	26	A
	2	DDT	0.23	1	A
Chinook	4	PCB	2.43	58	A
	3	PCB	1.17	48	B
	2	PCB	0.39	74	C
Chinook	4	Chlordane	0.16	8	A
	3	Chlordane	0.04	4	B
	2	Chlordane	0.0	8	B
Chinook	4	Dieldrin	0.21	8	A
	3	Dieldrin	0.04	4	A
	2	Dieldrin	0.01	8	A
Chinook	4	DDT	1.78	6	A
	3	DDT	0.29	3	A
Brown Trout	all	PCB	1.76	127	-
	all	Chlordane	0.13	45	-
	all	Dieldrin	0.10	45	-
	all	DDT	0.43	31	-
Coho	all	PCB	0.56	56	-
	all	Chlordane	0.07	28	-
	all	Dieldrin	0.03	28	-
	all	DDT	0.19	25	-

TABLE 6. Continued.

SPECIES	SIZE CLASS	CONTAMINANT	AVERAGE TISSUE CONCENTRATION (mg/kg)	N	GROUPIN
Perch	all	PCB	0.19	16	-
	all	Chlordane	0.01	7	-
	all	Dieldrin	0.02	7	-
	all	DDT	0.19	10	-
Walleye	all	PCB	0.67	15	-
	all	Chlordane	0.03	9	-
	all	Dieldrin	0.02	9	-
	all	DDT	0.11	9	-

CHAPTER 4

HAZARD ASSESSMENT AND THE CHARACTERIZATION OF CARCINOGENIC AND OTHER RISKS TO HUMANS EXPOSED TO PCBS

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PROLOGUE

The following report is a review of the toxicity of polychlorinated biphenyls (PCBs) with special attention to reports in the published literature that have relevance to human exposure to these contaminants via the consumption of sport caught fish. The document was produced by the National Wildlife Federation Great Lakes Natural Resource Center.

The review is not exhaustive, but emphasizes low-level, oral exposures which occur chronically. It is recognized that different PCB congeners and isomers have differing biological activity and therefore the toxicity of PCB mixtures will vary according to their composition. This report, while exploring some of the ramifications of this issue, primarily was limited to studies using PCB mixtures because the exposure data and tissue concentrations in Lake Michigan sport fish, have been reported only in terms of PCBs mixtures. This limitation in the exposure data lessens the sensitivity of the toxicological review and any resulting conclusions that may be drawn regarding potential human health effects.

INTRODUCTION

The environmental and public health significance of polychlorinated biphenyls (PCBs) stems from their bioavailability and persistent bioaccumulative characteristics. These characteristics are due in part to a high thermal stability, resistance to oxidation by acids, bases and other chemical agents, and lipid solubility (IARC, 1978). There are 209 possible isomers of PCB named according to the number and position of chlorine atoms on the biphenyl ring. The numbering of the carbon atoms in the ring is shown below.

Commercial PCB mixtures contain various combinations of congeners and isomers which determine the relative weight percent of chlorine in the product. Aroclors, produced in the United States, and Kanechlors, produced in Japan, were formulated into mixtures containing various degrees of chlorination. Table 1, from the IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (1978), presents the congener composition of some of the major commercial PCBs.

Dibenzofurans, compounds with similar toxic properties to PCBs, have been identified in commercial PCB formulations. This makes causal associations of PCBs drawn from epidemiological and toxicological studies more difficult. Concentrations which have been found in PCB mixtures are presented in Tables 2 and 3 taken from the IARC Monograph.

Certain properties of PCBs affect how they are distributed in the environment and in organisms. Individual PCB congeners increase in water solubility with decreasing chlorination and increasing temperature. Congeners with more than four chlorine substituents are relatively insoluble (U.S. EPA, 1987). The

octanol/water partition coefficient of a congener increases with increasing chlorination and ranges from 3.76 for biphenyl and 8.26 for decachlorobiphenyl at 25°C. The physical properties of these toxicants are also influenced by the placement of chlorine atoms on the biphenyl ring within a congener group.

Solubility as well as individual adsorption, evaporation, and biodegradation characteristics causes the composition of PCB mixtures in the environment to change with time (U.S. EPA, 1987). Lower chlorinated PCBs are primarily removed by degradation, volatilization and solubilization with a lesser degree of sedimentation. On the other hand, higher chlorinated congeners principally adsorb to sediments and biota, with a lesser degree of volatilization. These congeners are therefore more persistent compounds.

The high lipid solubility of the more highly chlorinated PCBs cause these compounds to bioaccumulate in aquatic organisms, wildlife and humans. Analytical studies of environmental samples and residues in laboratory animals treated with PCBs have found major differences between the composition in these samples and that in commercial mixtures (U.S. EPA, 1987). A congener specific high resolution glass capillary analysis of PCB congeners in Aroclor 1260 and human breast milk revealed that the two chromatographic patterns were not similar (U.S. EPA, 1987). Major components in both samples were 2,2',4,4',5,5'-hexachlorobiphenyl and 2,2',3,4,4',5'-hexachlorobiphenyl, 2,2',3,3',4,4',5-heptachlorobiphenyl, and 2,2',3,4,4',5,5'-heptachlorobiphenyl. The congener, 2,3,3',4,4',5-hexachlorobiphenyl, was a major component in the breast milk but was present in Aroclor 1260 in minor amounts. Four other major congeners identified in the human milk extract, 2,4,4'-trichlorobiphenyl, 2,4,4',5-tetrachlorobiphenyl, 2,2',4,4',5-pentachlorobiphenyl, and 2,3,4,4',5-pentachlorobiphenyl, were minor components

of Aroclor 1260. The U.S. EPA (1987) concluded that these congeners were derived from lower chlorinated formulations. Moreover, other major components of Aroclor 1260 were found to comprise only a minor portion of the PCBs in the human milk extract. These results demonstrated a structure-dependent bioaccumulation of specific congeners in human milk.

The primary environmental exposure for humans is the ingestion of PCBs in the diet. Fish are the main commodity in the United States contaminated with PCBs although prior to 1976, cheese, eggs and byproducts used in animal feed also contained significant concentrations (Jelinek and Corneliussen, 1976). Jelinek and Corneliussen (1976) estimated that an American teenage male had a daily dietary PCB intake of 15.0 ug/day in 1971 and 8.7 ug/day in 1975. The Food and Drug Administration (U.S. FDA) Comprehensive Fish Surveys for 1973, 1974, and 1975 found that freshwater fish contained the highest levels of PCBs. The U.S. EPA (1980) has estimated that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day. The current relative contribution of nonoccupational inhalation and dermal exposure was concluded to be small. Undoubtedly, nationwide PCB intake has decreased since 1975 due to more stringent regulatory measures taken in 1977 by the Environmental Protection Agency (IARC, 1978). It has also been recognized by the FDA however that the PCB intake of regular consumers of sport fish may be significantly different from the average (Jelinek and Corneliussen, 1976).

Contamination of the human population in the United States and other parts of the world is virtually universal. The National Human Monitoring Program of the EPA has detected PCBs in human adipose tissue and breast milk since the early 1970's (Kutz and Strassman, 1976). During 1973 and 1974, 35.1 and 40.3

percent of the tissues sampled contained levels of 1 ppm or more on a wet-weight basis. The New England, Middle Atlantic, South Atlantic, and East North Central Census Divisions consistently had frequencies of quantifiable levels exceeding the national frequency. The primary PCB isomers detected were the penta-, hexa-, and heptachlorobiphenyl compounds. All samples of human milk collected from Arkansas and Mississippi had trace amounts of PCBs.

Jensen (1987) estimated that the average background level of PCBs in blood plasma is close to 2 ppb. A literature review by Kreiss (1985) concluded that background serum PCBs levels in the United States are less than 10 ng/ml (ppb). On the other hand, populations with a documented intake of contaminated sport fish had serum PCBs concentrations more comparable to exposed industrial workers. Populations with occupational PCBs exposures have had reported geometric mean serum levels ranging between 21 ng/ml (ppb) to 119 ng/ml (ppb). Populations consuming contaminated fish in Michigan, Alabama, and Massachusetts were reported to have geometric means of 21.4 ng/ml (ppb) (median, 1980), 17.2 ng/ml (ppb) (1979), and 23.6 ng/ml (ppb) (1981), respectively.

A direct relationship between consumption of contaminated fish and elevated serum PCB levels has been documented. A population of Lake Michigan sport fish eaters had a range of 25 - 366 ug/kg (ppb) (median 56 ug/kg) in serum specimens in 1974 while a sample of Michigan farmers had a range of undetectable levels to 57 ug/kg (ppb) (median 6 ug/kg) (Humphrey, 1983). PCB concentrations in the Michigan farmers increased with age. The sample of 90 fish eaters ate more than 10.91 kg of fish per year (median 14.55 kg/yr) and had serum PCB levels significantly greater than people from the same communities who rarely ate such fish. The highest level of consumption

reported was 118 kg/yr. Individual serum PCB levels varied as a function of the quantity of fish eaten, type of fish eaten, and number of years of fish consumption. Humphrey estimated human exposure to be 46.5 mg PCBs per year based on eating cooked Lake Michigan fish contaminated with 0.4 to 5.4 mg/kg (ppm) PCB. Other nonoccupational exposures from ambient air and drinking water are included in Table 4.

A follow-up of the fish eaters indicated that in 1980 this group ate less fish (median 9.55 kg/yr) and had lower serum PCB levels (median 48 ug/kg). The range of serum values remained similar to the 1974 range however, and consumption by many individuals was still quite high (maximum 84 kg/yr).

Table 4. Comparison of Non-Occupational Sources of PCB Exposure.

Estimated Human Exposure	Exposure Source
7.8 ug/yr	Ambient air at 1.9 ng/m^3 air level PCB
2.9 ug/yr	Drinking water at 4 ng/l raw water PCB
46.5 mg/yr	Cooked Lake Michigan fish at 0.4 to 5.4 mg/kg (ppm) PCB

Source: Humphrey, 1983.

Typical average PCBs levels in milkfat have been reported to be 0.5 - 1.5 ppm using 12 peaks in the chromatogram for quantification (Jensen, 1987). Surveys of Michigan women have detected PCBs in all samples tested with a range of 0.3 - 5.1 mg/kg (ppm) (fat basis) in 1977-78 and 0.2 - 3.0 mg/kg (ppm) in 1980-81 (Humphrey, 1983). PCBs levels in milkfat may decrease during lactation, and with maternal age, weight and parity (Jensen, 1987).

Jensen has concluded that average PCBs levels in adipose tissue range from 0.5 - 10.0 ppm. An analysis of PCB congeners revealed the presence of the

following isomers in order of decreasing concentration; 2,2',4,4',5,5'-hexachlorobiphenyl > 2,2',3,4,4',5'-hexachlorobiphenyl > 2,2',3',4,4',5,5'-heptachlorobiphenyl > 2'3,4,4',5'-pentachlorobiphenyl (Jensen and Sundström, 1974).

PCBs levels in adipose tissues are higher in human males than females. A Canadian study was reported which analyzed autopsy specimens from two Ontario communities for several organochlorine compounds (Williams et al., 1984). A total of 91 samples were evaluated from Kingston, on Lake Ontario, and 84 specimens from Ottawa, a community outside of the Great Lakes basin. Patient age and weight distributions were not significantly different between the two populations. PCB residues were detected in 100% of the samples from both communities. Concentrations in males were significantly higher than those in the tissues of females residing near Lake Ontario; mean, 3708 ± 4620 ng/g (ppb) vs 1983 ± 1123 ng/g (ppb). Levels were also higher in males from Ottawa however the difference was not significant; 2167 ± 937 ng/g (ppb) vs 1718 ± 673 ng/g (ppb). Mean PCBs levels were also higher in samples from the Kingston community, although the difference was not significant.

It is difficult to make comparisons of tissue concentrations which have been measured at different points in time or by different investigators. Differences in analytical methodology, method of population selection and method of data reporting have caused direct comparisons in several instances to be impossible (Kreiss et al., 1985). Lawton et al. (1985) demonstrated a wide divergence in PCBs concentrations generated by alternative modes of reporting the same serum PCB data. The authors also noted that a great deal of variance was observed in results due to the choice of analytical method and variance in replicate readings of chromatograms.

PHARMACODYNAMICS OF PCBs

HUMAN

Pharmacodynamic data on PCBs in human beings are very scarce. Studies of exposed human populations suggest that PCBs accumulate in human tissues. The transfer of PCBs from mother to infant during gestation or via nursing may be a significant source of exposure to the developing fetus or infant.

The Michigan Department of Public Health, using 20 male volunteers, evaluated serum levels of chlordane, toxaphene, DDT and PCBs at various time points after one 3.3 gram meal of either lake trout or chinook salmon was consumed (MDPH, ND). Contaminant levels increased after the fish was eaten and reached a maximum after six hours. Serum concentrations rapidly decreased for the next 18 hours and then gradually to preconsumption levels by 168 hours. Serum levels in the eight subjects eating lake trout contaminated with 10.4 - 17.2 ppm PCBs rose from a concentration of 7.3 ppb (range, 3.3 - 39.0) to a maximum between 13 and 44 ug/l (ppb). Levels in the eight volunteers eating chinook salmon contaminated with 2.6 - 3.7 ppm PCBs rose from background levels to a maximum between 4 and 39 ug/l (ppb). Data was not presented for the four controls who ate fried cod containing undetectable levels of the toxicants. It is assumed from the observed behavior of PCBs in animals that the decrease in serum concentrations represented the metabolization and excretion of some PCB isomers and the redistribution of other isomers to adipose tissue.

Tissue concentrations of PCBs and PCDFs have been reported for patients in Japan and Taiwan who ingested rice oil contaminated with high concentrations of these chemicals. The poisoning incident in Japan occurred in 1968. Five

years after the poisoning was discovered, tissue concentrations in patients exhibiting a characteristic syndrome, termed Yusho, were 1.9 ± 1.4 ppm in adipose tissue (N=9), 0.08 ± 0.06 ppm in liver (N=8), and 0.0067 ± 5.3 ppm in blood (N=41). These levels were reported to be only twice those measured in the tissues of control subjects. In Taiwan, the recognizable symptoms were called Yu-Cheng, and blood concentrations one year after the exposure occurred were 0.099 ± 0.163 ppm (N=23) compared to 0.0012 ± 0.0007 ppm (N=29) in a Taiwanese control group (Masuda et al., 1985).

The ratio of adipose to liver PCBs concentrations increased over time. In 1969 a ratio of 14 was observed in the Yusho patients while in 1972 and 1977, the ratio had increased to 143 and 200 respectively. The concentration of PCBs in the adipose tissue measured at these three time points indicate that the compounds were redistributed to fat and then gradually eliminated. Concentrations were reported to be 2.8 ppm, 4.3 ppm, and 1.2 ppm in 1969, 1972, and 1977, respectively.

Maternal elimination of PCBs transplacentally and through nursing has been confirmed for human beings (Masuda et al., 1978; Schwartz et al., 1983; Jacobson et al.,1984; Rogan et al., 1986). Breastfeeding, in particular, may be a major route of elimination for certain PCB isomers in women.

A few studies of PCB transfer from mother to healthy infant have been conducted. Two investigations were reported in the Japanese population in the late 1970's. Masuda et al. (1978) collected paired samples of maternal and cord blood at normal deliveries, and maternal adipose tissue specimens, maternal blood, and cord blood samples at Caesarean births during 1973 to 1974 in Fukuoka, Japan. In addition, samples of infant blood, maternal blood and

breast milk were collected from healthy, breast-fed infants, two to three months old, and their mothers visiting the Kurume University School of Medicine during 1974 to 1975. A second group of samples were taken from babies, 4 - 12 months old, and their mothers. PCBs concentrations were quantified using a 1:1 mixture of Kanechlor 500 and Kanechlor 600 as a standard. A positive, but weak correlation was observed between maternal blood and cord blood ($r=0.244$, $N=60$). Mean cord blood levels, 0.61 ± 0.05 ppb, were significantly lower than maternal blood levels, 2.5 ± 0.14 ppb. Maternal blood concentrations were also significantly correlated with maternal milk measured on a fat basis ($r=0.289$). Furthermore, maternal blood concentrations were significantly correlated with maternal adipose tissue ($r=0.444$, whole basis; $r=0.451$, fat basis). Adipose tissue (mean, 600 ± 44 ppb) however, was not correlated with cord blood.

The correlation of maternal serum levels with cord serum levels indicated that PCBs are transferred from mother to infant during gestation. This was confirmed by the finding of detectable PCBs concentrations in adipose tissue, liver, and adrenal glands of 7 - 10 month old still-born fetuses ($N=14 - 16$). The significantly lower concentrations in cord blood led the authors to suggest the existence of a placental barrier to PCBs and to conclude that transfer via milk is more significant that placental transfer.

PCBs levels in the blood of infants in both age groups were significantly higher than levels in maternal blood, although there was no correlation between the levels in infant blood and maternal blood or milk. Mean PCBs concentrations in the blood of 2 to 3 month old babies were 2.4 ± 0.22 ppb compared to 1.8 ± 0.67 ppb in their mothers' blood. Mean concentrations in 4

to 12 month old babies (partially weaned) were 2.5 ± 0.17 ppb compared to 1.3 ± 0.64 ppb in their mothers' blood.

Kodama and Ota (1980) also found that cord blood was significantly correlated with maternal blood in a sample of volunteer women with normal deliveries sampled between 1974 and 1977 at a hospital in Aichi Perfecture, Japan. Cord blood levels were also approximately four times lower than maternal blood levels. These investigators conducted a time course study of maternal blood levels in 17 pregnant women at 8 and 4 months prepartum, and at delivery, one, three, five, and seven months postpartum. Maternal blood levels increased significantly between 8 and 4 months prepartum from 3.0 ppb to more than 4.5 ppb. It was suggested that the rise in concentration may be due to a corresponding increase in serum lipid that occurs as a normal part of pregnancy. After delivery, blood concentrations decreased markedly, especially between one and three months postpartum. PCBs levels in infant blood rose sharply during the first three months of life becoming significantly higher than maternal blood concentrations. The infant blood levels continued to increase during the first year, gradually decreasing thereafter. Blood concentrations in breast-fed infants were higher than bottle-fed infants at every sampling period.

A study of 313 women who ate contaminated Lake Michigan fish and their newborn infants correlated PCB concentrations in cord serum with levels in maternal blood (Schwartz et al., 1983, Jacobson et al., 1984). Median PCBs concentrations, quantified using an Aroclor 1260 standard, were 2.0 ng/ml (ppb) (whole basis, N=198) in cord serum and 4.6 ng/ml (ppb) (whole basis, N=196) in maternal serum. PCB levels in breast milk fat (median neonatal, 742.9 ng/g (ppb) fat basis; median 5 months, 641.7 ng/g (ppb) fat basis) were

also correlated with maternal serum. Again, concentrations were higher in milk and maternal serum than cord serum.

Rogan et al. (1986) reported on a study of contaminants in a nonrandom sample of 807 women in North Carolina (Rogan et al., 1986). The median PCBs concentration in breast milk at birth was 1.77 ppm (fat basis). These levels declined during lactation by 20% after six months and by 40% after 18 months. PCBs levels were also 12% lower in mothers who had breast fed previously. The authors concluded that excretion through nursing is a major factor in eliminating PCBs from the mother and implies a substantial exposure to the child. Rogan et al. also observed that maternal serum levels were higher than cord serum levels or placental PCB concentrations. The authors noted that fat concentrations are lower in cord serum and placenta and suggested that some of the difference in PCB concentration between cord serum and maternal serum may be attributable to the difference in fat content. A partial placental barrier to PCB transfer was also suggested.

Niessen et al. (1984) conducted an inventory of adipose tissue concentrations of chlorinated hydrocarbons in relation to intake of mother's milk in 50 randomly selected children aged one year (N=34), two years (N=14), and over three years (N=2) undergoing surgery at a hospital in Germany. The greatest body burden was always higher in children for which a high mother's milk intake was reported and the difference was statistically significant. The authors concluded that the concentrations measured in the children reflected primarily the long-term environmental exposure of the mother. This conclusion was supported by the observation that the body burden of PCBs and hexachlorobenzene was higher in German infants than in Turkish infants born and nurtured in Germany with mothers who had entered the country within the

last eight years. Turkey is a less highly industrialized country than Germany.

ANIMAL

The pharmacodynamics of several PCB congeners has been discussed in depth in the literature and it is possible to compare the behavior of certain congeners in more than one animal species. In addition, the importance of the degree of chlorination and position of chlorine atoms on the biphenyl ring in determining the relative ability of animal species to metabolize and excrete these compounds has been explored. This knowledge facilitates the selection of an appropriate animal model for extrapolating toxicity data to humans and provides some insight into the species variation in biological response to commercial PCB formulations.

Absorption

PCBs are almost completely absorbed from the gastrointestinal tract of rats and monkeys after oral administration of the experimental material. The administration of 5, 50, and 100 mg of 19 PCB isomers by gavage to male CD rats resulted in >90% absorption of all isomers at all dose levels (U.S. EPA, 1984). Jensen and Sundstrom (1974) fed rats a single dose of 20 mg/ml 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-HCB) dissolved in peanut oil in the diet. It was determined that approximately 15 mg PCB was consumed during the night. Seven percent of the total dose was excreted in feces during seven days after administration as unchanged biphenyl indicating that at least 93% of the isomer had been absorbed. Male Sprague-Dawley rats given a single 50

mg dose of radiolabeled 2,2',5,5'-tetrachlorobiphenyl (2,5-TCB) by gastric intubation excreted 10% of the total dose as unmetabolized compound over 14 days (Van Miller et al., 1975).

Monkeys have demonstrated a similar pattern of absorption. Male rhesus monkeys were dosed 1.5 g or 3.0 g Aroclor 1248/kg body weight by gastric intubation (Allen et al., 1974). Urine and feces were collected for two weeks. Greater than 90% of the total dose was determined to be absorbed from the gastrointestinal tract. Similar results were achieved when 18 mg 2,5,2',5'-tetrachlorobiphenyl (TCB)/kg body weight was administered by gastric intubation to seven adult male rhesus monkeys (Allen et al., 1975). After 14 days, over 12% of the total dose was recovered from feces as unmodified TCB.

It is possible that absorption is greater than 90% since the finding of unmodified parent compound in the feces does not exclude its absorption and subsequent excretion via the biliary tract. The effect of the dosing vehicle on gastrointestinal tract absorption has not been evaluated (U.S. EPA, 1987).

Excretion

Elimination of PCB isomers shows wide species variation due to its dependence upon the rate of metabolism. Metabolism of PCBs by rats, mice and monkeys is determined by the degree of chlorination as well as the position of chlorine atoms on the biphenyl ring. These parameters do not appear to be as important in dogs.

Matthews and Anderson (1975) studied the effect of chlorination on distribution and excretion in Sprague-Dawley rats. Rats were dosed 0.6 mg/kg (ppm) of 4-chloro-(1-CB), 4,4'-dichloro-(2-CB), 2,4,5,2',5'-pentachloro-(5-

CB), or 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) by i.v. injection into the tail vein. The percent of the total dose that was excreted in urine decreased with increasing chlorination and was 59.8%, 33.9%, 7.6% and 0.7% for mono-, di-, penta-, and hexa-CB respectively. The majority of the radioactivity in urine was excreted during the first 24 hours after administration. In contrast, the importance of the excretion of PCBs and metabolites in feces increased with increasing chlorination. Less than 10% of the total dose of the compounds was excreted unmetabolized and it was concluded that metabolism is a key factor in distribution and excretion.

The effect of chlorination on distribution and excretion was studied using the commercial formulations, Kanechlor-400 and Kanechlor-600 (Hashimoto, 1976). Male albino JCL-S.D. strain rats (12 animals/group) were administered a total dose of 7.14 to 7.85 mg/kg (ppm) Kanechlor-400 or Kanechlor-600 by gastric intubation over varying periods of time (Kanechlor-400 to 4 groups, once/week for 5, 10, 30, and 50 weeks; Kanechlor-600 to 1 group, once/week for 5 weeks). A higher percentage of radioactivity was excreted in urine after the last dose in rats dosed with Kanechlor-400 for five weeks than those given Kanechlor-600 (1.9 ± 0.96% vs 0.56 ± 0.15%). The authors concluded that the two mixtures have different water solubilities and different tendencies to be metabolized to more soluble compounds which would affect urinary excretion. Excretion in urine was mostly completed two days after each dosing in all groups.

The percentage of total dose in urine also increased with the length of the dosing period. Seven days after the final administration of Kanechlor-400, 1.9, 2.4, 4.4, and 4.9% of the dose was excreted in urine in groups dosed for 5, 10, 30, and 50 weeks respectively. It was concluded that this was due to the increased metabolism of PCB in the body with time. It was also noted that

since the total dose was equivalent in each dose group, the individual doses were smaller as the treatment period increased and may have had some effect on urinary excretion.

A much higher percentage of total dose was eliminated via feces. The percentage of the total dose measured in feces did not differ significantly with the period of administration of Kanechlor-400 (67.6 ± 6.6, 60.4 ± 5.0, 46.7 ± 13.8, and 53.6 ± 5.4) or between Kanechlor-400 and Kanechlor-600 (57.5 ± 7.8).

The importance of the presence of two adjacent unsubstituted carbon atoms on the biphenyl ring has also been demonstrated. Over 90% of a total i.v. dose of 2,4,5,2',5'-pentachlorobiphenyl, which has unsubstituted carbons at the meta and para positions on one biphenyl ring, was excreted over 42 days while the excretion of 2,4,5,2',4',5'-hexachlorobiphenyl (245-HCB) over the lifetime of the rat was calculated to be less than 20% (Matthews and Anderson, 1975). The comparison of two tetrachlorobiphenyl compounds, 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyl, with the penta- and hexa-chlorinated biphenyls illustrates this point further. Cumulative excretion of PCBs in feces over seven days occurred in the following decreasing order; 2,5,2',5'-TCB > 2,4,5,2',5'-PCB > 3,5,3',5'-TCB > 2,4,5,2',4',5'-HCB (Anderson et al., 1977). The first two compounds have unsubstituted carbon atoms while the latter two do not. Several authors have suggested that the presence of adjacent unsubstituted carbons facilitates rapid metabolism through the formation of arene oxide intermediates and subsequent excretion of the more polar metabolites.

Kato et al. (1980) studied the metabolism and excretion of four symmetrical hexachlorobiphenyls by male Sprague-Dawley rats administered 0.6 mg/kg by i.v. injection. Total excretion over seven days is presented in Table 5. Most of the excreted radioactivity was in feces and less than 1% of the total dose was in urine. 2,3,6,2',3',6'-hexachlorobiphenyl (2,3,6-HCB), the only compound with vicinal unsubstituted carbon atoms, was metabolized and excreted 7 to 19 times more rapidly than the other hexachlorinated biphenyls. Note that regardless of the amount of radioactivity excreted, less than 5% of it was unmetabolized compound.

TABLE 5. Total excretion of four symmetrical hexachlorobiphenyls in the rat over seven days.

Compound	% Total Dose	% Unmetabolized
2,3,6,2',3',6'-HCB	92.74	4.65
2,4,6,2',4',6'-HCB	13.46	1.75
2,4,5,2',4',5'-HCB	5.50	4.86
2,3,5,2',3',5'-HCB	4.49	2.96

Source: Kato et al, 1980.

Pharmacokinetic studies have been conducted for 2,3,6-HCB and 2,4,5-HCB using rats, monkeys, and dogs allowing the comparison of excretion and distribution across species. Table 6 and 7 summarize the cumulative excretion of the two isomers at various time points after an i.v. administration to rat, monkey and dog.

TABLE 6. Excretion of 2,3,6,2',3',6'-hexachlorobiphenyl in the rat, monkey and dog at various time points after i.v. administration of 0.6 mg/kg.

Species		Percent of Total Dose			
		24hr.	3 d	7 d	15 d
Rat[1]	feces			92.74	
	urine			<1	
Monkey[2]					
	feces	10			46
	urine	7.7			15
Dog[2]	feces	41	56		
	urine	11	14		

Source
(1) Kato et al., 1980.
(2) Sipes et al., 1982.

TABLE 7. Excretion of 2,4,5,2',4',5'-hexachlorobiphenyl in male rat, monkey and dog after i.v. administration of 0.6 mg/kg.

Species		Percent of Total Dose				
		24hr	7 d	15 d	42 d	90 d
Rat	feces	2.4[1]	5.5[2]		14[3]	
	urine	0.8[1]	<1[2]		<1[3]	
Monkey[4]						
	feces			6		17
	urine			<1		1
	total				17	
Dog[4]	feces	12		63		
	urine	<1		3		

Source
(1) Matthews and Anderson, 1975.
(2) Kato et al., 1980.
(3) Matthews and Tuey, 1980.
(4) Sipes et al., 1982.

Species variability in the elimination of the two isomers was quite pronounced. Dog and monkey were more similar to each other than the rat in the degree of excretion of 236-HCB and metabolites in the urine. The monkey and dog excreted 15% and 14% of the total dose in urine at 15 days and 3 days

respectively while the rat excreted less than 1% in 7 days. Excretion of 245-HCB in urine was similar in all three species. Rat and monkey eliminated less than 1% of the total dose while the dog excreted slightly more (3%).

The rate of excretion of 236-HCB was more rapid in the rat and dog than the monkey. Approximately 93% and 70% of the dose was present in the excreta of rat and dog after 7 and 3 days respectively. The monkey had eliminated only 61% after 15 days. In contrast, the rat eliminated 245-HCB at a rate more similar to the monkey. The dog excreted 66% of the total dose after 15 days while the rat and monkey excreted 14% and 17% respectively of the total dose after 42 days. It was estimated that only 20% (Matthews and Anderson, 1975) and 38% (Sipes et al, 1982) of the total dose would be excreted over the lifetimes of the rat and monkey. Elimination rate constants are presented in Table 8 for 2,3,6-HCB and 2,4,5-HCB which further demonstrates the variability in excretion of these isomers across species.

TABLE 8. Elimination Rate Constants for Two Hexachlorobiphenyl Isomers in Feces of Rats, Monkeys, and Dogs.

Species		Elimination Rate Constant (per day) 2,3,6-HCB	2,4,5-HCB
Rat[1]	Phase 1	0.93 (0.11)	0.59 (0.17)
	2	0.125 (0.021)	0.031 (0.003)
Monkey[2]	Phase 1	0.283 (0.031)	0.182 (0.011)
	2		0.006 (0.001)
Dog[3]	Phase 1	1.302 (0.324)	0.164 (0.015)

S.E. in parentheses
Source:
(1) Matthews and Tuey, 1980.
(2) Sipes et al, 1982a.
(3) Sipes et al, 1982b.

Less than 5-10% of the radioactivity present in excreta of the rat, monkey, and dog was the parent compound (Kato et al, 1980, Matthews and Anderson, 1975, Sipes et al, 1982a and 1982b).[1] Metabolism is therefore important for elimination of the polychlorobiphenyl isomers in all three species.

Biliary excretion is the major route of excretion for rat, mouse, monkey and dog (Matthews and Anderson, 1975; Sipes et al., 1982a and 1982b). The rates of biliary excretion vary however according to the species' ability to metabolize PCB isomers. Structure dependent metabolism therefore is the rate limiting step for elimination. Sipes et al (1982b) concluded that enterohepatic circulation was not significant in the dog however reabsorption of parent compound from the intestine after its secretion in bile may be more important in the monkey. It has been concluded that enterohepatic circulation plays a relatively minor role in rat excretion (Lutz et al., 1977; Matthews and Anderson, 1975b).

Total excretion of PCB isomers with various degrees of chlorination in the mouse is similar to that in the rat (Tuey and Matthews, 1980). However, the mouse excreted a larger proportion of the less chlorinated isomers in the urine than the rat. In addition, biliary clearance rates for the isomers studied in the mouse were greater than would have been predicted from the rat. The rates decreased with increasing chlorination while, in the rat, biliary clearance rates remained almost constant (Matthews and Tuey, 1980). Reabsorption of metabolites of the less chlorinated isomers from the intestine

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1Sipes et al. (1982b) measured 50% parent compound in the feces of monkeys at 24 hours. The percentage was below 5% at subsequent time points.

was also found to be more significant in the mouse which may account for its greater excretion in urine.

PCBs are transferred transplacentally and through nursing in monkeys (Allen et al.,1974), rats (Shain et al., 1986) and mice (Gallenberg and Vodicnik, 1987). The infant of a rhesus monkey fed 25 ppm Aroclor 1248 for two months had measurable levels of PCBs in its body when it was born eight months after treatment was discontinued (Allen et al., 1974). Levels in fat and adrenals were particularly high, 27.7 and 24.4 ug/g (ppm), respectively, indicating that transplacental transfer of PCBs in monkeys may be significant.

Placental exposure was relatively small compared to the amount of PCBs received through nursing in mice. The offspring of mice pretreated with 50 mg/kg 2,4,5,2',4',5'-hexachlorobiphenyl two weeks prior to mating had accumulated only 3% of the mother's dose on the day of birth however the dam's entire body burden was subsequently eliminated through lactation by the day of weaning (Gallenberg and Vodicnik, 1987). Lactation, therefore, is a very efficient route of elimination in nursing mothers and a potentially significant source of exposure for the breast feeding infant.

The accumulation of PCB congeners was analyzed in tissues of newborn and weanling rats to describe the effects of gestation and lactation (Shain et al., 1986). While detectable levels were found in all rat pups at birth and at 21 days of age, the older pups had greater congener concentrations in tissues. It was found that the same congeners were bioaccumulated during gestation and lactation indicating that lactation produced a higher effective dose of specific congeners. The bioaccumulation of individual congeners was

related primarily to molecular structure. The presence of chlorine atoms at the 4 position was associated with accumulation in tissues.

Distribution

PCB isomers exhibit a similar pattern of distribution in the tissues of various species (Van Miller et al, 1975, Allen et al, 1975, Matthews and Anderson, 1975, Matthews and Tuey, 1980, Sipes et al, 1982a and 1982b). The parent compound is rapidly cleared from the blood and is initially distributed to muscle and liver. Muscle has a large tissue mass and therefore receives a significant proportion of the initial dose, and liver has an intrinsic ability to extract xenobiotics. This is followed by metabolism and biliary excretion or redistribution of the parent compound and lipophilic metabolites into skin and adipose tissue. Final distribution is determined by the amount of lipid contained in a tissue or organ.

Lutz et al. (1977) developed a simple, flow-limited model for PCBs distribution. A flow diagram of the model is shown in figure 1. The model assumes that the tissue concentration of parent and metabolite maintains equilibrium with the venous blood.

The degree of chlorination and the position of the chlorines on the biphenyl molecule is a major determinant of the extent of PCBs metabolism and excretion (see section on excretion) or subsequent distribution in tissues. Less chlorinated isomers are more easily metabolized and excreted while more highly chlorinated isomers are not easily metabolized and accumulate in tissues.

Matthews and Tuey (1980) found that four hexachlorobiphenyl isomers were cleared from the liver of rats after a single i.v. dose in the following order

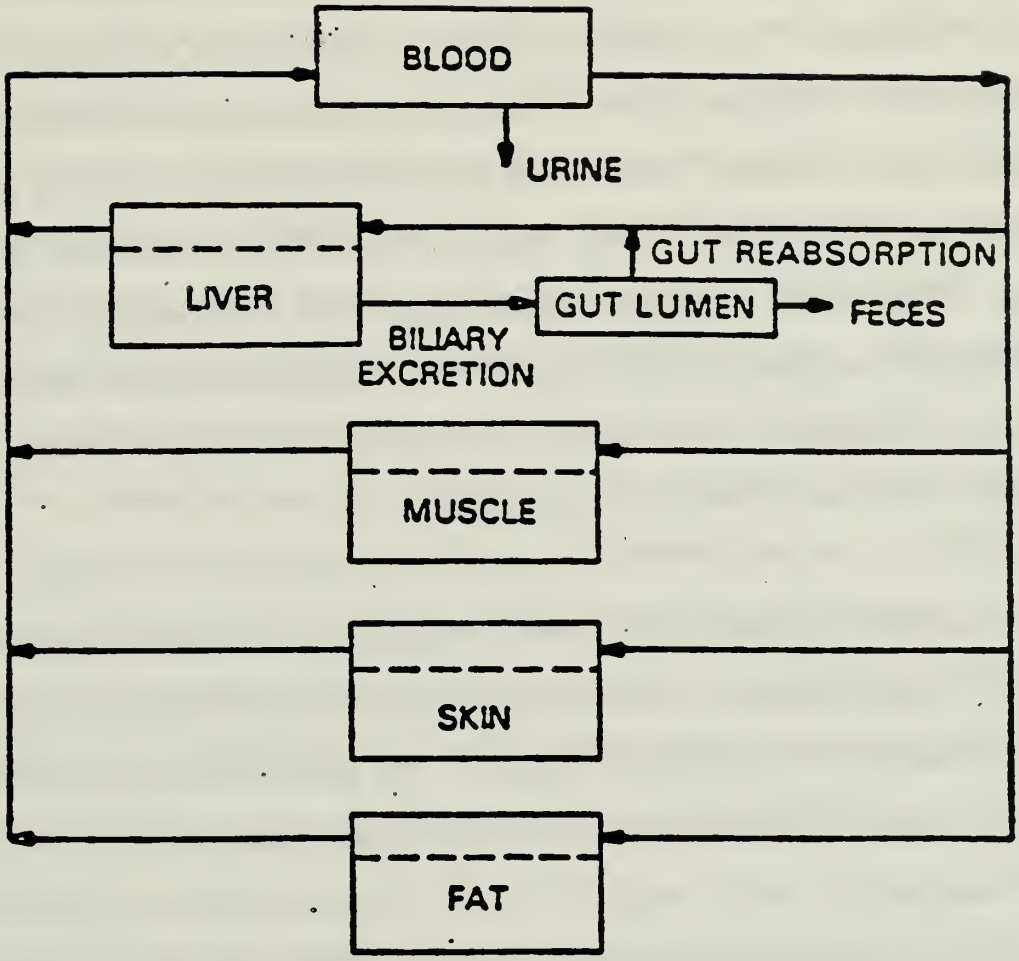

FIGURE I

PCB Pharmacokinetic Flow Diagram

Source: Lutz et al., 1977

of clearance rate; 236-HCB > 246-HCB > 245-HCB > 235-HCB with second phase half-lives of 2.4, 12.8, 33.3, and 33.7 days respectively. The rapid clearance of 236-HCB from liver was due to the more rapid excretion of metabolites in the bile. Clearance of the other compounds from liver was dependent on the slower redistribution to tissues of higher affinity and lower blood perfusion. 2,3,6-HCB was rapidly cleared from skin after its initial distribution relative to the other three isomers which accumulated in this tissue over 24 hours. After four days during which an initial decrease occurred, 235-HCB and 245-HCB showed no significant reduction in skin for the rest of the study. Adipose tissue also showed a decrease in 236-HCB concentration after 2 hours, however the concentrations of the other three hexachlorobiphenyls continued to increase for 14 days and remained at peak levels over the 42 days of observation.

Matthews and Anderson (1975) found that the more highly chlorinated isomers take a longer period of time to reach a peak concentration in skin and adipose tissue than less chlorinated isomers. The magnitude of the concentration in tissues varied to the degree of chlorination. However, this relationship was not observed for skin. Mono-CB, di-CB, and penta-CB were cleared from the skin with half-lives which increased with chlorine content. 245-HCB, however, showed no decrease in skin after four days. The magnitude of the concentration in adipose tissue increased with the degree of chlorination. The concentration of the hexachlorobiphenyl peaked after 7-14 days and remained constant for the remainder of the study while the other three isomers decreased in concentration at rates which varied inversely with degree of chlorination.

Similar results were attained for chronic administration of the commercial PCB mixtures, Kanechlor-400 and Kanechlor-600 (Hashimoto et al., 1976). After dosing rats once/week for five weeks, Kanechlor-600 tissue concentrations were generally higher than those of Kanechlor-400, even though the total dose of Kanechlor-600 administered was smaller. The highest concentrations were measured in adipose tissue regardless of dosing schedule for both compounds. Intermediate concentrations were measured in skin, adrenal gland, aorta, and sciatic nerve. Rats given Kanechlor-600 had twice the concentration of PCB in adipose tissue than rats given Kanechlor-400 for the same time period. The clearance rate from tissues was slower for Kanechlor-600, and the authors suggested that more highly chlorinated isomers were accumulating. Four groups were treated with Kanechlor-400 for five, ten, thirty, and fifty weeks, respectively. The concentrations in most tissues 30 days after the last dose was given were reported to vary with the period of administration. Animals dosed for a longer period of time had lower PCBs concentrations in most tissues than those with shorter administration periods. In addition, chronic administration seemed to affect the rate of clearance from tissues in that elimination became slower as the duration of treatment lengthened.

Rats treated for 52 weeks with various Aroclor mixtures containing different amounts of chlorine showed a similar distribution in tissues but differed in the extent of accumulation. Allen et al. (1976) fed Sprague-Dawley rats (24/group) a diet containing 100 ppm Aroclor 1248, Aroclor 1254 or Aroclor 1262. The control was fed an unmodified diet. Four rats per group were killed after 13, 26, 39 and 52 weeks of treatment. The surviving animals were fed the unmodified diet for an additional 13 weeks. The lowest concentrations of PCB isomers were measured in the tissues of rats fed Aroclor 1248. Aroclor 1262 concentrations were higher than Aroclor 1254 levels in fat, kidney, and

brain at all time points except in the kidney at 52 weeks. Fat levels of Aroclor 1262 were 6 to 6.5 times higher than Aroclor 1248 concentrations in fat over the entire 52 weeks. Levels of Aroclor 1254 were approximately 4 times higher than levels of Aroclor 1248 for 26 weeks and then decreased to levels twice as high from 26 to 52 weeks. Adipose tissue contained the highest levels for all PCBs over liver, kidney and brain. Steady state levels were reached in the tissues between 39 and 52 weeks. Concentrations in all tissues decreased during the 13 week recovery period in rats fed Aroclor 1248. In contrast, levels in adipose tissue during the recovery period were maintained at high levels in the rats fed Aroclor 1254 and Aroclor 1262 and were 6 and 17 times greater than the concentrations in Aroclor 1248 fed rats at 65 weeks.

A comparison of the distribution of a "pure" isomer with a commercial mixture with comparable chlorination indicates the importance of the action of the more highly chlorinated components (Allen et al, 1975). Male Sprague-Dawley rats fed 100 ppm Aroclor 1248 in the diet for four weeks had significant levels of PCB isomers in the adipose tissue, liver, kidney, and brain while those fed 100 ppm 2,5,2',5'-tetrachlorobiphenyl had appreciable levels in adipose tissue only. Adult male rhesus monkeys administered 18 mg/kg body weight Aroclor 1248 or the TCB by gastric intubation responded similarly. The tissue levels of monkeys dosed with Aroclor 1248 were higher than those of monkeys treated with the TCB. This finding does not rule out the potentially more important influence of chlorine position on distribution.

Metabolites are also distributed in body tissues. The proportion of radioactivity in the tissues accounted for by metabolites decreases with increasing chlorination. While animals dosed with mono-, di- and

pentachlorobiphenyl had maximum percentages between 80 to 96% and 52 to 98% metabolites in blood and liver respectively, the hexachlorobiphenyl group had 53% and 20% metabolites in blood and liver at 4 days (Matthews and Anderson, 1975). Skin and adipose tissue concentrations of the three less chlorinated compounds contained maximum metabolite percentages of 44, 19, and 6% while the hexachlorobiphenyl dose group had 3% metabolites in skin and less than 1% in adipose tissue. Matthews and Anderson (1975) measured highest percentages of metabolites in the blood for all of the isomers studied. The liver, muscle, skin, and adipose tissue contained decreasing percentages. This data confirms that less chlorinated isomers are more easily metabolized to more polar compounds and primarily excreted, while the more highly chlorinated isomers are much less easily metabolized and are stored primarily in skin and adipose tissue.

Matthews and Tuey (1980) determined that chlorine position also influences the degree to which PCBs are metabolized and sequestered in the tissues. Hexachlorobiphenyls with two vicinal unsubstituted carbons such as 2,3,6-HCB, had far greater percentages of metabolites in blood, liver, muscle, skin and adipose tissue. Up to one-half and one-third of the radioactivity in skin and adipose tissue was metabolites of 2,3,6-HCB whereas 2,4,6-HCB, 2,3,5-HCB, and 2,4,5-HCB had no greater than 3% metabolites in skin and 1% or less in adipose tissue.

Animal species show differing diposition patterns which relate to their ability to metabolize and excrete various PCB congeners. Tables 9 and 10 compare the distribution of an i.v. administration of 2,3,6,2′,3′,6′- and 2,4,5,2′,4′,5′-hexachlorobiphenyl in the tissues of three animal species.

TABLE 9. Tissue Distribution of 2,3,6,2',3',6'-hexachlorobiphenyl in rat, monkey and dog at various time points after administration by i.v. injection.

Percent of Total Dose

Tissue	Rat[1]		Monkey[2]			Dog[2]	
	1hr	14d	1hr	24hr	15d	1hr	24hr
Liver	12		25			25	9
Muscle	18		35			35	4
Skin							4
Adipose Tissue	10	<1	10	14	23	2-3	5

Source:
(1) Matthews and Tuey, 1980.
(2) Sipes et al, 1982.

TABLE 10. Tissue Distribution of 2,4,5,2',4',5'-hexachlorobiphenyl in rat, monkey and dog at various time points after administration by i.v. injection.

Percent of Total Dose

Tissue	Rat[1]			Monkey[2]			Dog[2]		
	1hr	24hr	42d	2hr	24hr	90d	30min	24hr	15d
Liver	25	4	<1	36			34		
Muscle	38	11	8	34			48		2
Skin	11	22	16		13	5		9	6
Adipose Tissue	5	23	85		35	45		33	16

Source:
(1) Matthews and Anderson, 1975
(2) Sipes et al, 1982b.

Distribution of 2,3,6-HCB in the rat and dog reflected their greater ability to metabolize and excrete this material. The rat liver contained a lower percentage of the total dose than the monkey and dog after one hour possibly indicating a higher metabolism rate of the compound. Adipose tissue of the rat and monkey contained the same percentage of total dose (10%) at one hour after administration while the dog adipose tissue levels were much less (2-3%). The monkey continued to accumulate the compound in adipose tissue over a 14 day period. Levels in the dog decreased slightly between 24 hours and 3 days while levels in the rat were less than 1% of the total dose at 14 days. Unmetabolized compound was identified in adipose tissue at levels of 70% and greater than 75% of the total radioactivity in that tissue in the rat and monkey respectively one day after dosing while the dog had levels about 20% lower. The liver of the dog contained less than 7% unmetabolized isomer at one day after administration while the rat and monkey livers contained 18% and less than 50% parent compound respectively.

Distribution of 2,4,5-HCB in the rat and monkey appears to be similar while the dog is much different. Rat and monkey accumulated the isomer in skin and adipose tissue to a greater extent than the dog. Levels in adipose tissue continued to increase over the study period in the rat (42 days) and the monkey (90 days). The dog, however, was able to decrease its concentration by approximately 50%. Unmetabolized compound comprised greater than 80% of the radioactivity in liver, muscle, skin, and adipose tissue in all three species at all time points. The one exception occurred in the liver of the dog which contained 80% metabolites at 24 hours. The data indicate that the dog has a unique ability to metabolize 2,4,5-HCB to a significant degree.

The pattern of distribution and excretion of PCB isomers with various degrees of chlorination and its dependence on metabolism rate is comparable in the mouse to the rat (Tuey and Matthews, 1980).

The distribution and excretion of poorly metabolized congeners in the tissues of the rat is influenced by its characteristic increase in adipose tissue mass during most of the species adult life. Wyss et al. (1986) studied the long-term pharmacokinetics of 245-HCB in rats fed a reduced diet which led to a constant body weight over 40 weeks. Thirty-four rats were injected with 0.6 mg/kg PCB 14 days after being put on a diet 50% of ad libitum intake. Only unmetabolized compound was detected in tissues and feces. Significant concentrations were detected in adipose tissue, skin and muscle which accumulated slowly reaching maximum values between two and four weeks. Similarly to rats fed ad libitum, adipose tissue and skin contained 68% and 15% of the dose respectively. In contrast to rats fed ad libitum which retain these high levels, after 280 days, the levels in rats with constant adipose tissue mass had decreased to 38% and 7% of the dose respectively. The elimination half-life of the terminal phase of the compound in the blood was 462 days.

Forty-three percent of the dose was excreted in feces over 280 days. The elimination half-life of the terminal phase was 478 days. Extrapolation to infinite time for these rats indicated that 99% and 2% of the dose would be excreted in feces and urine. Therefore, although excretion is complete, the reduction in body burden is still quite slow.

The increasing adipose tissue mass in rats fed ad libitum acts as a permanent storage site for PCBs. Concentrations in rats with constant adipose tissue

mass reach an equilibrium with blood levels so that blood clearance in these animals is slower. The relatively higher concentrations in the blood may deliver a higher effective dose over longer periods to target organs. The behavior of PCBs in the adult human may parralel either the increasing or constant adipose tissue scenarios reported in the rats depending on individual behavior patterns.

Metabolites

Phenols and their glucuronides are the major identified metabolites of PCBs (IARC,1978). Mono and dihydroxylated PCB metabolites have been identified in the excreta of mice, rats, rabbits, chickens, goat, cow, and rhesus monkey. Several authors have proposed the formation of an arene oxide intermediate as a common pathway for metabolism due to the identification of trans-dihydrodiols and methylsulphone derivatives of PCBs in the excreta of rodents. Dihydroxy PCB metabolites have also been identified in the urine of nonhuman primates (Hsu et al, 1975). Methylsulfone PCB metabolites were found in the human milk of a former employee in a capacitor factory (Safe, 1984). Methylthio and methylsulfone derivatives of PCBs were identified in the liver, lung, and adipose tissue of Yusho patients (Masuda et al., 1985).

Studies of unchlorinated biphenyl have indicated that the preferred route of biphenyl metabolism involves the formation of an arene oxide between the number 3 and 4 carbons of one biphenyl ring (Matthews, 1983). Therefore, PCB isomers with unsubstituted carbons at positions 3 or 4, and isomers with adjacent unsubstituted carbons would be expected to be more easily metabolized. This has been demonstrated by studies of hexachlorobiphenyls. 2,3,6-HCB was metabolized and excreted as rapidly as 4,4'-dichlorobiphenyl whereas 2,3,5-HCB and 2,4,5-HCB could not be completely eliminated over the

lifetime of the animal (Matthews, 1983). Matthews and Tuey (1980) reported that the excretion of metabolites of 2,4,6,2',4',6'-HCB and 2,4,5,2',4',5'-HCB in bile was as much as ten fold greater than was observed for 2,3,5,2',3',5'-HCB. 2,4,6-HCB and 2,4,5-HCB both have open meta positions which may offer a site of enzymatic attack for direct insertion of a hydroxyl group. This is a minor mechanism in the rat however.

Conclusion

Metabolism, distribution and elimination do not appear to be affected by the size of dose administered in rats or monkeys in the dose ranges administered. Male Sprague-Dawley rats treated with 0.6 or 6.0 mg 2,4,5,2',5'-pentachlorobiphenyl/kg cleared the material from the blood at equivalent rates (Matthews and Anderson, 1975b). Rhesus monkeys treated with 1.5 or 3.0 g Aroclor 1248/kg body weight by gastric intubation eliminated 5.75 and 5.6% of the total dose in excreta over 14 days (Allen et al, 1974).

Route of administration (i.v. vs oral) may have some effect on distribution and elimination. Differences in the percent of total dose were noted in tissues of rats administered similar doses by intubation or i.v. injection. The percent of radioactivity in adipose tissue of rats treated orally with 4-chloro, 4,4'-dichloro, or 2,4,5,2',5'-pentachlorobiphenyl was lower (Matthews and Anderson, 1975a). This difference in distribution was not apparent in the adipose tissue of rats treated with 2,4,5,2',4',5'-hexachlorobiphenyl. The percentage of the total dose excreted in bile was slightly higher and the amount excreted in the urine was slightly lower in rats treated orally. The authors concluded that the oral dose was metabolized to a greater extent in the liver reflecting its slower introduction via absorption and transport directly to the liver via the hepatoportal vein. This first pass effect would

be predicted to have more of an impact on the more rapidly metabolized isomers. Subsequently, these investigators (1975b) did not observe differences in the content of radioactivity in the major tissues of rats administered with 0.6 mg/kg 2,4,5,2',5'-pentachlorobiphenyl by oral intubation or i.v. injection. The percent of the total dose excreted was also similar in the two dose groups.

Acute and chronic administration appears to have little effect on the pattern of distribution (Allen et al., 1976, Hashimoto et al., 1976). Metabolism and urinary clearance may increase slightly as duration of exposure is lengthened (Hashimoto, et al., 1976).

Evidence suggests that the pharmacodynamics of PCBs in humans is similar to monkeys and quite different from dogs (Sipes et al,1985). The metabolism of 4,4'-di, 2,3,6,2',3',6'-hexa, and 2,4,5,2',4',5'-hexachlorobiphenyl by human, cynomolgus monkey and beagle liver microsomes was studied. 2,4,5-HCB was not metabolized by human or monkey liver microsomes but was metabolized by dog liver microsomes. 4-DCB and 236-HCB were metabolized by all three species. The metabolic constants and metabolic rates were similar for human and monkey and these two species were different from the dog for both isomers. In addition the metabolites generated in vitro were identical for the human and monkey microsomes. The authors pointed to epidemiological evidence to suggest that the in vitro data is applicable in vivo. The blood levels of 2,4,5-HCB in a population exposed to PCBs in Taiwan decreased only 10% over a 300-500 day period indicating a very slow elimination from the body. They also cited evidence from Jensen and Sundstrom that the PCB isomer found in greatest proportion in human adipose tissue is 2,4,5-HCB and 2,3,6-HCB. Moreover, the

relative metabolism rates *in vitro* corresponded to relative metabolic clearance recorded *in vivo*.

Human metabolism appears to be more similar to that of the rat and monkey. Metabolic rates measured *in vitro* for human liver preparations are in agreement with those of rat liver microsomes (U.S. EPA, 1987). Moreover, hepatic cytochrome P-450 concentrations are relatively similar in the human and the rat. The similarity of human disposition of PCBs to that in rat is suggested by the finding that the fat-to-blood distribution coefficient for 2,4,5-HCB in the rat agrees with the coefficient reported for humans (Anderson et al., 1977). The value of the ratio used by Lutz et al. (1977) was 400 while the ratio calculated from the adipose tissue and blood concentrations reported by Masuda et al. (1985) was 284.

SUBCHRONIC AND CHRONIC TOXICITY

Epidemiological evidence has indicated that microsomal enzyme induction and adverse reproductive outcomes may be caused in humans exposed to PCBs. The major targets for subchronic and chronic toxicity in mammals are the liver, immune system, endocrine system, reproductive system and the unborn fetus. Relatively low exposure levels of various commercial mixtures (5 ppm in diet) have increased liver weight and lipid content, elevated urinary coproporphyrin levels, and induced the mixed function oxidase system of rats (Bruckner et al., 1974; U.S. EPA, 1984). Hepatomegaly was observed in mice fed 3.75 ppm PCB (U.S. EPA, 1984). In addition, dietary levels of 1 and 5 ppm caused signs of thyroid toxicity and adrenal toxicity in rats (Byrne et al., 1987 and Byrne et al., 1988). Reproductive problems, teratogenicity and cancer are additional effects of chronic exposure observed in laboratory animals. Toxic effects resulting from subchronic and chronic exposure to PCB mixtures are presented in this section. Reproductive toxicity, teratogenicity, carcinogenicity and genotoxicity are discussed in subsequent sections.

HUMAN

Various symptoms and altered clinical parameters have been reported in humans exposed environmentally, occupationally, and accidentally to PCBs. These include higher serum triglycerides and cholesterol levels, the induction of hepatic enzymes, liver injury, chloracne, lassitude, loss of appetite, loss of libido, neurologic complaints, eye and upper respiratory irritation, effects on reproduction, and a higher risk of cancer. These human health effects have been reviewed recently by Kimbrough (1987).

Data concerning chronic, environmental exposure to PCBs is sparse. Fish consumption was found to correlate positively with PCB blood levels in a predominately black population in the southern United States (Kreiss et al., 1981). Serum concentrations of PCBs in subgroups of this population ranged from a mean of 1.50 to 20.6 ug/l (ppb) and were stated to be similar to other communities in the United States. PCB serum levels increased with age and were lower in females in each age group. This population was also exposed to DDT through fish consumption and residues in serum followed a similar pattern. Serum cholesterol was positively associated with log serum PCB level, independent of age, sex, fish consumption, body-mass index, and alcohol consumption. Definite and borderline hypertension rates in this population were 30% higher than expected based on national rates for a population of the same age, race, and sex composition. Log serum PCB values made a statistically significant contribution to explaining the variability of log systolic and log diastolic blood pressure in multiple regression analyses. Log DDT serum levels had no effect on blood pressure when included with log PCB in regressions controlled for age or obesity. Elevated blood pressure has not been found to be associated with PCB blood levels in Yusho patients (Akagi and Okumura, 1985; reported in Kimbrough, 1987).

Chase et al. (1982) did not find a significant correlation between PCBs concentrations in plasma or adipose tissue and cholesterol levels in a study of 120 employees at a railroad passenger car and locomotive maintenance facility. A significant correlation was found for plasma PCBs and serum triglycerides ($r=.56$, $p<0.0001$), however this did not hold for PCB levels in

adipose tissue and serum triglycerides. These relationships did not change after adjusting for age or length of employment. There was no significant difference among exposure groups in average levels of serum triglycerides, cholesterol, and high-density lipoproteins (HDL) although PCBs levels in plasma and fat were significantly elevated in the exposed group over the nominally exposed and nonexposed groups. Plasma PCBs levels averaged 33.4 ppb, 14.2 ppb, and 12.0 ppb in the exposed, nominally exposed, and nonexposed groups respectively. Average levels in adipose tissue were 5.6 ppm, 1.4 ppm, and 1.3 ppm in the same three exposure groups. The comparisons between exposure groups are not definitive however, because sample sizes were small for the nominally exposed (15) and nonexposed (19) groups, and while there were significant differences in age and length of employment between the exposed group and the other groups, the effect of these differences were not evaluated.

A positive correlation was observed between log serum PCBs and log triglyceride, total cholesterol, and log VLDL cholesterol in a population of switchgear shop employees involved in transformer maintenance functions (Emmett, 1985). The correlation disappeared after a linear adjustment was made for the potential confounding variables, age, race, smoking, alcohol intake, history of liver disease, glucose, family history of diabetes, high lipids and heart attacks. No correlation was noted between log serum PCBs or log adipose PCBs and log triglycerides, total cholesterol, log high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and log very low-density lipoprotein cholesterol.

The correlation of cholesterol levels with log serum PCB observed by some investigators has not been confirmed when expressed as PCB levels in serum lipids (Lawton et al., 1985; reported by Kimbrough, 1987). An effect on lipid metabolism has been suggested however an association between PCB serum levels and elevated triglycerides and serum cholesterol is possibly explained by the increased solubility of PCB in serum with higher lipid content (Kimbrough, 1987).

PCBs exposure is associated with hepatic enzyme induction in humans. An association of log serum PCB concentration with log gamma glutamyl transpeptidase (GGTP) did not disappear when PCB levels were expressed in terms of serum lipid (Lawton et al., 1985, reported in Kimbrough, 1987). This association, which indicates an induction of hepatic enzymes, has also been detected in several other cross-sectional studies (Kimbrough, 1987). Log serum GGTP was significantly associated with log serum PCB when evaluated independent of alcohol consumption and age in an environmentally exposed population (Kreiss, 1981). The induction of mixed-function oxidases by occupational PCB exposure has also been reported. Exposed workers have had higher serum GGTP levels (Chase et al., 1982) and a lower plasma antipyrine half-life than controls (Alvares et al., 1977, reported in Kimbrough, 1987).

A statistically significant correlation was observed by Emmett (1985) between log serum GGT and log serum PCBs ($p=0.029$). A significant negative correlation was also noted between urinary 17-hydroxycorticosteroid excretion and log adipose PCBs ($p=0.001$). Serum tetraiodothyronine (T_4) was significantly lower in the 54 exposed workers compared to the 54 controls

(8.24 vs 8.8, p=0.016), and the 24 hour urinary 17-corticosteroid excretion was also lower with marginal significance (6.43 mg/24 hour vs 7.41 mg/24 hour, (p=0.055). Antipyrine half-life, a direct measure of microsomal enzyme induction, was lower in the exposed group but with only marginal statistical significance (10.7 T/2 hour vs 12.42 T/2 hour, p=0.069). No correlation was found between antipyrine half-life and PCBs concentration. Geometric mean PCBs levels in the currently and previously exposed workers were 9.7 ppb in serum and 1.6 ppm in adipose tissue. Serum and adipose tissue concentrations in the comparison group were 4.6 ppb and 0.6 ppm respectively. These workers were also exposed to PCDFs.

Evidence of liver injury was found in most studies of workers with chloracne, a persistent skin condition that occurs as a result of extensive PCBs. One study reported abnormal liver function when chloracne was not present (Kimbrough, 1987). In the Chase et al. (1982) study of railroad employees, plasma PCBs levels were significantly correlated with serum glutamic oxaloacetic acid (SGOT) ($r=.26$, $p<0.05$) with an adjustment for the effect of age. Serum glutamic pyruvic transaminase (SGPT) and GGTP were not correlated with plasma or adipose tissue PCBs concentration. Although evidence of enzyme induction was found by Emmett's study (1985) of switchgear employees, no significant correlation was observed between adipose or serum PCBs levels and several indicators of liver injury (log total protein, log albumin, log bilirubin, log alkaline phosphatase (APH), log lactic dehydrogenase (LDH), log serum glutamic oxaloacetic transaminase (SGOT) and log serum glutamic pyruvic transaminase (SGPT)). The workers studied by Chase et al. may have been subject to higher exposures to PCBs. Fischbein et al. (1979), in a cross-

sectional study of capacitor workers, found a statistically significant association between abnormal SGOT levels and plasma levels of both lower and higher homologues of PCBs. Only 2.5% of the employees had abnormal SGOT levels however.

Maroni et al. (1981) documented liver involvement in 16 out of 80 capacitor manufacturing workers studied. None of these workers had chloracne, either at the time of examination or previously. Hepatomegaly was observed in all but two of these workers, and altered serum GGT activity, transaminases, and ornithine-carbamoyltransferase (OCT) were noted most frequently. Analysis of workers with and without hepatic involvement revealed a statistically significant positive association between prevalence of liver involvement and blood chlorobiphenyl concentrations, particularly trichlorobiphenyls. Mean serum concentrations of trichlorobiphenyl and total chlorobiphenyl were 215 ± 95 ppb and 524 ± 349 ppb in workers with liver abnormalities and 92 ± 64 ppb and 296 ± 160 ppb in workers without abnormal findings. Age and length of employment were not associated with hepatic involvement. Liver injury, indicated by above normal bromosulfopthalein levels, was found in four out of seven electrical workers who had blood Aroclor levels above 500 ppb (Ouw et al., 1976).

Chloracne has been reported in workers exposed to PCBs. Chloracne is characterized by cysts and comedone on the face and sometimes on genitalia, trunk, and extremities. Microscopically the lesions are similar to those found in the monkey. These are dilated hair follicles filled with keratin, partially or completely involuted sebaceous glands, proliferation of the

epithelium, and acanthosis. Symptoms of illness have included lassitude, loss of appetite, and loss of libido (Kimbrough, 1987). Maroni et al. (1982) diagnosed chloracne attributable to PCBs exposure in some of the workers in his study population. Fischbein et al. (1979) found a high prevalence of reported dermatologic symptoms in 326 capacitor manufacturing workers. A history of dermatologic complaints was reported in 45% of male workers and 55% of female workers, and skin rash was reported by 39% of all workers. A history of acne after beginning work at the plant was given by 11% of the workers. Physical examinations also revealed a high prevalence of abnormal dermatological findings. Erythema, swelling, dryness, and thickening was found in 41% and 38% of males and females respectively with acneiform eruptions in 5% of those examined. Palpebral hyperpigmentation and edema, characteristic PCB-associated effects, along with injected conjunctiva were present in 15% of the workers. Chloracne and other dermatological complaints were found more often in PCB-exposed occupational groups compared to controls by other investigators as well (Chase et al., 1982; Ouw et al., 1976).

A high prevalence of neurologic symptoms were reported by 326 exposed capacitor manufacturing workers (Fischbein et al., 1979). Thirty-nine percent of male workers and 58% of female workers gave a history of neurologic symptoms including headache, dizziness, depression, memory loss, fatigue, nervousness, sleeplessness, and somnolence. Routine neurologic examination did not reveal any abnormalities however.

Eye and upper respiratory irritation were also reported in workers in a capacitor plant (Warshow et al., 1979). An abnormal forced vital capacity

measurement (FVC) was observed in 14.9% of males and 13.1% of females and restrictive impairment was reported in 13.2% of males and 9.0% of females. Employees with exposure to asbestos, talc, or textile dust were removed from the spirometric studies. Thirty-five percent and 30% of males and females, respectively, were smokers or exsmokers however the authors stated that a decrease in FVC is not characteristic in smokers in the absence of airway obstruction. Significant airway obstruction was measured in seven out of the 34 workers with a reduced FVC.

Reproductive and carcinogenic effects have also been reported and are discussed in subsequent sections.

Two major poisoning incidents have occurred each involving over 1000 people and exposure through the ingestion of rice oil contaminated with PCBs, polychlorinated dibenzofurans (PCDFs) and polychlorinated quaterphenyls (PCQs) (Kimbrough, 1987). The first incident occurred in Japan in 1968. Although the oil was not analyzed until later, it was found to contain 1000 ppm Kanechlor-400, 5 ppm PCDFs and 866 ppm PCQs. The average dose to Yusho patients was calculated to be 157 ug/kg body weight/day PCB, 0.9 ug/kg body weight/day PCDF, and 148 ug/kg body weight/day PCQ. Severity of symptoms was positively associated with the amount of contaminated rice oil consumed.

Patients had chloracne, dark-brown pigmentation of the nails, itching, pigmentation of the skin, swelling of the limbs, pigmented mucous membranes, eye discharge, hyperemic conjunctivae, jaundice, swelling of the upper eyelids, a feeling of weakness, numbness of the limbs, and fever. Babies

showed fetal PCB syndrome, a dark-brown pigmentation of the skin and pigmented mucous membranes. The pigmentation disappeared by 2 - 5 months of age. Other symptoms in infants included small size, edema of the face, spotty calcifications in the skull, and early eruption of teeth at birth. Adults with clinical symptoms later developed respiratory symptoms which persisted for more than 15 years.

It is generally felt that the clinical manifestations observed were primarily due to the PCDFs in the yusho oil (Kimbrough, 1987; Rogan et al., 1988). A recent analysis of PCDF and PCB isomers has suggested, however, that certain PCB isomers may contribute more to the overall toxicity of the mixture present in the tissues of Yusho and Yu-Cheng patients than other PCDF isomers (Olafsson et al., 1988). Only a small number of Yusho patients have died of cancer, although the latency period for the development of cancer may be longer than the time that has occurred since the initial exposures.

The second episode (Yucheng) occurred in Taiwan in 1979. Rice oil and patients' blood contained Kanechlor-400 or Kanechlor-500 at concentrations as high as 65 and 108 ppm respectively. Over 2000 patients were identified and the period of PCB ingestion was determined to be 3 to 9 months. The oil was also contaminated with PCDF and PCQ and symptoms were similar to those observed in the Yusho outbreak. The 24 hour urinary excretion of the heme precursors, delta-aminolevulinic acid and uroporphyrin, were significantly elevated in Yu-Cheng patients. In addition, the ratio of uroporphyrinogen to coproporphyrin was greater than one in a majority of the Yu-Cheng patients which is a condition that may be unique to hexachlorobenzene and PCB poisoning (Chang et al., 1980).

Immunotoxicity was reported for Yucheng patients. Delayed immune response was evaluated in 30 Yucheng patients and compared with those of 50 controls (Chang et al., 1982). Mean age in both groups was 14 years of age. Only 43% of the exposed group had an induration with a diameter of 5 mm or more 24 or 48 hours after subcutaneous injection of streptokinase and streptodornase into the forearm compared to 80% of the controls. The percentage of patients with a positive response decreased with increasing severity of chloracne in patients. The degree of chloracne was associated with the whole blood PCB concentrations which ranged between 15.5 and 98.4 ppb in the patients. Concentrations in controls were below detection limits (< 1.0 ppb). Decreased concentrations of IgA and IgM but not IgG were also reported to be associated with PCB in serum (Chang et al., 1981). In addition, the percent of total T cells, active T cells, and T mu cells (helper T cells) was significantly decreased in Yucheng patients while the percent of B cells and T gamma cells (suppressor T cells) was not significantly decreased. It was suggested that the decrease in thymus-dependent cells (T cells) may be responsible for the observed diminished delayed type skin hypersensitivity response to streptokinase in Yucheng patients.

Finally, blood levels of PCB, PCDF, and PCQ were negatively correlated with lowered nerve conduction velocity in 110 Yucheng patients evaluated between 1979 and 1980 (Chen et al., 1985). The linear correlation coefficient was not statistically significant however. Motor and sensory nerve conduction velocities were significantly slower ($p < 0.01$) in Yucheng patients than in a group of normal controls.

ANIMAL

Hepatic Effects

A statistically significant increase in relative liver weight occurred in all treated male and female Sherman rats fed 20 ppm or more Aroclor 1254 or Aroclor 1260 in the diet for eight months (Kimbrough et al., 1972; reported in U.S. EPA, 1984). Females fed 20 ppm Aroclor 1260 did not exhibit this response although the female control group for this dose level had unusually large relative liver weights. Hepatocellular enlargement with foamy cytoplasm containing inclusions was noted in the 20 ppm dose groups for both mixtures. Accumulation of pigment, lipid accumulation and extensive foci of adenofibrosis were noted in the livers of the 100 ppm dose groups. An increase in smooth endoplasmic reticulum, cytoplasmic inclusion of lipid-containing vacuoles, atypical mitochondria and structures of concentrically arranged membranous whorls surrounding lipid-containing vacuoles were ultrastructural changes noted in these livers. Adenofibrosis (bile duct proliferation) was more pronounced in the livers of rats fed Aroclor 1254.

Groups of six male Sprague-Dawley rats fed 0, 5, or 25 ppm Aroclor 1242 for two, four, or six months did not show a difference in body weight gain or food consumption (Bruckner et al., 1974). Liver weight expressed as a percent of body weight was elevated in the 25 ppm group after four months. Liver lipid (mg/g wet weight) was elevated after only two months and remained elevated in the 25 ppm and 5 ppm groups after six months. Urinary coproporphyrin excretion was significantly increased by 5 ppm in the diet for six months and some animals exhibited high urinary coproporphyrin levels at two and four

months. Histopathological changes involving increased lipid deposition, lipid vacuolation, and smooth endoplasmic reticulum proliferation were reported. A subchronic dose of 5 ppm in diet was concluded by the EPA to be a LOAEL in rats (U.S. EPA,1984).

Concentrations of 2.5 and 26 ppm Aroclor 1254 in the diets of female Wistar-derived rats from mating to weaning of offspring produced significantly increased liver weights expressed as a percent of body weight (Overmann et al., 1987).

Similar results were observed in female CD rats exposed to Aroclor 1254 in the diet for 20 weeks (Zinkl, 1977; reported in U.S. EPA, 1984). Decreased body weight gain, and increased relative liver weights occurred in a dose-related manner in rats exposed to 30 ppm or more. Liver lesions were similar to those described by other investigators and were noted in the 30 ppm group becoming more pronounced in the 100 ppm group. The LOAEL was concluded to be 10 ppm for Aroclor 1254 in these rats (U.S. EPA, 1984).

In an experiment comparing the relative toxicity of Aroclor 1221, Aroclor 1242 and Aroclor 1254 in male BALB/C mice for six months, hepatomegaly was produced in mice fed 3.75, 37.5 and 375 ppm Aroclor 1254 and mice fed 375 ppm Aroclor 1242 which persisted throughout a three month observation period after exposure ceased (Koller, 1977; reported in U.S. EPA, 1984). Liver lesions were noted in mice fed 37.5 ppm or more Aroclor 1254 or 375 ppm Aroclor 1242. The LOAEL was concluded to be 3.75 ppm (U.S. EPA, 1984).

Long-term administration (greater than one year) of various PCB mixtures to rodents has resulted in liver toxicity and tumorigenesis (reproductive and developmental toxicity were not evaluated). All studies reported a lowered body weight gain and a higher liver weight expressed as percent of body weight. These effects became more pronounced with increasing dose. The lowest dose level evaluated was 25 and 50 ppm Aroclor 1254. At these dose levels, body weight gain became different from controls at 50 ppm Aroclor 1254 for males and 25 ppm for females (NCI, 1978). Enlarged livers were reported in rats at 25 ppm. Liver pathology, including oval cell proliferation, bile duct proliferation, fatty changes and hepatocellular hypertrophy, was observed in these rats at doses as low as 100 ppm.

Hepatomegaly has also been induced in mice in a dose-related manner by PCB exposure beginning at 100 ppm (Ito et al., 1973). Other findings reported in mice were similar, although oval cells and bile duct proliferation were less prevalent in treated livers and fibrosis was more common. Slightly increased smooth endoplasmic reticulum with rare mitochondrial changes in cytoplasm was also noted (Kimbrough and Linder, 1974).

The administration of 2 mg/kg/day Kanechlor 400 (without PCDF contamination) in the diet of a cynomolgus monkey for 20 weeks produced enlarged hepatocytes with eosinophilic cytoplasm, the proliferation of epithelial cells of bile ducts, and inflammatory change in stroma (Hori, 1982). Ultrastructural changes noted were the proliferation of smooth endoplasmic reticulum and the formation of cytoplasmic vacuoles resulting from the destruction of rough endoplasmic reticulum. The liver of a rhesus monkey that died after consuming

450 mg PCB contained focal areas of necrosis and enlarged hepatocytes with numerous small lipid droplets distributed in the abundant eosinophilic cytoplasm (Allen et al., 1974). Hepatic cells in the other treated animals fed 25 ppm Aroclor 1248 over a period of 10 months showed a proliferation of the smooth endoplasmic reticulum, and a majority of the Kupffer cells contained fat droplets which altered the position of cytoplasmic organelles and nuclei.

Biochemical Changes

Reduced hematocrit (statistically significant) occurred in male Sprague-Dawley rats fed 25 ppm at two months and reduced hemoglobin levels (statistically significant) were noted in the rats fed 5 and 25 ppm (Bruckner et al., 1974). The levels remained lower at four and six months but were not significantly different from controls. Mixed-function oxidase activity was induced at a level that was statistically significant after two months in the 25 ppm group and after four months in the five ppm group. The increased activity was dose-dependent. N-demethylase activity was significantly induced by 25 ppm Aroclor 1242 after four months.

Male Sprague-Dawley rats fed 100 ppm Aroclor 1248, Aroclor 1254 or Aroclor 1262 in the diet for 52 weeks followed by a 13 week recovery period, developed distinct modifications in the serum lipids (Allen et al., 1976). Total serum lipids and serum cholesterol were increased in all treated groups and an increase in serum triglyceride was observed in the Aroclor 1254 exposed group at 52 weeks. Liver weight increases and pathological alterations in the liver occurred in the treated groups and were similar to those described by other

investigators. However, body weight gain was not different relative to controls and no appreciable differences in hemoglobin, hematocrit, and white blood cells were noted. The degenerative changes in the liver continued to increase throughout the 13 week recovery period. The authors suggested that the increased serum lipids were due to an increased synthesis of lipid within the smooth endoplasmic reticulum associated with liver hypertrophy. They hypothesized that PCBs are sequestered in the cytoplasmic membranes stimulating membrane development then ultimately causing membrane dysfunction and cell degeneration.

Female CD rats exposed to 30 ppm Aroclor 1254 in the diet for 20 weeks developed elevated serum cholesterol levels. SGOT and beta globulin values were also increased in the 30 ppm group while serum gamma globulin was reduced in the 100 ppm group (U.S. EPA, 1984).

Dietary concentrations of 2.5 and 5.0 ppm caused changes in biochemical parameters in nonhuman primates (Barsotti and Allen, 1975, Barsotti et al., 1976, Allen and Barsotti, 1976, and Allen et al., 1979). Serum concentrations of triglyceride, cholesterol and total lipids decreased progressively over eight months. The decrease in total lipids was statistically significant at eight months. A shift in the albumin/globulin ratio was noted and a progressive increase in SGPT activity became significant at 8 months. The changes in triglyceride and cholesterol became significant at 12 months. Two of the adult females died (one in each dose group). Nonhuman primates fed higher PCB concentrations in the diet (25 ppm) experienced similar biochemical changes as well as hematological changes in cases of more severe toxicity (Allen et al., 1974).

Dermatological Effects

monkeys are quite sensitive to the effects of PCBs exposure. Rhesus monkeys (six per group) fed 0 and 25 ppm Aroclor 1248 in the diet for two months responded with a variety of toxic effects over a total period of 10 months (Allen et al., 1974). Skin lesions were the initial and primary toxic manifestation. Animals began to lose hair and develop obvious edema of the lips and eyelids within 4 to 6 weeks and this lesion became progressively more severe with time. Animals developed extensive alopecia and small pustules involving hair follicles were obvious around the mouth, cheeks, and neck. Skin, liver and bone marrow also showed microscopic changes. Hair follicles on the face were filled with keratin and there was hyperplasia of the follicular epithelium and inflammation.

Female rhesus monkeys exposed to 2.5 and 5.0 ppm Aroclor 1248 for seven months showed weight loss, skin lesions on the face and neck, and increase in serum SGPT, and reproductive problems (Barsotti and Allen, 1975, Barsotti at al., 1976, Allen and Barsotti, 1976, and Allen et al., 1979). Food intake in pregnant monkeys was reported to be similar for exposed and control monkeys however both treated groups lost an average of 15.1% of their initial body weight. PCB intoxication was apparent at two months indicated by loss of hair, acne on the face and neck, and erythema and swelling of eyelids and was exhibited by all the females at six months. Skin biopsies revealed hair follicles with prominent keratinization. All exposed males (5.0 ppm in diet) had a slight to moderate periorbital edema and congestion at 14 months. It was suggested that the sex difference may have been due to the greater quantity of adipose tissue in males in which PCBs could be sequestered.

Skin lesions were reported for three cynomolgus monkeys dosed with 2 mg/kg/day Kanechlor 400 containing 400 ppm PCDF for 20 weeks and 5 mg/kg/day for 8 and 4 weeks (Hori et al., 1982). No lesions occurred in a monkey fed 2 mg/kg/day Kanechlor 400 containing no furans.

Few reports of skin lesions have been reported in rodents. Female CD rats exposed to 30 ppm or more Aroclor 1254 in the diet for 20 weeks were reported to develop skin lesions on the ears, tail and dorsum of the nose (U.S. EPA, 1984). Skin lesions were also reported for female Swiss Albino mice exposed at much higher levels (200 ppm) Aroclor 1254 (U.S. EPA, 1984).

Gastrointestinal Tract Toxicity

Damage to the gastrointestinal tract has been reported in monkeys. A rhesus monkey which died after ingesting a total of 450 mg Aroclor 1248 over four months had an acute hyperplastic gastritis with widespread focal hemorrhage and ulcerations (Allen et al., 1974). Microscopically, the morphological features of the gastric mucosa were disrupted by numerous large mucin-filled cysts.

Urinary Tract Toxicity

There has been some discussion of effects on the kidney due to PCB exposure. A slight vacuolization of convoluted tubules in the kidney was observed in male Sprague-Dawley rats fed 25 ppm Aroclor 1242 in the diet after four and six months but these effects were not reported for the 5 ppm group (Bruckner et al., 1974). A mild lesion was noted in the kidney of a cynomolgus monkey

fed two mg/kg/day Kanechlor-400 for 20 weeks (Hori et al., 1982). Slight cytoplasmic vacuolization was observed in the epithelial cells of the renal convoluted tubules.

Endocrine System Toxicity

Endocrine effects have been demonstrated in rats at relatively low chronic levels of PCBs. Female Sprague-Dawley rats (ten animals per group) were fed 0, 1, 5, 10, and 50 ppm Aroclor 1254 in the diet for at least five months (Byrne et al., 1987). Blood samples were taken before treatments began and at intervals during the treatment period. Serum thyroxine (T4) levels were significantly reduced in the 10 and 50 ppm groups by day 14 compared to the control group and to pretreatment levels. Serum T4 levels also became significantly decreased in the 1 and 5 ppm groups at day 35. A significant decrease in serum triiodothyronine (T3) levels occurred in the 10 and 50 ppm groups by day 20 compared to controls and pretreatment levels. The decrease became significantly lower in the 5 ppm group by day 40. Rats in all treatment groups responded to a TSH challenge by increasing T4 and T3 levels in serum. The level of response was significantly less than controls in the 10 ppm group.

The disappearance rate of injected doses of L[125I] T4 was significantly decreased in all treatment groups and its severity was dose related. The T4 distribution space (TDS) increased with increasing dose and became 8 times the control value in the 50 ppm group. The T_4 production rate (T4PR) was significantly decreased in all treated groups and was dose-related. These parameters indicated that PCB treatment decreased the rate of T4 degradation

without increasing its metabolic clearance, expanded the pools in which T4 was distributed and decreased the rate of T4 production.

The authors concluded that these responses were due to primary hypothyroidism. The relatively smaller response to TSH challenge in the treated groups would not be expected if TSH secretion was impaired and this provides evidence of a primary effect. Peripheral tissue damage was indicated by the expanded TDS. This effect was not considered to result from hypothyroidism but from the damage of plasma membranes by PCBs and expansion of T4 and T3 dispersal into the interstitial fluid or other nonvascular spaces. Liver toxicity would preclude a mechanism for hypothyroidism through increased hepatic catabolism or renal clearance of serum T4 and T3.

The adverse effects were induced by a PCB dose level lower than that which caused liver toxicity. Liver weight was slightly elevated in the 10 and 50 ppm dose groups however thyroid weight was not significantly increased. Food consumption was not affected by treatment and body weights were not significantly different.

The same investigators observed a suppression of circulating levels of the adrenal cortex hormones B, DHEA and DHS in rats after long-term administration of relatively low doses of Aroclor 1254, Aroclor 1242 and Aroclor 1016 (Byrne et al., 1988). Concentrations of 1, 5, 10, or 50 ppm of these PCB mixtures were fed to female Sprague-Dawley rats for five months. PCB doses were 8.35, 47.9, 83.64, and 418.59 ug/100g body weight/day. Aroclor 1254 significantly decreased corticosterone levels in serum compared to control and pretreatment

levels in all dose groups except 1 ppm. B levels in the 50 ppm group were five times lower than control and pretreatment levels by the end of the study. Serum dehydroepiandrosterone (DHEA) levels were significantly decreased in both treatment groups after a transitory increase during the first month in rats treated with Aroclor 1254. All treatment levels of Aroclor 1242 and Aroclor 1016 significantly decreased serum DHEA levels by the end of the five months although the depression did not begin until much later. Levels of dehydroepiandrosterone sulfate (DHS) were also significantly decreased by chronic treatment of all three Aroclor mixtures. As the animals aged, control DHS increased steadily while it did not in the treated groups with the exception of an anomalous spike on day 80. Adrenal weights were significantly lower than the controls at all dose levels in the Aroclor 1254 treated groups however liver weights were not affected. Food consumption was not significantly different between and among control and treatment groups. Inhibition of hormone levels was greatest in Aroclor 1254 treated rats and became less pronounced in rats fed PCB mixtures with decreasing percent chlorination.

The authors ruled out the unavailability of precursor-substrate cholesterol for adrenal steroidogenesis as the reason for decreased hormone levels as well as an enhanced degradation and hepatic clearance. The authors could not state whether or not the hormone suppression is at the adrenal or hypothalamopituitary axis because although decreased adrenal weight corresponded to the reduced hormone levels, the impact of PCB treatment on secretion of adrenocorticotrophic hormone (ACTH) by the pituitary was not known.

Immunotoxicity

Earlier studies have demonstrated immunotoxicity induced by PCBs in chickens, guinea pigs, mice, rats and monkeys (Safe, 1984). Effects in mice and rats include thymic and splenic atrophy, suppression of antibody response, decreased resistance to host infections, depression of T-cell responsiveness to mitogens, and delayed hypersensitivity.

Monkeys exhibited lower globulin levels and lower anti-SRBC antibody titers after chronic exposure to PCBs. Three pregnant cynomolgus monkeys were dosed with 100 ug/kg body weight/day and 400 ug/kg body weight/day Aroclor 1254 beginning on day 60 of gestation (Truelove et al., 1982). All treated monkeys had a substantially lower antibody production compared to the one control animal after a second sheep red blood cell (SRBC) challenge. This assessment was made after 148 days of treatment, approximately 50 days post partum. The only other sign of maternal toxicity was the loss of finger nails after 233 days (100 ug/kg/day) and 242 days (400 ug/kg/day). The authors suggested that immunologic function may be a relatively sensitive measure of toxicity.

Primary immune response was depressed in a cynomolgus monkey dosed with two mg/kg/day Kanechlor 400 (PCDF absent) for 20 weeks (Hori et al., 1982). The monkey was immunized with SRBC on days 16, 44, and 72 of feeding and showed an antibody level of 100 compared to 1280 in control monkeys. Antibody production was not stimulated after the second or third immunization. Similar patterns were observed in assays for hemaglutinating activity and IgG levels.

More recent work evaluating the effect of PCBs on natural killer cell cytotoxicity has confirmed the immunotoxic effects of PCBs on rats (Talcott et al., 1985). Male Sprague-Dawley rats were exposed to 50 and 500 ppm Aroclor 1254 in the feed for ten weeks. Natural killer (NK) cell cytotoxicity was compared to a positive and non-treated control group. Both dose levels significantly depressed splenic NK cell cytotoxicity in vivo. A reduction in splenic cellularity was also observed in rats fed 500 ppm Aroclor 1254.

Concentrations of 0.4 or 20.0 ug/ml PCB significantly suppressed NK cell cytotoxicity in an in vitro assay. It was concluded that the suppression was caused by a mechanism other than a direct cytocidal effect on the lymphocytes because cell viability in treated cultures was always greater than 85%. The authors reported that no visual signs of toxicity and no changes in body weight were observed during treatment. It was postulated that suppressed immunity may increase carcinogenic ability by impairing the ability of the host to recognize and reject neoplastically transformed cells however the authors recognized that other factors may also be involved.

Conclusion

A number of adverse health effects have been reported in humans exposed to PCBs. Other than the finding of hypertension in an environmentally exposed population, the effects were similar to those reported in experimental animals. A causal conclusion regarding PCBs exposure and health effects in humans is impossible to make because the study populations were exposed to other toxicants and exposure was difficult to quantify.

Hepatomegaly and an increase in liver lipids was observed in rodents at dietary PCB concentrations as low as 3.75 ppm - 5 ppm. The severity of liver lesions increased with increasing PCBs concentration in diet. Hepatotoxicity was also produced in nonhuman primates although the dose levels used also produced severe illness in the animals. The induction of liver enzymes was observed in rodents fed five ppm PCBs in the diet. Hepatic enzyme induction has also been documented in occupational workers.

Biochemical changes have been noted in both experimental animals and humans exposed to PCBs. An increase in serum lipids and serum cholesterol occurred in rodents fed PCBs at a level which also induced liver pathology (30 ppm). Concentrations of serum triglyceride, cholesterol, and total lipids decreased in nonhuman primates experiencing severe PCB toxicity. An increase in serum lipids and cholesterol was recorded in human populations exposed to PCBs in the environment or through their occupation. The data in humans is conflicting however, as some studies did not observe this effect. Reductions in hemoglobin were observed in rats fed 5 ppm PCBs. Reductions in hemoglobin have also been reported in rhesus monkeys and PCB-poisoned patients. Further, PCB-induced porphyria has been reported in PCB-poisoned patients and rats. Increased urinary coproporphyrin levels were a relatively sensitive indicator of PCB exposure in rats.

The dermatological symptoms noted in humans involved in the poisoning incidents in Japan and Taiwan, and workers exposed at higher PCB concentrations, are quite similar to those induced at high dietary concentrations in nonhuman primates after exposure to PCB mixtures. An effect

on the immune system, observed in Yucheng patients, has also been produced experimentally in rodents and nonhuman primates. Moreover, a decrease in T_4 levels in blood caused by dosing rats with PCBs, has been reported in workers exposed to PCBs.

A LOAEL for thyroid toxicity and alterations in adrenal hormones in rats appears to occur at 1 ppm (0.08 mg/kg body weight/day) in the diet for Aroclor 1254, Aroclor 1242, and Aroclor 1016. These effects were more sensitive indicators of toxicity than increased liver weight which occurred at higher dose levels in the same experiments (Byrne et al., 1987 and Byrne et al., 1988). A LOAEL for liver toxicity was shown by Bruckner et al. (1974) to occur at a dietary concentration of 5 ppm (0.2 mg/kg body weight/day) Aroclor 1242. Nonhuman primates are extremely sensitive to the effects of PCBs. Severe toxicity and death are produced at very low doses compared to other species.

ADVERSE REPRODUCTIVE EFFECTS AND TERATOGENICITY

HUMAN

Babies of parents exposed to contaminated rice oil in Japan and Taiwan exhibited a fetal PCB syndrome consisting of a dark-brown pigmentation of the skin caused by an increase in melanin pigment in the epidermis and mucous membranes. This condition disappeared between two and five months of age (Kimbrough, 1987). These infants showed facial edema, spotty calcifications in the parietal and occipital areas of the skull, and the teeth in some had erupted prematurely. Consumption of the rice oil contaminants has been

estimated at 157 ug/kg body weight/day PCBs, 0.9 ug/kg body weight/day PCDF, and 148 ug/kg body weight/day PCQ.

In 1985, a field survey was conducted of all living children known to have been in utero during or after the period of oil contamination in Taiwan (Rogan et al., 1988). Information was obtained on 128 children. A control population was selected from 96 families who lived in the same neighborhoods and data was obtained for 115 children. Medical histories revealed that children of exposed mothers had lower birth weight (2749 ± 46 g vs 3228 ± 40 g), had subsequently had a much higher rate of bronchitis, and were behind in reaching 32 out of 33 developmental milestones. Physical examinations showed that exposed children averaged 93% (95% confidence interval (CI), 90-96) of control weight and 97% (95% CI, 96-99) of control height, adjusted for age and sex. Many of the symptoms present at birth in the exposed children were still present and abnormalities consistent with bronchitis were identified in several children. Marked differences were noted in eyebrow flare, hypertelorism and clinodactyly. Exposed children were delayed compared to controls in the age at which they performed certain tasks and neurologists identified a developmental or psychomotor delay in 12% (10) of the exposed compared with 3% (3) of the controls. The performance of exposed children in age-appropriate behavioral assessments and tests of cognitive development were inferior to that of control children. The authors concluded that many of the adverse effects were consistent with an acquired (neuro)ectodermal dysplasia although it is impossible to separate *in utero* injury and the effect of continued internal exposure. A blind study design was not used which may have biased the observed effects in a positive direction.

Workers in a capacitor factory in Japan which had used Kanechlor-300 and Kanechlor-500 prior to 1973 were found to have whole blood PCB concentrations 10 to 100 times higher than the general population just after use of these commercial mixtures had ceased (Hara, 1985). Workers showed symptoms of PCB intoxication during their exposure and mild symptoms were still present in 1973. The duration of PCB handling and PCB concentrations in breast milk were correlated with blood PCB levels. The health of the children of these workers appears to have been affected. Body weights of female infants of the mothers whose jobs involved handling PCBs was lower than normal for their age and sex. The infants of employees who did not handle PCBs and the infants of women not employed at the factory did not have lower than normal body weight. In addition, the number of complaints including fatigue, catching cold, weak digestion, coughing, expectoration, and itchy skin obtained by questionnaire was higher among the children of exposed female workers and increased in incidence with the length of breast feeding. Children given a medical examination once a year for five years exhibited some more mild but "typical" Yusho symptoms such as decayed nails, gingival pigmentation, mottled enamel, and dental caries. Incidence was not related to PCB levels in the children's blood however. Blood PCB levels in female workers who handled PCB at the plant until 1972 averaged 21.5 ng/g (KC-300) and 99.0 ng/g (KC-300 and KC-500). The effect of potential confounding variables was not discussed by the authors making a causal association between handling PCBs and health problems in the workers' children difficult.

A study of workers in two capacitor manufacturing plants in New York found an association with cumulative occupational PCB exposure and lower birth weight

and gestational age of infants (Taylor et al., 1984). The birth certificates of 388 children born to 354 women between 1958 and 1977 were reviewed and various parameters were compared by exposure category. Female workers who worked in one or more areas with direct contact with PCBs for a minimum of one year at any time prior to the birth of the infant (51 births to 39 women) were included in the high exposure category. Women employed in all other areas were considered to have low exposure (337 births to 280 women). Previous industrial hygiene surveys had found air concentrations of PCBs in the high exposure areas to be approximately 10-fold greater than in the low exposure areas. The commercial mixtures, Aroclor 1254, Aroclor 1242, and Aroclor 1016 had been used at the plant.

Birth weights, adjusted for gestational age, were 58 g lower for infants of mothers in the high exposure category than for those in the low exposure category. Mean gestational age in the high exposure group was reduced by 6.6 days compared with the low exposure group. Exposure to PCBs was associated with low birth weight and gestational age even after other parameters influencing birth weight (year of birth, maternal age, parity, parents' education and sex of the infant) were included in the analysis. When compared to controls from the community matched for maternal age, parity, and year of birth, the average birth weight of infants in the low exposure group was 66 g greater while infants in the high exposure group averaged 95 g less although this relationship was not statistically significant. Other factors which negatively affect birth weight were not evaluated in this study including tobacco use, underlying medical conditions, maternal height, and previous history of low birth weight. The authors concluded that the effect on birth

weight probably resulted from a shortened gestational period instead of retarded intrauterine growth. These conclusions were termed "tentative" because of the small number of observations, lack of information on potential confounding variables, and use of a binary exposure measure.

The effects of low level chronic exposure are likely to be subtle and may occur in the absence of symptoms typically associated with high level acute exposure. Jacobson et al. (1983) used a longitudinal design to investigate a model of parameters related to contaminated fish consumption and effects in pregnant women and their newborn offspring. The investigators studied 313 mothers and their babies delivering at three Grand Rapids hospitals and one hospital in Muskegan. Participants were eligible to join the study if they were 18 years of age or older, had completed the tenth grade, and met certain criteria for Lake Michigan fish consumption. Individuals in the exposed category had a cumulative exposure of 11.8 kg (26 pounds) or more within a six year period and those in the unexposed category reported no Lake Michigan fish consumption in the past or during their pregnancy. Fish eaters and controls did not differ significantly in socioeconomic status, maternal age, education, marital status, or sex of infant, and both study groups did not differ significantly on these variables compared with a community group matched for hospital and approximate date of delivery. The fish species eaten were weighted for relative PCB content with lake trout and salmon having a unit weight of one. Thus, lake trout equivalent consumption was calculated and expressed as PCB-kg. The mean annual rate of PCB-fish consumption reported was 6.7 PCB-kg (14.7 pounds) per year with a range of 1.2 to 41.7 kg/year. This is equivalent to 2 to 3 salmon or lake trout meals per month at 0.2

kg/meal or approximately 7 perch meals per month (0.2 kg/meal). Average length of fish consumption in the exposed group was 16.1 years.

Cord serum PCB concentrations were correlated with maternal serum levels indicating the transfer of PCBs across the placental barrier. Maternal serum levels were also correlated with neonatal breast milk concentrations. In addition, the concentration in maternal serum was positively correlated with all three of the fish consumption measures used. The mean PCB level in maternal serum was 6.1 ng/ml in the exposed women and 4.1 ng/ml in the unexposed group. Finally, there was a significant correlation between fish consumption and PCB levels in neonatal breast milk (865.6 in exposed vs 622.2 ng/g in non-exposed, fat basis). The fish consumption parameters were not correlated with cord serum PCB levels however. Cord serum PCB levels averaged 2.5 ng/ml, much lower than the concentration in breast milk. These results were concluded to provide empirical support for the use of maternal reports of fish consumption as reliable indicators of PCB exposure, predicting transfer of PCBs *in utero* and in the breast milk.

Infants of fish eating mothers averaged 190 grams less in birth weight, 0.6 cm less in head circumference, and 4.9 days less in gestational age than infants of non-fish eating mothers (Fein et al., 1984). These differences were significant when controlling for maternal prepregnancy weight, type of delivery, alcohol and caffeine consumption, and use of cold medicines. The values decreased with increasing dose. Head circumference was significantly smaller even after birth weight and gestational age were treated as confounders. Cord serum PCB level predicted lower birth weight, smaller head

circumference, and shorter gestational age based on reported last menstrual period when controlling for sex of infant and three potential confounding variables (maternal age, weight gain during pregnancy, and type of delivery). Infants with cord serum values at or above the laboratory's detection limit averaged 160 grams less in birth weight, 0.7 cm less in head circumference, and 8.8 days less in gestational age. Head circumference remained significantly smaller when birth weight and gestational age were controlled. Socioeconomic status, maternal smoking, PBB exposure, and parity were found to be unrelated to exposure and therefore were excluded as potential confounders of the observed associations between fish consumption and the adverse outcomes reported.

Rogan et al. (1986) did not find an association between PCB levels in milk fat at birth and birth weight, head circumference, or hyperbilirubinemia in 912 infants in North Carolina. Cord serum PCB concentrations were reported to be twice as high as those which were correlated with adverse effects in Michigan infants.

Two case control studies found higher concentrations of PCBs in the blood of women with premature delivery or suffering from toxemia of pregnancy compared to women with normal, third trimester pregnancies (Wassermann et al., 1980; Wassermann et al., 1982). The differences in concentration were statistically significant. The composition of PCB isomers in the blood of control women, a low PCBs group, and a high PCBs group differed. High levels of DDT and metabolites were also measured in the premature delivery group. It is not possible to draw conclusions regarding the nature of the relationship between

the presence of the xenobiotics in the blood and the reproductive problems studies because various demographic characteristics and other potential risk factors in the cases and controls were not described by the authors.

PCBs exposure may adversely affect male reproductive ability. A cross-sectional study of human semen abnormalities and toxicant concentrations found an association between decreased motility in samples with low sperm counts and PCB concentration (Bush et al., 1986).

Bush et al. (1986) found that sperm motility was inversely correlated with three PCB congeners in infertile human males. Sperm samples from 170 fertile, subfertile and infertile men who had presented themselves at the Albany Medical Center in New York because of fertility problems or for vasectomy were examined. The samples were examined for 74 PCB congeners, DDE, mirex, and hexachlorobenzene. A mean PCB concentration of 5.8 ng/g wet weight comprising 32 congeners was measured in the samples. The authors reported that these congeners corresponded to those most frequently found in blood and milk from women in the same region. No difference in concentration was found for total PCBs, p,p'-DDE, mirex, or hexachorobenzene between fertility groups. Although not significant for all samples minus vasectomized samples, the linear regression of some congeners as predictors for sperm motility index found an association in the subfertile and infertile groups. The probabilities that the associations were due to chance was low. The regression predicted substantial reductions in motility with each 1 ng/g increase in concentration. Step-wise regression confirmed the association for two of the congeners, 2,4,5,3',4'-pentachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl. The

third, 2,4,5,2',3',4'-hexachlorobiphenyl was not tested in step-wise regression because of its colinearity with the other two compounds. Although the predicted effect was dramatic, the authors cautioned that the proportion of the total variance attributable to the regression (R^2) was only 9-16% for the three congeners. The statistical power of the procedure to detect a "real" significant effect was >0.99. It was noted that the three congeners are ubiquitous in the human population and are obvious candidates for causing the observed effects on sperm however these compounds may just indicate the presence of other more toxic compounds such as dibenzofurans or dioxins. Potential confounders were not evaluated in the study and the cross-sectional study design requires that the hypothesis that PCB exposure negatively affects sperm motility be examined in greater depth.

Behavioral and neurological deficits have been identified in the offspring of human beings (Jacobson et al.,1984, Jacobson et al., 1985, and Rogan et al., 1986).

The Jacobson cohort of infants indirectly exposed to PCBs prenatally and throughout nursing (Jacobson et al., 1984, 1985) exhibited "worrisome" behavior assessed using the Brazelton Neonatal Scale at birth and showed deficits in visual recognition memory at seven months of age. The investigators tested a multiple effects model of teratological exposure which suggests that neonatal deficits associated with intrauterine exposure to small doses of a potentially teratogenic agent will vary across individuals. This variation may be caused by genetic predisposition or the presence of a prior sensitizing condition. Behavioral outcomes were assessed using both direct

and indirect measures of exposure. The sample included 242 infants born to women who reported moderate consumption of Lake Michigan fish and 71 infants whose mothers ate no Lake Michigan fish. The Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered to 287 newborns on the third day after birth (except for four cases). Seven summary clusters were evaluated and thirty-six potential confounding variables were examined for their association with the exposure parameters. Contaminated fish consumption was correlated with caffeine and alcohol consumption before and during pregnancy. Cord serum PCB level was correlated with alcohol and caffeine consumption during pregnancy, weight gain during pregnancy, and maternal age.

Contaminated fish consumption was associated with five NBAS clusters with the strongest relationships for autonomic maturity, number of abnormal reflexes, and range of state. Maternal contaminated fish consumption was highest among the infants classified as worrisome on these three clusters. The range of state cluster included a flat or depressed category and exposure was highest among those infants classified in this category. A range of state item, lability of states, was predicted for by fish consumption controlling for confounders in the regression analysis. A higher percent (42%) of the exposed infants were hyporesponsive compared to 17% of the unexposed infants. Contaminated fish consumption also predicted two of the three autonomic items, motor maturity and amount of startle, controlling for the four potentially confounding variables. In addition, abnormally weak reflexes in the abnormal reflex cluster were predicted for by fish consumption. Controlling for the size and gestational age of the newborn in the statistical analysis did not appreciably alter the relation of contaminated fish consumption to NBAS

performance. Therefore, the authors concluded that the behavioral outcomes were not byproducts of PCB effects on birth size and gestational age.

Cord serum PCB level adjusted for its confounding variables did not predict the behavioral outcomes. Cord serum values were not available for 36.7% of the sample and values in the most highly exposed category were under-represented. This may be one reason for the lack of association. In addition, the possibility was raised that the effects were caused by the presence of toxins other than PCB in the contaminated fish.

Stepwise multiple regression indicated that neonatal behavior was affected in some individuals whose birth size and gestational age were not affected and that some individuals whose physical development was affected did not exhibit the behavioral deficits. It was concluded that chronic low-level exposure may be associated with a variety of relatively subtle deficits. The authors cautioned that neonatal deficits are frequently transitory however, and their long-term developmental implications are uncertain.

The behavioral effects were confirmed by Rogan et al. (1986) using the same seven BNAS cluster scores as Jacobson et al on a cohort of 930 newborns in North Carolina. An association with maternal PCB body burden measured as concentration in milk fat at birth and the tonicity and reflex cluster scores was identified. Higher PCB levels (3.5 ppm and greater) were associated with less muscle tone and activity and with hyporeflexia. Ten out of 54 variables screened had a relationship to PCB exposure and were included in the analysis as confounders. This did not alter the significant association of PCB exposure with the behavioral outcomes measured.

Jacobson et al. continued their study of behavioral outcomes in the sample of infants of mothers who ate contaminated fish by evaluating their response in a recognition memory test at the age of seven months. Visual recognition was defined as the percent of total fixation paid to a novel target compared to a familiar target. An overall score was assigned for three situations. Several potential confounders were evaluated and socioeconomic status, maternal age and parity were found to be correlated with cord serum PCB levels. Pregravid maternal weight, use of acetaminophen and antacids during pregnancy were associated with fish consumption. Socioeconomic status, maternal age, maternal height, alcohol, smoking, caffeine, antibiotics, breast milk PCB level, maternal employment and age at 7-month visit were correlated with three components of postnatal exposure; breast milk PCB level, number of weeks of nursing, and an interaction term weighting breast milk level by weeks of nursing.

Visual recognition scores were not related to social class, maternal age or education, parity, or infant's age at time of assessment. Scores were also unrelated to examiner differences or to the neonatal variables found to be associated with prenatal PCB exposure, including birth size, gestational age, and performance on the NBAS. Controlling for the potential confounders, cord serum PCB level was the strongest predictor of poorer visual recognition memory, but it was also predicted by contaminated fish consumption. The association with cord serum PCB level remained significant when the neonatal mediating variables and relevant confounding variables were included in the regressions. This indicates that the effects of intrauterine exposure are not

by-products of small birth size, gestational age, or neonatal behavioral performance. This effect was dose dependent; the percent fixation on the novel target decreased with increasing cord serum PCB concentrations.

The combination of postnatal exposure variables from nursing were unrelated to recognition memory performance at seven months. Therefore, the results of this study support the premise that intrauterine exposure may be particularly harmful. The developing fetus lacks protective barriers to molecular transport such as the blood-brain barrier and the ability to metabolize xenobiotics. The investigators believed that infant recognition memory may be a valid predictor of later intelligence in apparently normal populations because it is sensitive to a range of at-risk conditions and is able to predict later IQ test scores. They concluded that the potential of this test to predict long-term damage from toxic exposure is yet to be confirmed in older age groups.

ANIMAL

Table 11 presents the study design of experiments evaluating reproductive and developmental effects due to exposure to PCB mixtures in monkeys, rats, mice and mink.

Table 11. Animal Studies on Reproductive and Developmental Effects of Maternal Exposure to PCBs.

Species	PCB[1]	Number/ Group	Conc. in Diet (ppm)	Dose Rate mg/kg/ day	Dosing Period	Duration of Study	Reference
Rhesus monkey	A-1248	12 8 8	Control 2.5 5.0	0 0.1 0.2	18 mo.	2 yr.	Allen & Barsotti, 1976; Barsotti et al., 1976
Rhesus monkey	A-1248	? 8 8	Control 0.5 1.0	0 0.01 0.02	18 mo.	?	Allen et al., 1979
Rhesus monkey	A-1016	8 8 8	Control 0.25 1.0	0 0.007 0.03	87 wks.	87 wks.	Barsotti & Van Miller, 1984
Cynomol-gus monkey	A-1254	1 2 1	Control 2.5 10	0 0.1 0.4	238-267 days	NR	Truelove et al., 1982
NMRI mice	C-A60	NR	Control 5 ppm	0 0.8	72-76 days	NR	Orberg & Kihlstrom, 1973
ICR Swiss mice	A-1254	4-6/ group	Control 1 10 100	0 0.2 2 20	g.d. 6-18 or 108 days	12 days 108 days	Welsch, 1985

Table 11 continued.

Species	PCB[1]	Number/ Group	Conc. in Diet (ppm)	Dose Rate mg/kg/ day	Dosing Period	Duration of Study	Reference
White-footed mice	A-1254	F_o F_o 101 F_1 26 F_1 25	Control 10 Control 10	0 2 0 2	46 days 12.5-17.5 mo.	12.5-17.5 mo.	Linzey, 1988
Swiss Webster mice	A-1254	 18 24 32	Control 10 100 250	0 1.16 116.69 291.69	18 wks.	18 wks.	Talcott & Koller, 1983
C3H/HeN mice	K-500	NR	Gavage	50	2 x/wk. for 3 wks.	20.5 wks.	Takagi et al., 1987
Sherman rats	A-1254 A-1260	10 M 20 F/ group	Control 1 5 20 100 500	0 0.06 0.39 1.5 7.4 35.4	vari-able	3 gen-erations	Linder et al., 1974
Sherman rats	A-1254 A-1260	10 M 10 F/ group	Gavage	0 10 50 100	g.d. 7-15	1 gen-eration	Linder et al., 1974
Holtzman rats	A-1254	7/ group	Control 25 50 100 150 200 300 400	0 1.29 2.57 5.14 7.71 10.28 15.42 20.56	g.d. 6-15	10 days	Spencer, 1982
Sprague-Dawley 1974 rats	K-300 K-500	NR	Control 20 100 500	0 1.1 6.2 28.6	g.d. 0-21	21 days	Shiota, 1976

Table 11 continued.

Species	PCB[1]	Number/ Group	Conc. in Diet (ppm)	Dose Rate mg/kg/ day	Dosing Period	Duration of Study	Reference
Wistar rats	A-1254	15 15 14 15	Control 2.5 26 269	0 0.13 1.34 13.8	6 wks.	53 wks.	Overman, et al., 1987
344 & Sprague-Dawley rats	A-1254	5/ group	Gavage	0 0.5 1.5 5.0 15 50 150 500	g.d.15 one dose	2 days	Wong et al., 1987
Mink	A-1016 A-1221 A-1242 A-1254	8 F 2 M/ group	Control 2 Control 2	0 0.3 0 0.3	10 mo.	10 mo.	Aulerich & Ringer, 1977
Mink	A-1254	20 10	Control 2.5	0 0.38	1 mo. prior to mating through partur-ition	same as dose period	Aulerich et al., 1985

1. A- Aroclor K- Kanechlor C- Clophen
2. Dose rate calculated assuming food consumption of:
   4.2% of body weight, monkeys;
   5.14% body weight, female rat;
   20% body weight, female mice
3. NR = not reported

The reproductive system of monkeys is very sensitive to the biological action of PCB exposure. Diets containing 2.5 ppm and 5.0 ppm Aroclor-1248 caused maternal intoxication and a lower conception rate among female rhesus monkeys maintained for 52 weeks (Barsotti and Allen, 1975). Females had higher levels of urinary ketosteroids and a decided increase in the length of menses and the amount of menstrual bleeding. Males exhibited only moderate periorbital edema and erythema and exposure had no impact on reproductive performance.

Female rhesus monkeys fed the same diets for six months prior to breeding, throughout gestation, and for three months following delivery exhibited a similar response (Allen and Barsotti, 1976; Barsotti et al., 1976). Menstrual cycles were altered at four months, becoming longer in duration and with increased bleeding. All of the eight females fed 2.5 ppm in the diet conceived but only five infants were carried to term. Six out of eight females fed 5.0 ppm conceived, four were aborted early in gestation, and one stillborn infant was delivered due to hypoxia during a difficult delivery. All the 12 control females conceived and delivered normal infants. The mean birth weight of the six exposed infants was 399 grams compared to 507 grams in the control group. The infants were small and had focal areas of hyperpigmentation of the skin. Weight gain was consistent, but lower than controls, and signs of PCB intoxication were present after two months. These included the development of acne on the face, swelling of the eyelids, loss of eyelashes and increased pigmentation of the skin. PCBs concentrations in three milk samples ranged from 0.154 to 0.397 ppm and were 16.44 ppm in the fat of a fourth sample. Skin concentrations in the infants were 1.0 to 4.8 ug/g tissue, 86.4 to 136.8 ug/g tissue and 4.19 ug/g tissue at birth, 3 months, and 8 months respectively. The level in fat was measured to be 19.71 ug/g tissue. Half of the infants died, and necropsy revealed toxic effects on

the thymus and spleen, bone marrow, liver and gastric mucosa in addition to the effects on facial skin, eyelids and hair follicles of the eyelashes. The health of the surviving infants improved after they were weaned at 4 months of age although retarded growth rate was reported to persist.

Maternal food intake was reported to be similar for exposed and control monkeys, however both treated groups lost an average of 15.1% of their initial body weight. PCB intoxication was apparent at two months indicated by loss of hair, acne on the face and neck, and erythema and swelling of eyelids and was exhibited by all the females at six months. An alteration of several clinical parameters was also evident. Two of the adult females died (one in each dose group). All exposed males (5.0 ppm in diet) had a slight to moderate periorbital edema and congestion at 14 months but experienced no alteration in sperm counts or breeding success.

The same females were again bred to control males one year after their removal from the experimental diets (Allen et al., 1979; Allen et al., 1980). All conceived and seven out of eight females in the 2.5 ppm group and five of the seven females in the 5.0 ppm group gave birth to live infants. The infants were small compared to their controls. Birthweights were 480 ± 83 grams and 440 ± 55 grams in the 2.5 and 5.0 ppm groups, respectively, compared to 516 ± 65 grams in the control group. The difference was statistically significant between the high dose group and controls ($p<.05$). During the next four months of nursing, the infants had slower growth, developed focal areas of hyperpigmentation and became ill more frequently. PCBs levels in whole milk averaged 0.05 ug/g (0.02 - 0.19 ug/g) at the time of weaning. PCBs

concentrations in subcutaneous tissue increased from undetectable at birth to an average of 3.31 ug/g at three months. Two infants in the 5.0 ppm group died and were found to have signs and symptoms characteristic of PCB intoxication in addition to small thymuses and rudimentary lymph nodes and spleens. Therefore, although a marked improvement in physical condition was observed one year after adult monkeys were removed from their PCB diets, severe ill effects were produced in their young.

Adverse reproductive effects were produced by lower dietary concentrations (0.5 and 1.0 ppm) of Aroclor 1248 administered three times per week for approximately 18 months (Allen et al., 1979). Consumption was equivalent to 0.01 and 0.02 mg/kg/day. Females were bred to control males after a seven month exposure period. No effect was noted on menstruation, hormone levels, or conception. Six out of eight females in the 0.5 ppm group and seven out of eight of the females in 1.0 ppm group gave birth to live infants. The infants were reported to be "somewhat smaller" than controls and gained weight less rapidly. Birth weights were 463 grams and 466 grams in the 0.5 ppm and 1.0 ppm dose groups. Birth weights were not reported for the control offspring. These weights may be compared to 507 ± 59 grams in the control group of the previous study. Focal areas of skin hyperpigmentation developed during nursing. The offspring were weaned at four months. In addition, the authors reported that these infants showed behavioral patterns similar to the infants exposed to higher levels of PCBs.

Fetotoxicity was also produced in rhesus monkeys (8 monkeys per dose group) consuming diets containing 1.0 ppm Aroclor 1016 (0.03 mg/kg/day) seven months prior and throughout gestation and a four month nursing period. This effect was not observed at a dietary level of 0.25 ppm (0.007 mg/kg/day; Barsotti and

Van Miller, 1984). No changes in food intake and general appearance was observed during the first seven months and no hematological or serum chemistry changes were noted. In addition, reproductive success was not altered and all infants were carried to term.

Birth weights of infants in the 1.0 ppm group were significantly lower than controls ($p<.001$). Neonatal weights were 422 ± 29 g, 491 ± 24g and 512 ± 64g in the 1.0 ppm, 0.25 ppm, and control groups respectively. At weaning, the infant weights in the 1.0 ppm group remained lower than controls (864 ± 97g vs 896 ± 90g) although the difference was not statistically significant.

A dose rate of Aroclor 1254 which produced only slight maternal toxicity resulted in adverse reproductive outcomes in cynomolgus monkeys although the small numbers make causal associations difficult (Truelove et al., 1982). Four pregnant monkeys which had previously exhibited normal menstrual cycles and delivered normal infants were dosed beginning on day 60 of gestation. Concentrations of 100 ug/kg body weight/day and 400 ug/kg/day Aroclor 1254 in apple juice were given to two monkeys and one monkey respectively three times per week. One additional monkey was given the dosing vehicle only. One treated monkey in each dose group lost its finger nails after 233 days (100 ug/kg/day) and 242 days (400 ug/kg/day) respectively. In addition, all three of the treated monkeys had a substantially lower antibody production compared to that of the control after a second SRBC challenge (148 days of treatment). Immunologic function, therefore, may be a more sensitive measure of toxicity than dermal effects. Both monkeys dosed 100 ug/kg/day delivered dead male infants after 105 and 108 days of dosing. A female infant, delivered by the 400 ug/kg/day mother, died at 139 days of age of acute confluent bronchopneumonia. This infant was below normal birth weight while the control

infant was within normal standards. Weight gain of the treated infant was retarded between 90 and 130 days. The exposed infant exhibited a reduced antibody production following SRBC injections and it was suggested that its death resulted from immunological deficiency.

Mink are also highly sensitive to PCB toxicity. Aroclor 1254 has caused reproductive failure when included in the diet at concentrations as low as 2 ppm over a period of eight months (Aulerich and Ringer, 1977). A long-term low level feeding trial was conducted using diets containing 2 ppm Aroclors 1016, 1221, 1242, and 1254. Dose groups contained eight female and two male mink. Body weight gain, hemoglobin or hematocrit values of treated mink were not significantly different from controls. Aroclor 1254 was the only PCB which exerted a negative effect on reproduction. Kits were whelped by only two out of seven females. Only one live kit was produced which weighed less than the average of kits from the other treatment groups.

Similar results were observed in mink fed 2.5 ppm Aroclor 1254 for approximately three months prior to and throughout gestation (Aulerich et al., 1985). Body weight gain was not significantly different from controls however liver weights were significantly increased (474 ± 21g vs 388 ± 21.2g). The liver enzymes, cytochrome P-450, benzo[a]pyrene (BaP) hydroxylase, and ethoxyresorufin O-deethylase, were significantly induced. Only one of the ten mated females whelped and the offspring, one kit, was stillborn. Six of the other females showed evidence of uterine implantation sites at necropsy. Plasma progesterone concentrations were significantly reduced, and it was suggested that the hormone levels were not high enough to maintain pregnancy (1.3 ± 0.34 ng/ml vs 3.4 ± 0.2 ng/ml).

Different mice species have shown varying sensitivities and responses to PCB mixtures. Altered estrus cycles and decreased implantation were produced in NMRI mice dosed 0.025 mg/day (1 mg/kg/day, 0.025 kg body weight) Clophen A60 for 72 - 76 days (Orberg and Kihlstrom, 1973). Continuous administration of Aroclor 1254 at concentrations from 1 ppm in the diet (0.125 mg/kg/day) to 100 ppm (12.5 mg/kg/day) to ICR Swiss mice did not produce adverse effects on fetal liver weight or skeletal development by gestation day 18 (Welsch, 1985). Maternal conception rate was reduced in the 100 ppm mice. In contrast, chronic dosing schedules of 10 ppm Aroclor 1254 in the diet (2 mg/kg/day assuming food consumption of 20% of body weight) produced reduced weight gain and decreased survival in first and second generation white-footed mice, and a decreased conception rate in the first generation (Linzey, 1988).

Orberg and Kihlstrom (1973) fed 23 sexually mature NMRI-strain mice 0.025 mg Clophen A 60 dissolved in 0.1 ml peanut oil daily by means of a glass tube inserted into the cage. The 22 control animals were given the peanut oil vehicle alone. The length of the estrus cycle was measured over 62 days and was found to be significantly longer in the 14 experimental mice compared to the 11 controls. Mean cycle length was 8.7 ± 4.3 days and 6.6 ± 2.5 days in mice fed Clophen A 60 and control mice respectively ($p<0.0005$). All 45 females were mated with untreated males at the end of 62 days. A significant reduction in the frequency of implanted ova was observed in the treated mice on day 8-10 of pregnancy. These animals had been exposed for 72 - 76 days. Eighty-seven percent of ova were implanted in the control group compared to only 79.5 percent in the PCB treated group ($p<.025$). The authors concluded that the results were probably caused by an increased metabolism of sex hormones. PCB residue levels were 44 to 424 ppm in the liver (fat weight basis) in the exposed group but did not exceed 5 ppm in the control group.

Welsch (1985) subjected female ICR Swiss mice to two dose regimens of Aroclor 1254 to study the effect of enzyme induction on the teratogenic potency of two model teratogens. Animals were provided 1, 10, and 100 ppm Aroclor 1254 in the diet beginning on gestation day 6 (acute) or were fed the treated diet beginning at about 30 days of age and maintained on the diet for 90 days. The mice were then bred and continued on the treated diet through gestation (chronic). Dams were challenged with a single intraperitoneal dose of a teratogen on gestation day 11 and were sacrificed on gestation day 18. The Aroclor 1254 was evaluated for contaminants and was found to be pure. The group of mice administered Aroclor 1254 alone showed no obvious signs of toxicity although there were subtle effects. Maternal liver to body weight ratio was increased significantly at all three chronic diet levels and at 100 ppm administered beginning on day 6 of gestation. Demethylation of aminopyrine was stimulated and 7-ethoxyresorufin was induced at 10 ppm in the mice fed the chronic dose regimen. Acute and chronic intake of 100 ppm Aroclor 1254 caused the induction of cytochrome P-450, demethylation of aminopyrine, AHH activity, and 7-ethoxyresorufin. Maternal conception rate was altered in the chronic 100 ppm group (15-20 mg/kg body weight). The uterine contents and morphology of fetuses including skeletal development were not affected on gestation 18. The number of resorptions was not different from controls and no malformations were observed.

Cumulative effects of Aroclor treatment were observed in white-footed mice followed for three generations (Linzey, 1988). Diets of 10 ppm Aroclor 1254 (2 mg/kg/day assuming a food consumption of 20% of body weight) were provided to 101 mice in three age groups; wild-caught adults, lab-raised mice paired at 12 weeks of age and lab-raised mice paired at 16 weeks of age. The parents,

which were fed control and treated diets throughout gestation and lactation, produced 246 litters. These were separated from the parents at 28 days of age or upon the birth of a subsequent litter. The offspring were fed the same diets as their parents. A group of 25 treated and 26 control pairs in the second generation assigned at 12 weeks of age were followed for 8 to 13 months.

Body weight at birth in the second generation did not differ significantly from controls. By four weeks of age however body weights of treated young were significantly lower than those of controls (11.01 g vs 12.79 g), and by 12 weeks control young averaged 2.6 g heavier than treated pups (17.08 g vs 19.68 g). Uterus and ovary weights of treated females were significantly less than controls at 8 and 12 weeks of age. Testis weights of males did not differ among groups. Of the 25 treated pairs in the second generation, only four litters were delivered (17 pups) by one pair compared to 28 litters (119 pups) born to seven out of the 26 control pairs. This 4% reproductive success rate in treated mice was much reduced compared to the parental generation, of which 42% produced litters. Average litter size was similar (4.2 treated vs 4.4 controls). Only one treated offspring survived to 12 weeks of age while 99 (83%) of the control young survived. This mouse weighed as much as a four week old control mouse. The reproductive success of second generation control mice was also poorer than their parents (27% vs 61%). It was suggested that this was caused by the restriction of the experimental colony to a single food item for an extended period of time.

A study investigating the immunotoxic effects of PCBs in mice observed no significant adverse reproductive effects (Talcott and Koller, 1983). Groups

of female Swiss-Webster mice were fed 10 ppm (1.169 mg/kg/week, 18 mice), 100 ppm (116.69 mg/kg/week, 24 mice), or 250 ppm (291.69 mg/kg/week, 32 mice) Aroclor 1254 in the diet for 12 weeks. The mice were then bred and exposure continued throughout gestation and lactation (18 week total exposure). Offspring were weaned at 3 weeks of age. No obvious illness was noted in treated dams and the frequency of pregnancy was not affected. The average number of pups per litter was reduced in the treated groups compared to controls, but the difference was not statistically significant. Maternal body weights were increased in all treatment groups compared to controls, and the difference was significant in the 250 ppm group. Liver weights expressed as a percent of body weight were significantly greater in the 250 ppm group, and slight to moderate hepatocellular hypertrophy was noted in the livers of dams in the 100 and 250 ppm groups. Treatment had no effect on the body or liver weights of progeny. Spleen-to-body weight ratios in offspring were decreased in all of the treatment groups compared to controls, and the difference was significant in the 250 ppm group. No liver lesions were observed histopathologically. Tests for the suppression of humoral immunity, delayed-type hypersensitivity, or phagocytosis activity of peritoneal macrophages in vitro were negative in all treatment groups.

Another study which tested the effect of Kanechlor-500 on filial immunocompetence found an age-dependent suppressive effect on helper T cell activity (Takagi et al., 1987). This effect was particularly intense in offspring exposed prenatally. Inbred C3H/HeN mice were administered 50 mg/kg Kanechlor-500 in olive oil twice a week for three weeks by gastric intubation. Females were mated with untreated males four weeks after the last administration of PCBs. Prior to nursing, fostering and cross-fostering was accomplished so that some of the pups were exposed to the test chemical only

in utero and some only through nursing. Although B cell activity was not affected, helper T cell activity, identified by a reduced production of anti-dinitrophenyl (DNP)-IgG plaque forming cells *in vitro*, was suppressed. The suppression was 50% of controls in four week old mice exposed only postnatally and was returned to normal in mice 7 weeks of age. Suppression was more pronounced in the mice exposed only prenatally with a response 20% of controls at 4 weeks of age and 40-50% of controls at 7 weeks of age. Response was returned to normal by 11 weeks of age. The more gradual recovery of suppressed helper T cell activity suggests that prenatal exposure to PCBs delays the maturation of the filial T cell activity. This data suggests that immunotoxicity is a sensitive indicator, and that intrauterine exposure may be more important than postnatal exposure for some developmental effects. The authors could not rule out the possibility that contaminants in Kanechlor-500 were the responsible agent for the observed immunosuppression.

Teratogenicity has been reported in mice exposed to the isomer, 2,3,5,2',4',5'-hexachlorobiphenyl during days 6-15 of gestation (U.S. EPA, 1984). Significant incidence of cleft palate was observed in fetuses of dams dosed at 2 mg/kg/day or greater. The occurrence was dose-related. Maternal toxicity was noted at doses of 8 mg/kg/day or greater. In addition, hydronephrosis incidence was significant and dose-related at 4 mg/kg/day or greater. Liver nodes occurred at significant levels at dose levels of 1 mg/kg/day or greater.

Research of interactive effects indicates that certain PCB isomers enhance the teratogenic potency of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (Birnbaum et al., 1985). Another PCB isomer, 2,3,4,5,3',4'-HCB, caused a statistically significant increase in the incidence of fetuses with cleft palate per litter

when administered in combination with 2,3,7,8-TCDD when compared to the effect of TCDD alone. The TCDD (12 ug/kg) and PCB (40 mg/kg and 80 mg/kg) were given by gavage to pregnant C57BL/6N mice on day 11 of gestation. The PCB isomer alone did not cause cleft palate in the offspring. Similar increases in cleft palate incidence were observed when TCDD (3 ug/kg) and the PCB isomer (10 mg/kg and 20 mg/kg) were administered to the dams on days 10 - 13 of gestation. A clear dose related effect occurred. The isomer, 2,4,5,2',4',5'-HCB had no significant effect on TCDD-induced cleft palate formation.

Hydronephrosis was caused by 2,3,4,5,3',4'-HCB when given to mice on gestation days 10 -13. The effect occurred in a dose-related manner although the renal lesions were much less severe than those caused by TCDD. This isomer did not enhance the effect of TCDD when administered in combination. 2,4,5,2',4',5'-HCB caused no renal damage and had no effect on TCDD-induced hydronephrosis. These studies show that certain PCB isomers, presumably those that induce cytochrome P-448 and bind to the Ah locus, are able to cause teratogenic effects in mice and enhance the teratogenic potency of 2,3,7,8-TCDD.

Chronic administration of PCBs to rats caused a reduced litter size and decreased survival in offspring of dams fed 20 ppm (1.5 mg/kg/day) or more Aroclor 1254 and 500 ppm (35.4 mg/kg/day) Aroclor 1260. A response was noted in the liver at the lowest concentrations tested, 1 ppm (0.06 mg/kg/day) Aroclor 1254 and 5 ppm (0.39 mg/kg/day) Aroclor 1260 (Linder et al, 1974). Similar effects were observed when rats were dosed with PCBs on specific days during gestation (Wong et al., 1987, Sager et al.,1987, Spencer, 1982, Shiota, 1976).

Linder et al. (1974) conducted a two generation reproduction study in Sherman strain rats using Aroclors 1254 and 1260. Aroclor 1254 was fed in the diet in concentrations of 0, 1, 5, 20, and 100 ppm, and levels of Aroclor 1260 were 0, 5, 20, 100 and 500 ppm. The parent generation was started on the treated diets at 3-4 weeks of age and exposure was continuous through mating, gestation and lactation until the rats were killed. The F1b generation was fed the treated diet upon weaning. Groups of 10 males and 20 females were fed at each dietary level in both generations. The F0 rats were pair-mated at 3 and 7 months of age to produce the F1a and F1b generations respectively. Breeding stock F1b rats were selected at weaning from all available litters and were pair-mated when 3 months old to produce the F2a generation. The F1b rats fed 0, 20, and 100 ppm Aroclor 1254 were mated a second time when eight months old to produce the F2b generation.

Litter size was decreased in the F1 generation. Reduced litter size was statistically significant at 100 ppm in the F1a generation and at 20 ppm in the F1b generation demonstrating the effect of duration of exposure. This fetotoxic effect was statistically significant in both the F2a and F2b generations at 20 ppm. The cumulative toxicity of PCBs exposure was expressed in the decreased number of litters born in both F2 generations at 100 ppm and possibly 20 ppm (F2b). Survival to weaning was reduced in the F1b and F2a generations at 100 ppm. Survival in the F1b mice was 73.6% compared to 90.4% in controls and 77.8% in the F2a mice compared to 97.5% in controls. Only 3.5 pups/litter were born in the F2b generation which may have given these offspring a better chance of survival to weaning accounting for the 100% survival in this group. Mean body weight was decreased in both F1 generations (six grams less than controls) in the 100 ppm dose group.

Concentrations of 1 and 5 ppm Aroclor 1254 in the diet had no adverse impact on reproduction however the livers of offspring showed a response to treatment. Both sexes of 21 day old weanlings in the F1 and F2 generations had increased liver to body weight ratios beginning at the 5 ppm dose level. This effect was observed in F1 generation males at 1 ppm. Liver damage was noted in weanling rats at 20 ppm and to a greater extent at 100 ppm. Liver cells were enlarged with vacuolated cytoplasm due to lipid accumulation. Slightly enlarged hepatocytes in some livers was the only microscopic change noted at 5 and 1 ppm although the livers of rats dosed at these lower dietary levels were not examined systematically. The liver to body weight ratio was still increased in 10 month old F1 rats of both sexes fed 100 ppm but only in males fed 20 ppm. Among the eight livers examined from male rats dosed with 20 ppm, eight contained enlarged hepatocytes, three contained inclusions, seven had foamy cytoplasm and one contained pigment. While liver weights of female F1b rats dosed 20 ppm were not increased, out of the seven animals examined, seven contained enlarged hepatocytes, two contained inclusions, five had foamy cytoplasm and seven contained pigment. At 5 and 1 ppm the cytoplasm of many liver cells were reported to be vacuolated or foamy. The authors concluded that these changes are indicative of lipid accumulation. These effects on the liver cells grew more pronounced in male and female F1 and F2 rats exposed to 100 ppm. In addition, adenofibrosis and hepatic nodules were noted in males (3 out of 10 and 1 out of 10 livers examined respectively) and females (5 out of 10 and 7 out of 10 livers respectively). Hepatic nodules were also noted in 3 out of 7 livers of female rats in the 20 ppm group. Hematologic signs were altered in F1b rats at 100 ppm and F1b adult males in this dose group had an increased testes to body weight ratio.

Dose levels of 0, 5 (0.39 mg/kg/day), 20 (1.5 mg/kg/day), and 100 ppm (7.4 mg/kg/day) Aroclor 1260 produced no effect on reproduction through two generations. Increased liver to body weight ratios were observed in 21 day old pups in all generations in the 5 ppm dose group or higher. The effect on liver weight was still present in 5 to 7 month old F1b males at all dose levels and in F1b females at 100 ppm. Concentrations of 20 and 100 ppm Aroclor 1260 caused an increase in liver weight in F0 males after 8 months exposure. Enlarged hepatocytes and vacuolated cytoplasm were observed with increasing frequency in the F2 weanlings between 5 and 100 ppm. This effect on liver cells became apparent in the F1 weanlings at 100 ppm and hepatic nodules were observed in F1 adults at this dose level. An increase in testes to body weight ratios was observed in F1b males at 20 ppm and the higher ratio became significant at 100 ppm. Body weight gain was comparable to controls in all the test groups.

The author stated that the effects on liver weight in weanlings was probably due to exposure via nursing and concluded that 1 ppm may be very near a threshold for liver weight increase in weanling rats. Lipid accumulation was observed in hepatocytes at this dose level as well. Fetotoxicity indicated by decreased litter size and altered mating performance was observed in rats dosed with 20 ppm Aroclor 1254 in the diet over extended periods.

Fetotoxicity was apparent in the litters of dams dosed with 100 ppm Aroclor 1254 in the diet from days 6 through 15 of gestation (Spencer, 1982). Female Holtzman strain albino rats showed a decrease in placental protein and placental glycogen on gestation day 16 which became significant at 150 ppm and 400 ppm respectively (mean of 7 rats). Fetotoxicity was demonstrated by a decrease in fetal survival rate per liter, significant at 300 ppm (28%

survival vs 79.54% in controls), and a reduction in average fetal weight at birth per litter, significant at 100 ppm (6.19 g vs 7.02 g in controls). The fetal weight difference was observed in the absence of maternal toxicity which became manifest at 200 ppm. Maternal body weight gain was less than controls beginning at 200 and 300 ppm (+25 g and +20 g respectively vs +29 g in controls) and reductions in chow consumption started at 300 ppm. The authors suggested that the results indicated that Aroclor 1254 causes imbalances in hormonally-mediated biochemical effects in pregnant rats. Systemic, via nutritional deficiencies, and direct action on fetal growth and development was observed.

Fetotoxicity was not apparent in Sherman strain rats administered 100 mg/kg/day Aroclor 1254 by gastric intubation on days 7 - 15 of gestation (Linder et al., 1974). However, survival to weaning was decreased compared to controls (30.1% vs 98.2%) and the mean body weight of weanlings was 7.1 g less than controls. Toxicity was not evident in the offspring of dams dosed with 50 mg/kg/day Aroclor 1254 or 100 mg/kg/day Aroclor 1260. It was concluded that although the litters were grossly normal at birth, ingestion of PCBs via milk contributed to increased mortality.

Toxic effects were noted in fetuses of Sprague-Dawley rats consuming 0.9 - 1.3 mg/kg/day (20 ppm) and 5.3 - 7.0 mg/kg/day (100 ppm) Kanechlor-300 and 23.2 - 37.7 mg/kg/day (500 ppm) Kanechlor-300 and 500 on gestation days 0 to 21 (Shiota, 1976). No significant difference was observed between treated rats and controls in number of implants and number of resorptions although the resorption rate was relatively high in both control groups. A dose-related decrease in body weight in live fetuses was significant at 20 and 100 ppm for Kanechlor-300 and at 500 ppm for Kanechlor-500. Observed malformations were

not dose-related and therefore probably were not due to treatment. Mean body weights in offspring of dams allowed to litter naturally were less than controls although the difference was not statistically significant. Only four litters per dose group were examined however. Fetal pup survival was not adversely affected. The lowest fetotoxic dose was estimated to be 1.1 mg/kg/day Kanechlor-300 and 28.6 mg/kg/day Kanechlor-500. The fetotoxic dose for Kanechlor 500 also produced maternal toxicity indicated by a statistically significant reduced weight gain on gestation day 21 and decreased food consumption during the first two weeks of gestation.

A more recent study using Aroclor 1254 found similar effects in the offspring of Wistar-derived rats exposed to 0.02 (control, 15 rats), 2.5 (15 rats), 26 (14 rats), or 269 ppm (15 rats) PCB in the diet from mating to weaning of offspring (Overmann et al., 1987). Pregnancy success, pup birth weight, and dam body weight and food intake were not altered by the 2.5 or 26 ppm dose rate. Pup growth prior to weaning was reduced in the 26 ppm group and was slightly reduced in the 2.5 ppm group. Exposure to the 269 ppm diet reduced the number of litters delivered by impregnated rats, lowered pup birth weight, and most pups died within seven days.

Dams sacrificed at the weaning of their pups were found to have significantly increased liver weights expressed as a percent of body weight in the 2.5 and 26 ppm groups. Increased liver weight was also observed in 21 day old pups in the 26 ppm dose group but not in 150 day old offspring. Absolute spleen weight in 21 day old weanlings decreased with increasing PCB concentration and was significantly smaller in 150 day old rats exposed to 2.5 ppm. Absolute thymus weight also decreased with increasing dose and became significantly reduced in 21 day old rats in the 26 ppm group. A histopathological analysis

of these organs was not reported. Brain PCB levels at birth were 2 and 22 times higher than control values in the 2.5 and 26 ppm groups respectively and the concentrations increased with age in all dose groups. At weaning, the brain levels were 8 and 30 times higher than controls respectively and remained substantially elevated above control levels in the 26 ppm group at 150 days of age. The concentrations in pup brains at weaning were twice the levels in the brains of their dams. Behavioral performance was also altered and these effects are included in the section on behavioral teratology. Low concentrations of polychlorinated dibenzofuran were detected in the Aroclor 1254 in this study.

The results observed by Shiota (1976) and Overmann et al. (1987) were less severe than those observed for similar dose levels of Aroclor 1254 which caused fetal resorptions and abortions and decreased infant survival during a two generation study (Linder et al., 1974). This may be a function of a shorter duration of treatment, differences in strain sensitivity, or differences in the isomer composition of Kanechlor and Aloclor. The number of breeding females was not stated in the two shorter studies and the number of litters in each dose group was not large (8 - 11). Both studies demonstrated that reproductive toxicity is inversely related to the degree of chlorination of the PCB mixture.

The P-450 mono-oxygenase system in fetal livers was induced at PCB dose levels approximately one order of magnitude higher than those which induced metabolic activity in maternal liver and placenta (Wong et al.,1987). Small groups (4-5/group) of female pregnant F344 and Sprague-Dawley rats were treated with a single dose of Aroclor 1254 in corn oil by gavage on day 15 of gestation. Dose rates were 0, 0.5, 5.0, 50, and 500 mg/kg body weight. An additional

group of Sprague-Dawley rats were dosed 0, 1.5, 15, 50, and 150 mg/kg body weight. The dams were killed after 72 hours. Mean fetal liver weights, pooled for each dose group, increased with increasing dose for both sets of Sprague-Dawley rats. The positive correlation was statistically significant. No difference in fetal resorptions between control and treated groups was observed for either strain. In F344 rats, aryl hydrocarbon hydroxylase (AHH) activity was induced in maternal livers by 5-50 mg/kg and increased with increasing dose. Placental AHH activity was induced at the same dose levels but was much lower than hepatic activity and appeared to plateau with increasing dose. Fetal liver AHH activity was first detected between 50 and 500 mg/kg. The dose dependency of enzyme induction was also observed in the Sprague-Dawley rats. Maternal and placental induction was significantly higher than controls at 15 mg/kg while enzyme induction in fetal liver did not become significantly greater until 150 mg/kg. O-deethylase activity (7ECD) was 2-5 times higher than the corresponding AHH activity. 7ECD induction was present in one set of Sprague-Dawley rats at 1.5 mg/kg and was not present in the other set at 5 mg/kg. The presence of a placental barrier to PCB transfer was suggested as an explanation for the higher dose requirement for transplacental induction of fetal livers compared with maternal tissues. Transplacental mono-oxygenase induction by PCBs has also been demonstrated in Sprague-Dawley rats dosed with 25 mg/kg/day Aroclor 1254 during gestation days 14-19 (Wong et al., 1987). Hepatic mono-oxygenases have been induced in adult rats with doses of PCBs at 0.5 to 10 ppm in the diet.

Adverse effects on reproductive performance in males have not been demonstrated in several rodent studies. However, a recent study designed to differentiate between the effect of reduced weight gain and PCB toxicity on

reproduction in rats has demonstrated that early postnatal exposure to PCBs negatively affected male fertility (Sager et al., 1987).

Sager et al. (1987) exposed female lactating Holtzman strain rats to oral doses of Aroclor 1254 on days 1, 3, 5, 7, and 9 at a rate of 8, 32, and 64 mg/kg. Two control groups, one normal and one at 70-75% of the food intake of the normal control, received oral treatments of peanut oil. Males, weaned at 23 days of age, were mated with normal females at 130 days of age. One group of dams were killed and autopsied on day 11 or 12 of gestation. Another group of dams, mated to the same treated males, were killed and autopsied on day 2 or day 4 of gestation to quantify the 2-4 cell stage of development or morula/blastocyst stage respectively. Dams autopsied on gestation day 11 or 12 had fewer implants, fewer embryos, and a reduced proportion of implanted ovulated eggs compared to both control groups. The reductions were significantly different at all dose levels except the number of implants/corpora lutea at 8 mg/kg (reduced but not significant). Body weights at mating and autopsy were comparable in all dose groups. Reduced fertility was not seen in the nutritionally deprived control group. Dams receiving doses of 32 mg/kg and 64 mg/kg had significantly fewer eggs in the expected stage of development. In addition, the average number of blastocysts found in one uterine horn on day 4 was significantly reduced in these dose groups. Females could either support normal embryonic development to the 2-4 cell stage but not to the blastocyst stage, or not to either stage. The difference in the number of 2-4 cell embryos became no longer significant when the number of 1 cell stage embryos was included in the analysis. This suggested that fertilization/initial development may be occurring, but may be delayed. The sperm counts in the treated males were not significantly different among groups at autopsy. The authors suggested that motility may be a factor.

Reproductive failure occurred in a male rhesus monkey exposed to 5 ppm Aroclor 1248 for 18 months (Allen et al., 1979). This animal developed clinical signs of PCB intoxication between the 12th and 18th month, experienced a decreased sperm count, and failed to impregnate females after repeated breedings. Spermatogenesis was absent but completely recovered one year after exposure was discontinued.

Behavioral Deficits

PCBs exposure has resulted in behavioral and neurological deficits in the offspring of monkeys (Bowman and Heironimus, 1981), mice (Storm et al., 1981) and rats (Shiota, 1976 and Overmann, 1987).

Infant rhesus monkeys whose mothers had been fed 2.5 ppm Aroclor 1248 in the diet exhibited locomotor hyperactivity at six and twelve months of age. The hyperactivity was positively correlated with levels of PCB residues assayed in the body fat of the infants. The same animals were tested again at 44 months of age and were found to be hypoactive relative to controls (Bowman and Heironimus, 1981). The locomotor activity of three offspring of mothers fed 2.5 ppm PCBs (one male and two females) and three non-exposed controls (two males and one female) was assessed in an activity chamber quadrasected by two photobeams five days per week for 24 days. The number of photobeam breaks were tabulated by 15 minute periods during 90 minute sessions. The PCB group averaged one half or less the locomotor activity of the controls at nearly every one of the 15 minute periods in each four day block of sessions. The observed hypoactivity was statistically significant. No statistically significant difference was noted between the adaptive response of the two groups over time although the controls reached the final activity pattern at a

relatively faster rate. The investigators stated that subsequent experiments using a larger sample size followed up to two years of age, had shown similar responses in activity. The appearance of hypoactivity was interpreted to be a delayed, chronic effect occurring nearly four years after PCBs exposure and three years after clearance of measurable PCB residues from the animals' mesenteric fat. It was suggested that the response could be irreversible since it appeared subsequent to sexual maturity.

Mice exposed to low levels of PCBs pre- and postnatally exhibited a significantly altered pattern of behavioral response to a learning task and to a novel environment (Storm et al., 1981). Ten adult female ICR strain mice per dose group were fed diets containing 0, 11, and 82 ppm Aroclor 1254 beginning three days prior to mating. The resulting litters were reduced to eight pups each and were weaned at 21 days of age. Offspring were fed the same diets as their parents. One-half of the litters were evaluated in conditioned avoidance response training over 13 days beginning at 23 days of age and the rest participated in open field observation at 27 days of age. No significant litter effects were noted for any of the behavioral measures, and therefore, the data for individual mice was used in the statistical analysis. Both groups of treated mice on day 1 of the conditioned avoidance response trials took longer to respond to the stimulus over 20 trials than the controls. A steeper decline in response latency over the 13 day period was also observed in these animals so that the difference in response latency between exposed and control animals disappeared during the course of the training. Treated mice in the open field test tended to traverse more squares than controls during the entire 30 minutes except for the first 5 minute period. All three groups showed habituation to the new environment however the rate of decline in activity was less in the experimental group than the

control group. The direction of alterations observed in this study was reported to be similar but more subtle than those observed in previous experiments using higher dose levels of PCBs.

Spinning syndrome, a permanent motor disturbance, was produced in 24 offspring of 60 CD-1 mice dosed orally on gestation days 10 through 16 with 32 mg/kg/day of the PCB isomer, 3,4,3',4'-tetrachlorobiphenyl (Chou et al., 1979). Three dopaminergic drugs were able to the modify the spinning behavior of the mice.

Sprague-Dawley JCL strain offspring of rats exposed to 0, 20 mg/kg or 100 mg/kg Kanechlor 500 on either days 8-14 or 15-21 of gestation were reported to show no significant differences in behavior in an open field test conducted at 12 weeks of age (Shiota, 1976). However, the data as presented indicate that treated males were more active. The number of animals participating in the tests was small and the data was not evaluated for differential effects by litter. Food consumption was reported to be suppressed in dams in the high dose group.

Overmann et al. (1987) used better statistical techniques although the number of litters tested was also relatively small with 11 (control), 10 (2.5 ppm), and 8 (26 ppm) litters comprised of 8 pups/litter. Pups in the 26 ppm group were significantly slower than controls in completing a 135 degree turn at 7 and 8 days of age. This functional alteration occurred before an effect on pup body weight was noted. The appearance of auditory startle response was slightly delayed in the 2.5 and 26 ppm groups and the development of air righting ability, an indication of neuromuscular maturation, was slightly delayed in the 26 ppm group. Both PCB doses altered the duration of maximal electroshock seizure phases in rats postweaning, tested at either 40 or 330

days of age. The authors concluded that a persistent, probably irreversible, functional change in the nervous system had resulted from excess perinatal PCB exposure.

SUMMARY

Epidemiological studies of the children of mothers exposed to PCBs through Lake Michigan fish consumption have revealed physical and behavioral deficits (Jacobson et al., 1983, Fein et al., 1984, Jacobson et al., 1984, and Jacobson et al., 1985). These effects occurred in a sample with a mean consumption level of 6.7 kg per year of lake trout equivalent fish. A dose-response relationship for fish consumption and low birth weight, smaller head circumference and delayed motor maturity was demonstrated. Lower visual recognition memory scores were also measured in the children of fish consumers and were correlated with cord serum PCB levels. This may signify an irreversible effect due to intrauterine exposure. Low birth weight and shorter gestational age were associated with other environmental and occupational exposures, as well as poisoning incidents. These effects were also observed in the offspring of nonhuman primates. Altered behavior which may also be irreversible has been observed in adolescent rhesus monkeys exposed pre- and postnatally to Aroclor 1248 (0.5, 1.0, and 2.5 ppm in the maternal diet; Bowman and Heironimus, 1981; Allen et al., 1979).

Reproductive toxicity was produced by low-level PCBs exposure in nonhuman primates and mice. Rhesus monkeys fed 2.5 and 5.0 ppm Aroclor 1248 for six months experienced changes in the length of menstrual cycles and hormone levels (Allen and Barsotti, 1976; Barsotti et al., 1976). The estrus cycle was lengthened in NMRI mice fed 5 ppm (0.8 mg/kg/day) Clophen A 60 in the diet

for 62 days. In addition, implantation frequency was reduced in NMRI mice exposed to PCBs at the same dose level for 72 - 76 days.

Fetotoxicity and behavioral teratological effects have been produced in mice and rats exposed prenatally and through nursing. White-footed mice may be the most sensitive strain among mice tested (Linzey, 1988). Second generation offspring had a depressed body weight gain at four weeks of age and lower uterus and ovary weights at eight and twelve weeks in a three generation continuous feeding experiment. This dose group (10 ppm in diet) also produced a lower number of litters when offspring were paired at twelve weeks of age. Altered behavioral responses occurred in ICR strain mice at a similar dose level (11 ppm during gestation; Storm et al., 1981).

An adverse effect of PCBs exposure was observed on helper T cells activity in mice exposed prenatally by Takagi et al. (1987). This effect was also reported in human patients exposed to PCBs through consumption of rice oil (Chang et al., 1982). Similarly to the deficits reported in exposed human offspring, prenatal exposure appears to be primarily responsible for the PCBs-induced immunosuppression reported in mice.

Rats fed 20 ppm (1.5 mg/kg/day) produced a lower number of litters and smaller litter sizes than controls (Linder et al., 1974). Liver damage was observed in the offspring. Dietary levels as low as 1 ppm (0.06 mg/kg/day) increased liver to body weight ratios in second generation 21 day old males. The effect on the liver was present in both sexes in the second and third generations and in five to seven month old second generation males in the 5 ppm (0.32 mg/kg/day) dose group. This data indicates that a LOAEL for increased liver weight in rat offspring may occur at 1 ppm in the diet. Enlarged hepatocytes

with an indication of lipid accumulation were observed in these offspring as well. Fetotoxicity appears to occur at dietary dose levels of 20 ppm and greater in rats while a toxic response occurs in the livers and spleens of offspring exposed prenatally and via nursing at lower dose levels (Linder et al., 1974 and Overman et al., 1987).

A dose level of 8 mg/kg caused reproductive deficits in male rats exposed postnatally through nursing. It was suggested that sperm motility may be affected (Sager et al., 1987). Low concentrations of three PCB congeners in human sperm were associated with decreased motility in infertile men (Bush et al., 1986).

A NOAEL of 0.25 ppm was reported for Aroclor 1016 fed to rhesus monkeys where 1.0 ppm in the diet for seven months prior to and throughout gestation to four months postpartum resulted in significantly lower birth weights (Barsotti and Van Miller, 1984). The offspring of rhesus monkeys exposed to 0.5 and 1.0 ppm Aroclor 1248 in the diet were smaller, gained weight less rapidly than controls, and exhibited behavioral abnormalities (Allen et al., 1979). These PCB concentrations had no adverse effect on female reproductive capacity and are the lowest dose levels reported for effects on nonhuman primates. Higher dietary levels produced severe maternal toxicity and adverse effects in exposed offspring. Severe reproductive toxicity was observed in mink at the lowest dose tested. Complete reproductive failure was produced in eight mink fed 2.0 ppm Aroclor 1254 in the diet for ten months (Aulerich et al., 1977).

CARCINOGENESIS

HUMAN

Evidence for carcinogenicity in humans is limited but suggestive. An excess of malignant tumors has been reported among Yusho patients with the gastrointestinal tract and lymphatic and hematopoietic tissue most affected (Kuratsune et al., 1987). An investigation of causes of death in this population reported in 1984 confirmed the existence of a statistically significant excess of deaths from malignant tumors (SMR = 165) and reported six cases of primary carcinomas of the liver (SMR = 492). Occupational studies have identified melanoma and pancreatic cancer (Bahn et al., 1976), hepatic and rectal tumors (Brown and Jones, 1982 and Brown, 1987) and cancer of the GI tract and lymphatic tissue (Bertazzi et al, 1987) associated with PCB exposure.

A total of 1761 out of 1821 officially registered Yusho patients (887 males and 874 females) were followed by Kuratsune et al. (1987) from the date of registration to the end of 1983. The average duration of follow-up was reported to be 11 years. Observed deaths were compared with the expected number of deaths calculated by applying national age, sex, and cause specific death rates in 1970, 1975, and 1980 to the person-years at risk. A total of 120 deaths were recorded (79 males and 41 females).

Death from cerebrovascular disease was less than expected in males and females and was statistically significant in females. Cancer mortality, all sites, was significantly greater in males but not females. Deaths from liver cancer was higher than expected in males (SMR 5.59) and females (SMR 3.04) and the

difference was statistically significant in males. The excess liver cancer mortality in males remained significant when death rates were compared with those in Fukuoka and Nagasaki prefectures where 45% and 40%, respectively, of the Yusho patients reside. A significant increase also remained when four cases occurring in Fukuoka prefecture at least nine years after poisoning were compared with expected deaths in that area (expected 1.04, SMR 3.85). This apparent association of exposure with poisoning is clouded by an unequal geographic distribution of liver cancer deaths between the two prefectures. The authors stated that it was too early to draw conclusions which seems reasonable in light of the relatively low number of deaths which had occurred and the relatively short period of time that had elapsed.

A study of workers employed in a capacitor manufacturing plant in Italy identified an excess in the number of deaths from cancer in both sexes. The target sites involved were the lymphatic tissue and the GI tract (Bertazzi, 1987). The workers had been exposed to PCB mixtures containing 54% chlorine (1946-1970) and 42% chlorine (1964-1980). All male and female workers employed between 1946 and 1978 for at least one week in the plant were included in the study. Specific job descriptions were not available for all study participants. Vital status for workers no longer employed was obtained from the Vital Statistics Bureau at the place of residence or birth. Mortality data was examined for the period 1946 - 1982 and was obtained from death certificates. The total study population was 544 males and 1556 females, a total of 41,010 person-years.

Cancer deaths among males were significantly greater than expected based on national or local population mortality rates (SMR = 183). The risk of cancer of the digestive tract was also significantly greater than expected (SMR =

274). A greater but statistically insignificant incidence in hematologic neoplasms was also detected in males (SMR = 263). Overall mortality in females was greater than that expected in the local area (SMR = 206) and was accounted for by cancer and accident mortality. Total malignant tumors (SMR = 226) and hematologic neoplasms (SMR = 377) were significantly greater. No pattern or trend was noted for duration of exposure, latency, and year of first exposure.

Personal and environmental characteristics of the study population were reported to be similar to the local population and therefore can not completely explain the difference in cancer mortality. Of the GI tract cancers in males, two of the six cases were not employed directly in production and one had been hired at age 59, was exposed for less than 5 months, and had a latency of 7 years. The authors concluded that the hematologic neoplasms were possibly associated with exposure because all three cases were production workers with a potential for high exposure during the period of their employment (7, 9, and 12 years). Two of the four females with lymphatic cancer were employed for only 2.4 and 8.4 months and had latencies of 0.2 and 2 years making a causal association questionable. Two of the male hematologic neoplasms were leukemia which has also been associated with exposure to electromagnetic fields. There was a potential for this type of exposure in the capacitor plant during the testing of large power capacitors. Other exposures and life-style behaviors having an impact on cancer incidence were not evaluated. This information deficit, plus the lack of a defined control group, and lack of a time-related trend in incidence do not allow for a definite causal association for PCB exposure. The authors concluded that although a causal association was not possible, the results were similar to those of previous investigations and do support the possibility of a

carcinogenic risk of PCBs to humans with primary target organs being the GI tract and lymphatic tissue.

The National Institute for Occupational Safety and Health conducted a retrospective cohort mortality study of 2,567 workers in two capacitor manufacturing plants (Brown and Jones, 1982). Workers who had accumulated at least three months of employment in areas of potential exposure were included in the study population unless they were also potentially exposed to trichloroethylene, a degreaser sometimes used in the manufacturing process. While mortality from all causes of cancer was not increased among the exposed group, the standard mortality ratios (SMR) for cancer of the liver (4 observed vs 1.19 expected) and rectum (3 observed vs 1.07 expected) were elevated. Cirrhosis of the liver was also higher than expected. The observed increases were not statistically significant except for rectal cancer in females at one plant. The number of deaths was quite low however. The investigators were not able to evaluate alcohol consumption, a potential confounder for cirrhosis of the liver. The incidence of liver cancer did not correlate with increasing latency period in this study, although an increased incidence of rectal cancer was correlated with latency.

An update of mortality in the study population expanded by 21 additional workers who qualified for inclusion in the cohort was reported for seven more years through December 31, 1982. There were 132 more deaths and the number of person-years was increased to 16,527. The SMR for rectal cancer dropped to 211 (4 observed vs 1.9 expected) and it was no longer statistically significant. Two deaths from cancer of the liver, gall bladder, and biliary tract occurred among females at one of the plants during this time. The SMR for the cohort was 263 (5 observed vs 1.9 expected) and was statistically

significant. A relationship was not demonstrated between cancer incidence and length of employment, or the time since first employment in "PCB-exposed" jobs. The authors suggested that this was due to the small number of deaths observed and a lack of information regarding other exposures.

On the other hand, all four deaths among females at the one plant occurred after 15 years of latency. Moreover, all workers who died from liver or biliary tract cancer at this site were first employed at the plants in the 1940's and early 1950's when exposures may have been higher, and when more highly chlorinated PCB mixtures were probably used. Experiments in rodents have shown that the more highly chlorinated PCBs are more potent carcinogens (Ito et al., 1973, Ito et al., 1974, Schaeffer et al., 1984). The authors suggested that the greater incidence in females was due to four possible reasons. (1) Females at that plant accounted for the largest segment of the total cohort (41%) and 52% of the person-years over 20 years of latency. (2) Airborne exposure levels may have been greater at the plant. (3) Risk factors not accounted for in this study may have influenced cancer incidence. (4) Finally, the excess risk in females may have been caused by a sex-dependent carcinogenic promoting effect which has been observed in rats.

A retrospective study of 142 male Swedish capacitor manufacturing workers did not find excess cancer incidence (Gustavsson et al., 1986). Participants were employed for at least six months between 1965 and 1978. PCBs were used as a dielectricum between 1960 and 1978. The mean exposure time of this population was 6.5 years. Information was obtained on 21 deaths from death certificates between 1965 - 1982 and seven cancer cases reported to the National Cancer Registry between 1965 - 1980. No excess in cancer or mortality was observed. The sample population was small, and the length of follow-up was too short to

evaluate the carcinogenic potential of PCBs in this group of workers. The median follow-up period was 13 years with a range of between 4 to 22 years. Mortality from cancer of the liver, gall bladder, and biliary tract did not occur in the NIOSH study until 15 years of exposure had occurred in four out the five cases observed. This would have an effect of reducing the sensitivity of the study design.

Bahn et al. (1976) reported an increased incidence of malignant melanoma among workers who had handled PCBs at a petrochemical plant in the northeastern United States. Aroclor 1254 had been used at the plant for nine years ending in the late 1950's. Information on cancer incidence was obtained from the plant's medical director. Two melanomas were reported among 31 men believed to be heavily exposed. This incidence was significantly higher than the 0.04 cases expected. Although association of skin cancer with PCBs exposure has biologic plausibility because of other dermatologic symptoms seen in humans, there was no analysis of the PCB exposure, other potential chemical exposures, or potential confounding factors.

ANIMAL

The primary target site for tumorigenesis associated with PCBs is the liver in rats (Ito et al., 1974, Kimbrough et al., 1975, Kimura and Baba, 1973, NCI, 1978, Norback and Weltman, 1985 and Schaeffer et al., 1984) and mice (Ito et al., 1973 and Kimbrough and Linder, 1974). These studies on rats and mice have reported a dose-related occurrence of foci of hepatocellular alteration, hyperplastic nodules, neoplastic nodules, and hepatocellular carcinoma. In addition, adenofibrosis (Ito et al., 1974, Kimbrough et al., 1975, Norback and

Weltman, 1985), adenoma (NCI, 1978), and adenocarcinoma (Norback and Weltman, 1985) were reported in rat liver, and hepatoma and adenofibrosis (Kimbrough and Linder, 1974) were reported in mouse liver.

Carcinogenic effects may also be associated with PCB exposure in other organs. NCI (1978) concluded that carcinomas observed in the gastrointestinal tract may have been related to treatment in male and female rats. Morgan et al. (1981) detected an increased incidence of intestinal metaplasia and adenocarcinoma in the glandular stomach of male and female rats.

The higher chlorinated PCB mixtures are associated with a greater carcinogenic response in both rats (Ito et al., 1974, Schaeffer et al., 1984) and mice (Ito et al., 1973). Limited data suggest that females may be more sensitive to the carcinogenic stimulus than males (Norback and Weltman, 1985).

Carcinogenic potential has been evaluated in five strains of rats and two strains of mice. Table 12 summarizes the study designs used in each investigation.

Noritaka and Baba (1973) fed Donryu strain rats (ten males and ten females) a powdered diet mixed with Kanechlor-400 dissolved in olive oil. Control groups of five males and five females were fed the diet mixed with olive oil. The concentration in the diet varied over the treatment period increasing from 38.5 to 616 ppm over 181 days. During 56 days of treatment, at the highest dose level, a large body weight decrease was observed and the concentration was subsequently reduced to 462 ppm for the remainder of the study. The last 275 days of the 456 day treatment period were interspersed with two 28 day

Table 12. Animal Studies of Carcinogenicity Resulting from Dietary Exposure to PCBs.

Species/ Sex/ Reference	PCB[1]	Dose (ppm)	Dose Rate[2] (mg/kg/ day)	Number/ group	Number at Risk	Duration of Study (weeks)
Donyru rats M & F Noritaka & Baba, 1973	K-400	0 38.5- 616	NA	5 10	NR	23- 80
Wistar rats M Ito et al., 1974	K-500 K-400 K-300	0 100 500 1000	Control 4 20 40	29 29 29 29	K-5 K-4 K-3 18 25 16 22 26 8 19 13 10 15	28- 52
Sherman rats F Kimbrough et al., 1975	A-1260	0 100	Control 5	200 200	173 184	84
Fisher 344 rats M & F NCI, 1978	A-1254	0 25 50 100	Control 1.13 2.25 4.5	24 24 24 24	NR	105
Sprague-Dawley rats M & F Norback & Weltman, 1985	A-1260	0 100 for 16 mo., then 50 for 8 mo.	Control 3.75	140 126	81 93	116
Wistar rats M Schaeffer et al., 1984	C-A30 C-A60	0 100 C-A30 100 C-A60	Control 4 4	139 152 141	53 87 85	119

Table 12 continued.

Species/ Sex/ Reference	PCB[1]	Dose (ppm)	Dose Rate[2] (mg/kg/ day)	Number/ group	Number at Risk	Duration of Study
dd mice M Ito et al., 1973	K-500	0	Control	6	NR	32
	K-400	100	16.6	12		
	K-300	250	41.6	12		
		500	83.3	12		
BALB/cJ mice M Kimbrough & Linder, 1974	A-1254	0		100	58	44
		300 (6 mo.)	Control 50	50	24	
		300 (11 mo.)	50	50	22	

1. K- Kanechlor A- Aloclor C- Clophen
2. Calculated based on the following assumption of consumption as percent of body weight (Hallenbeck and Cunningham, 1986).
   Male rats: 4%
   Female rats: 5%
   Male mice: 16.66%
   Female mice: 20%
3. Defined as the number of animals alive at a time designated by the author and considered to be at risk of developing cancer.

periods of no exposure to the test substance resulting in a total feeding period of 400 days. Results were presented in relation to the total approximate amount (mg) of Kanechlor-400 ingested. The time of sacrifice or death was not stated by the authors but appears to have occurred between 159 to 530 days for treated males, and 244 to 560 days for treated females. Information was not given regarding the selection of tissues for pathologic evaluation.

The liver was the site of toxic action reported in males and females. All rats ingesting 700 mg or more showed hypertrophy of the liver. Fatty degeneration was observed in the livers of all treated male and female rats

but was noted in only two female control rats. Multiple adenomatous nodules were noted only in females ingesting 1200 mg or more. Female rats with a positive response ingested an average of 1450 mg Kanechlor-400, and the average experimental period for this group was 481 days. The average total dose and experimental period for female rats indicating a negative response was 1000 mg and 341 days. Nodules were not observed in the control groups or in treated males which had ingested comparable or even greater amounts of Kanechlor-400.

Lung abscesses, pneumonia, spleen atrophy, and intracranial abscesses were found frequently in the experimental group but not in controls. The authors suggested that this indicated a reduced resistance to infection.

The results of this study are compromised by the very small sample sizes used and an observation period only two-thirds the expected lifetime of the animals. Moreover, the data on body weight gain plus the other toxic effects noted by the authors, indicate that the maximum tolerated dose (MTD) was exceeded. The effects on the liver were consistent with other chronic studies and seem to indicate that the female is more sensitive. However, this study cannot stand alone in determining the carcinogenic potential of PCBs.

A study of male Wistar strain rats evaluated the effects on the liver produced by three PCB mixtures of varying degrees of chlorination; Kanechlor-300, Kanechlor-400 and Kanechlor-500 (Ito et al., 1974). A total of 290 male rats were divided into ten groups and were treated with 1000, 500, or 100 ppm of one of the PCB mixtures in the diet. One group was fed the basic diet only. The total number of animals allocated to each group was not stated. The rats were sacrificed after 28 to 52 weeks. Any rats which had died during the

first 25 weeks were not included in the final evaluation. The liver was the only organ or tissue for which evaluation of gross and histological changes was described although it was stated that no remarkable changes were seen in other organs.

Body weight gain decreased with increasing dose in all the treatment groups, and the severity was more pronounced in the Kanechlor-500 group. Rats in the Kanechlor-400 group fed 500 and 1000 ppm had a percentage increase in body weight gain that was extremely low. The "effective" number of rats available for evaluation was also much lower in these dose groups, and they were sacrificed earlier indicating possible problems with survival in these groups. Survival was not discussed by the authors. Data on body weight gain indicate that doses of 500 and 1000 ppm of all Kanechlor mixtures as well as 100 ppm Kanechlor-400 were above the MTD. An increase in liver weight, expressed as percent of body weight, was also dose related. The response was comparable in the groups fed Kanechlor-400 and Kanechlor-500 and was twice as high as that in the group fed Kanechlor-300 at 1000 ppm.

Oval cell proliferation, bile duct proliferation, fatty changes and cell hypertrophy were described in all treated groups but not in the controls. Hypertrophic changes of liver parenchymal cells in centrolobular areas was stated to be clear in the Kanechlor-400 and Kanechlor-500 groups dosed with 1000 ppm. Cholangiofibrosis was observed in the rats fed 1000 ppm in all treated groups and incidence was highest in the Kanechlor-500 group. Nodular hyperplasia was observed in all treated groups with the highest occurrence in the rats fed 1000 ppm Kanechlor-500. No hepatocellular carcinomas were observed although the authors suggested that they may be induced after a longer treatment period. The nodular hyperplasia incidence of 3/25 and 1/22

for rats dosed with 100 ppm Kanechlor 500 and 300 respectively is suggestive of a preneoplastic effect. This study was weakened limited by the apparent low numbers in each dose group (29), some excessively high dose levels, and a duration of study less than lifetime.

Kimbrough et al. (1975) treated 200 female Sherman strain COBS rats with 100 ppm Aroclor 1260 mixed with cornstarch in the diet for approximately 21 months. An equal number of controls were fed ground rat chow. The rats were housed ten per cage. Body weights were recorded as combined weights in the cages weekly until six months of age, biweekly until 12 months, and then monthly. Individual weights were recorded at the beginning of the experiment and upon sacrifice. Animals were sacrificed at 23 months of age. Histopathology was conducted on all major organs.

A slight decline in body weight gain was observed in the test group beginning after three months. The mean final body weights in the treated groups was significantly less than that of controls (392 g vs. 420 g, $p<0.001$) as was the difference in weight gain (323 g vs. 350 g, $p<0.001$) although the difference was not great enough to indicate that the MTD was exceeded. Food consumption was reported to be comparable in both groups. PCB intake was reported to be 11.6 mg/kg/day during the first week, 6.1 mg/kg/day at three months, and 4.3 mg/kg/day at 20 months.

Foci of altered cytoplasm, neoplastic nodules, and hepatocellular carcinoma were observed in the rats. Neoplastic nodules occurred in 144 out of 184 treated rats while incidence in the controls was zero. Hepatocellular carcinoma was also present in 26 rats out of 184 rats in the treatment group while one rat in the control group had this lesion. Foci of altered cytoplasm

were observed in 182 out of 184 treated rats and 28 out of 173 controls. Adenofibrosis was noted in the livers of a few treated animals.

The hepatocellular carcinomas were primarily well-differentiated trabecular types which showed severe disruption of normal liver architecture. The hepatocytes ranged from normal to enlarged, acidophilic, or diffusely basophilic cells with large, hyperchromatic nuclei and prominent nucleoli. Foci of coagulative necrosis were occasionally observed in cancerous areas, but fibrosis was not present. No definite intravascular invasion or metastases were noted. Neoplastic nodules were spherical and well demarcated with enlarged cells. Cytoplasm was ground glass appearing, diffusely basophilic, or clear. Enlarged hyperchromatic nuclei, double nuclei, and mitotic figures were often present. Normal liver architecture was absent within nodules which also compressed the surrounding tissue. The foci in the treated group were different from those in controls and were reported to be generally similar to the cells in the neoplastic nodules without the architectural alterations characteristic of the latter.

The authors concluded that "neoplastic nodules are part of the spectrum of response to hepatocarcinogens and must be included in the evaluation of tumorigenesis". The incidence of neoplastic lesions was not evaluated statistically and no analysis of survival was conducted. Animals were caged in rather high numbers although this did not appear to negatively affect the animals' health as indicated by food consumption. Individually weighing the animals during the study would have improved the experimental protocol. The study is also weakened by the use of only one dose group and one sex. It does, however, lead to the conclusion that Aroclor 1260 is carcinogenic in female Sherman rats.

A National Cancer Institute study evaluated the carcinogenic potential of Aroclor 1254 in Fisher 344 rats (NCI, 1978). Male and female rats, 24 per group, were fed 0, 25, 50, and 100 ppm Aroclor 1254 in the diet for 105 weeks. Control and treatment diets both contained 3% corn oil. Rats were housed three to a cage and were given food and water ad libitum. Statistical tests were based on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52.

Mean body weights of male and female rats were lower than controls at week 10 in the high dose group and at week 20 in the mid-dose group. Males body weights were comparable to controls in the low dose group, but female body weights were less than controls during the second year. Clinical signs including alopecia, amber-colored urine, facial edema, exophthalmos, and cyanosis were noted in the high dose group at week 72, and in the mid-dose group at week 104. Males exhibited a dose-related trend in decreased survival that was statistically significant; 92% of control males, 83% of the low dose, 58% of the intermediate dose, and 46% of the high dose males survived to the end of the bioassay. Survival in females was not affected by treatment.

No proliferative lesions in the hepatocytes were found in control rats. An increasing incidence of nodular hyperplasia was observed with increasing dose in both males and females. Adenomas were also observed in the high dose (both sexes) and mid-dose (females) groups. Hepatocellular carcinoma was noted in males only in the mid and high dose groups. The incidence of hepatocellular neoplasms was not statistically significant.

The hepatocellular carcinomas were characterized by large foci of proliferating hepatocytes involving several lobules which compressed the surrounding normal liver tissue. Hepatocytes sometimes contained two or more nuclei. The sinusoidal architecture was lost, and frequently mitotic figures were present. Adenomas involved several lobules of swollen, severely vacuolated hepatocytes still maintaining the general sinusoidal architecture of the liver. The foci of nodular hyperplasia involved two or more hepatic lobules and contained hepatocytes with tinctorial properties that were distinctly different from those of the surrounding liver tissue.

A low incidence of lesions of the gastrointestinal tract was observed in treated male and female rats with zero incidence in controls. These included adenocarcinoma in the stomach and cecum, adenoma in the stomach, and carcinoma in the jejunum. Although not statistically significant, the investigators concluded that the lesions may be related to treatment. Stomachs were examined histologically only when a lesion was detected grossly in treated animals although the stomachs of controls were examined routinely.

Stomach tissues from all the treated rats were examined histologically, and the original slides were reevaluated in a later investigation by Morgan et al. (1981). Stomachs were sectioned through the junction of the pyloric region of the stomach and duodenum, a common area for gastric neoplastic lesions in humans and rats as well as at any sites which had stained positive for alkaline phosphatase (AP) activity. The incidence of lesions increased with increasing dose. Concentrations of Aroclor 1254 in the diet of 0, 25, 50, and 100 ppm resulted in ratios of rats with stomach lesions to total rats in the dose group of 3/47, 5/48, 8/48 and 17/48 respectively. Significant differences were not present between males and females. The lesions were

focal intestinal metaplasia and adenocarcinoma, and their incidence is summarized in Table 13.

In addition to the two adenocarcinomas identified in the 100 ppm dose group, regions of severe dysplasia were identified in two additional foci of intestinal metaplasia, however adenocarcinoma could not be identified with certainty. In some cases, intestinal metaplasia was observed to disrupt the muscularis mucosa, had features of adenocarcinoma, and resembled areas of invasive carcinomas. The likelihood of observing six adenocarcinomas in 144 rats based on the incidence in the control and historical incidence in F344 rats was calculated to be 0.05 (significant at $p<0.05$) and 1.3×10^{-10} (significant at $p<0.001$).

Table 13. Stomach Lesions in Male and Female F344 rats Exposed to Aroclor 1254 in Their Diets

Concentration (ppm)	Total Rats Examined	Intestinal Metaplasia	Adeno-carcinoma
0	47	3	0
25	48	4	1
50	48	5	3
100	48	15	2

The authors concluded that Aroclor 1254 exposure leads to the induction of intestinal metaplasia and probably leads to the induction of adenocarcinoma in the glandular stomachs of F344 rats.

Malignant lymphoma and leukemia were observed in multiple organs, lymph node and spleen in both sexes in the NCI study. A significant positive dose-

related trend in incidence of leukemia and combined leukemia and lymphoma was found in males however the Fisher's exact test for a difference between dose groups and control was not significant.

The low incidence of hepatocellular carcinoma in male rats, and the incidence of neoplastic nodules in both sexes appears to be dose-related although the lesions were not statistically significant under the conditions of this study. The statistical power of the study to detect carcinogens of low potency and slow development may not have been sufficient. It is generally agreed that 50 animals per dose group per sex should be used in a long-term bioassay of rodents (OSTP, 1986). An evaluation of this study by the U.S. EPA (1987) concluded that a true difference in incidence of 35% would be required to have a 90% chance of statistical significance ($p=0.05$) using 24 animals per group. The difference of 10% (2 out of 20 animals at risk) observed in this study would require 117 animals per dose group. The results of the NCI study are consistent with the 14% incidence reported by Kimbrough et al. (1975).

All liver sections from the NCI bioassay were reviewed by Ward in 1983 (Ward, 1985). Hepatocellular foci and adenomas were classified as either eosinophilic or basophilic. Eosinophilic foci were found only in exposed rats. Incidence was dose-related in males. The increase compared to controls was statistically significant for all dose groups. Twenty-nine percent, 33%, and 43% of male rats had the lesion in the low, intermediate, and high dose groups. Incidence was not dose-related in females however the increase compared to controls was statistically significant in all dose groups. The percent with the lesion in the low, intermediate, and high dose groups was 54%, 62%, and 41%. Both control and exposed rats had basophilic foci and incidence did not appear to be related to treatment.

The number of animals with adenomas and carcinomas is presented in Table 14. Adenomas were primarily eosinophilic, and the two hepatocellular carcinomas were composed predominantly of eosinophilic hepatocytes. A dose-related trend in tumor incidence in liver adenomas and carcinomas combined was produced in male rats ($p<0.01$).

Table 14. Hepatocellular Tumors in F344 Rats Fed Aroclor 1254 Diets as Classified by Ward (1985).

	Hepatocellular Adenoma (Number with Lesion)				
Dose (ppm)	Eosino-philic	Baso-philic	Vacuo-philic	Carci-oma	All Tumors
Males					
0	0	0	0	0	0
25	1	0	0	0	1
50	2	0	0	0	2
100	3	1	1	2	7 ($p<0.05$)
Females					
0	0	0	0	0	0
25	0	0	0	0	0
50	3	0	0	0	3
100	1	0	1	0	2

Ward proposed that the data provide evidence that Aroclor 1254 is a tumor initiator. Similar numbers of basophilic foci were observed in control and exposed rats, while eosinophilic foci and tumors were produced only in exposed rats which suggests that they were formed *de novo*. In addition, the metaplastic lesions found in the stomachs of exposed rats differed morphologically from the few lesions in controls.

The long-term hepatocarcinogenic activity of Aroclor 1260 was demonstrated by Norback and Weltman (1985) in Sprague-Dawley rats. The treated group, 70 males and 70 females, received 100 ug/g Aroclor 1260 dissolved in corn oil in the diet for 16 months and then 50 ug/g for an additional 8 months. The control group, 63 males and 63 females, received a basal diet with added corn oil for 18 months and basal diet alone for an additional 5 months. Survivors in both groups were fed the basal diet for months 25 through 29 of the experiment. Partial hepatectomies were conducted on ten rats (two rats of both sexes in controls and 3 rats of both sexes in treated group) at 1, 3, 6, 9, 12, 15, and 18 months. A similar group of ten was sacrificed at 24 months with the remaining animals sacrificed at 29 months.

Macroscopic evaluation of liver tissue at selected time points revealed early and progressive alterations. Hepatomegaly was observed at the first month, and by 18 months, female livers averaged 12% of body weight in contrast to 4% in the controls. Small tan areas, neoplastic nodules, hepatocellular carcinoma and adenofibrosis were also observed. The hepatocellular carcinoma had ill-defined borders which compressed adjacent parenchyma and contained some hemorrhagic or necrotic portions. Some contained cystic areas with clear fluid. The neoplastic nodules also compressed the surrounding parenchyma. The lesions were classified microscopically as centrolobular cell hypertrophy

(first observed at one month), foci (observed at three months), areas of cell alteration (observed at six months), neoplastic nodules (observed at 12 months), trabecular carcinoma (observed at 15 months), and adenocarcinoma (observed at 24 months). Other lesions were diagnosed as simple and cystic cholangioma that first appeared at 18 and 23 months respectively, and adenofibrosis, first observed at 22 months. Carcinomas contained large hepatocyte-like cells containing large abnormal nuclei with clumped peripheral chromatin and huge nucleoli. Numerous mitotic figures were present in some cells. There was no evidence that any of the carcinomas had metastasized to the lungs.

Liver slices were analyzed for gamma glutamyl transpeptidase (GGT) activity at each time point. GGT activity was negative in control hepatocytes and in the areas of centrolobular cell hypertrophy of treated rat hepatocytes. Some of the hepatocytes described as foci were strongly positive, while the neoplastic nodules and hepatocellular carcinomas contained positive cells admixed with negative cells. Adenocarcinoma cells were also positive.

Animals that survived for 18 months or longer were evaluated for hepatocellular neoplasms. Treated males showed a 4% (2/46) incidence of trabecular carcinoma and zero incidence of adenocarcinoma. Treated females exhibited a 40% (19/47) incidence of trabecular carcinoma and 51% (24/47) incidence of adenocarcinoma. These lesions were not found in control rats of either sex. Neoplastic nodules and no other neoplastic lesions were observed in 11% (5/46) males and 4% (2/47) females in the treated group. No neoplastic lesions were noted in 39/46 males and 2/47 females. One female control rat also contained a neoplastic nodule.

The authors concluded that the tumors met the morphologic criteria for malignancy, but their biologic behavior was relatively unaggressive. No metastases were found and mortality was not increased, possibly due to the late appearance and slow growth of hepatocellular carcinoma. It was suggested that the sex differences may be related to sex-linked differences in enzymatic activation and deactivation, or the presence of androgens or estrogens which compete with the carcinogen for metabolism. Although only one dose level was used, this study design had some strong attributes including the inclusion of both sexes, the large number per dose group, the long duration (more than 2 years), the detailed pathological descriptions, and the use of Sprague-Dawley rats which have a low incidence of spontaneous liver tumors.

Schaeffer et al. (1984) tested the hepatocarcinogenic effect of two different chlorinated commercial PCB mixtures in male rats when administered by continuous feeding over a period of more than 800 days. Three groups of male Wistar rats were fed either the basic diet only (139 rats; Group 1), the basic diet with 100 ppm Clophen A 30 (152 rats; Group 2), or the basic diet with 100 ppm Clophen A 60 (141 rats; Group 3). The Clophen mixtures were analyzed for contamination, and furans were stated to be absent. Necropsies were performed on all that died or were killed when moribund. After day 801, randomly selected animals from all three groups were killed each day until day 832. Some animals in groups 1, 2, and 3 were not available for necropsy. These "lost" animals numbered 8, 14, and 12 in groups 1, 2, and 3 respectively. It appears that more treated rats were "lost" and therefore not evaluated for lesions than the control rats, although the difference may not be significant. Statistical significance was analyzed using Fisher's exact test.

Total mortality over the first 800 days was lower in the treated groups (Group 2: 43% and Group 3: 40%) than in the control (62%), and the difference was significant ($p < .05$). Hepatocellular carcinomas were found in 21% (9/44) of the rats necropsied by day 800 in Group 3, 2% (1/51) of those in Group 2 and in none (0/78) of the control rats. This accounted for 7 (9/129), 1 (1/138), and 0 (0/131) percent of the entire sample not lost to necropsy. Incidence of thymoma and other neoplasias was significantly less in the treated groups compared to the control group.

Hepatocellular carcinoma was the most common lesion observed in Group 3 rats killed after day 800. Incidence was 1.9 (1/53), 3.4 (3/87), and 61.2 (52/85) percent in Groups 1, 2, and 3 respectively. In contrast, thymoma, other neoplasias and Wistar-nephritis was found at much lower incidence in the treated groups than in the control. Liver cells contained hypertrophic cells with hyperchromatic nuclei and foamy or vacuolated cytoplasm. Foci of hepatocellular alteration and neoplastic nodules were regularly observed in the treated rats.

Preneoplastic and neoplastic lesions exhibited a time-dependent sequence of development. Foci and nodules were observed after day 500. Foci incidence was low in controls until day 800 after which incidence rose to 32% (17/53). Neoplastic nodules and hepatocellular carcinoma remained low in incidence in the control group with 4 (2/53) and 2 (1/53) percent respectively. Preneoplastic and neoplastic lesions in Group 2 remained constant at 50-60% between day 500 and 800 rising to 100% in animals killed after day 800. The incidence of foci predominated at all time intervals, but an increase in neoplastic nodules and hepatocellular carcinoma was observed as the exposure period increased. Preneoplastic or neoplastic lesions were observed in 100%

of Group 3 necropsied after day 500. There was a time-dependent trend from foci to neoplastic nodules to hepatocellular carcinoma.

Clophen A 60 was concluded to have a definite and Clophen A 30 a weak carcinogenic effect on rat liver. Carcinomas exhibited a latency of 700 days and incidence was highest in the Clophen A 60 group. The high incidence of preneoplastic and neoplastic lesions in the liver of treated animals was stated to indicate a carcinogenic effect of both PCB mixtures, however the experiment could not allow a distinction between initiating and promoting activity. The low incidence of neoplastic nodules in the control rats was felt to be consistent with the observations that neoplastic nodules are rare in untreated rats, except in aged rats, and that they are only induced earlier by hepatocarcinogens. Foci of cellular alteration were observed in treated and control rats after 500 days, but incidence was higher in the treated groups. The authors also stated that although foci are known to appear in old untreated rats, increased numbers encountered in bioassay studies are accepted as an indication of possible carcinogenicity. The reduced mortality rate noted in the treated rats was explained by the authors as a possible interaction of PCBs with the immune system.

A relatively short bioassay using dd strain mice resulted in the induction of liver neoplasias after 32 weeks of treatment. Ito et al. (1973) treated male dd mice, 12 animals per group, with 100, 250, or 500 ppm Kanechlor-500, Kanechlor-400 or Kanechlor-300. One group of untreated mice served as a control. Mice were housed individually and given food and water ad libitum. Mice dying during the experiment were excluded from the final effective number of animals. The average daily dose was calculated by the U.S. EPA (1980) to

be 16.5, 41.3, and 82.5 mg/kg/day for dietary concentrations of 100, 250, and 500 ppm respectively.

Increased liver weights were noted in the treated groups in comparison to the control group, and severity notably increased with increasing dose in the Kanechlor-500 group. Liver neoplasias were observed in seven out of twelve mice administered 500 ppm Kanechlor-500. These were histologically classified as hyperplastic nodules (7/12) and well-differentiated hepatocellular carcinomas (5/12). No cirrhotic changes were noted in livers, and no metastatic changes or neoplasms in other organs were seen in mice with or without liver nodules. These lesions were not observed in any other dose group.

Histopathologic evaluation found focal hypertrophy of the centrolobular liver cells in non-neoplastic areas. There was occasional nuclear enlargement, and only rare mitotic figures, fatty changes, and necrotic signs in cells. Oval cells and bile-duct proliferation were also rare. Non-neoplastic areas also contained a slightly increased amount of smooth endoplasmic reticulum but mitochondrial changes were rare. Hyperplastic nodules were relatively clearly demarcated. Nuclei were relatively small, and mitotic findings were rare. Cytoplasm had elongated, irregularly-shaped mitochondria and a greatly increased amount of smooth endoplasmic reticulum.

Although sample size was quite small, and the observation period extended over only a small portion of the animals' lifetime, neoplastic lesions were induced in 58% of mice fed 500 ppm Kanechlor-500. The lack of response in other dose groups does not signify a negative response given the short time period,

however it does indicate that the PCB mixtures with less chlorination are less potent in mice.

Long-term administration of Aroclor 1254 was associated with the induction of hepatomas in BALB/cJ inbred male mice (Kimbrough and Linder, 1974). Two groups of mice (50 per dose group) were fed 300 ppm (49.8 mg/kg/day) of the PCB mixture mixed with cornstarch in ground rat chow. One group was fed PCB for six months and then switched to the normal rat chow, while the other group was fed the treated diet for 11 months. Two control groups (50 per group) were included. Animals were caged in groups of five. Unfortunately approximately half the mice were lost due to fighting. After six months, the mice were caged in pairs, and the lost mice were not included in the evaluation of liver pathology. Organs were routinely examined grossly, and abnormal appearing liver tissue was examined microscopically.

Mice in the 11 month group had large livers comprising 25% of body weight which may have been responsible for a slight, statistically significant increase in overall body weight. Body weights were increased over controls in the six month group when the treatment diet was discontinued but were comparable at the end of the study.

Liver cells were enlarged in the experimental group exposed for 11 months and contained enlarged, hyperchromatic, and atypical nuclei. The cytoplasm was either smooth or vacuolated. Some livers had extensive areas of coagulation necrosis and fibrosis. Nine out of 22 mice (40%) had hepatomas described as tan nodules. These were well-circumscribed and surrounded by compressed hepatic parenchyma or strands of fibrous tissue. Metastases were not seen upon gross inspection. Livers in the six month group had enlarged cells that

often had enlarged, atypical, hyperchromatic nuclei. Many cells had vacuolated cytoplasm. Two-thirds of the livers were noted to have slight-to-moderate diffuse, interstitial fibrosis. One hepatoma was observed in this group. No hepatomas were observed in the control groups.

Hepatomas and adenofibrosis were concluded to be induced in this study. This mouse strain was stated to only rarely develop hepatomas spontaneously. The number of surviving mice was greatly reduced by the fighting early in the experiment, and the mice were not allowed to complete their life spans. Even so, carcinogenicity was demonstrated in this mouse strain after 11 months.

Summary of Carcinogenicity

Hepatobiliary cancer is a consistent finding among epidemiological studies of PCBs exposure. The epidemiological studies of the association of occupational PCB exposure with mortality and carcinogenicity do not lead to a definite causal determination however. All of the studies were limited by a small number of deaths in the cohort available for analysis, insufficient length of follow-up, and a lack of information regarding potentially confounding variables. Furthermore, it was not possible to obtain a quantitative estimate of the PCB dose received by the workers. Although inconclusive, the risk of mortality from cancer of the liver, gall bladder and biliary tract appears to be elevated among employees exposed to PCBs. This general finding is supported by the finding of an association of PCBs with liver cancer in rodents and lends credibility to the use of rodent studies to infer hypotheses regarding health effects in humans.

Preneoplastic and neoplastic lesions in rats are summarized in Table 15 and 16. Incidence is presented for males and females by the commercial mixture used and the concentration in the diet.

The studies which investigated effects on both sexes resulted in contradictory indications of relative sensitivity. Hepatocellular carcinoma was not induced in female rats treated with Aroclor 1254 but was induced in males (NCI, 1978). In contrast, females were found to have a much higher incidence of this cancer (40%) compared to males (4%) after treatment with Aroclor 1260 (Norback and Weltman, 1985). In addition, Noritaka and Baba (1973) induced adenomatous nodules in the livers of females which had ingested 1200 - 1500 mg Kanechlor-400 and not in males which had ingested equivalent or greater amounts. The average experimental period for these females was longer than for the males. More weight should be given to the results of the Norback and Weltman study because of larger sample sizes and a longer observation period, however a definite finding of greater sensitivity in the induction of cancer for females is not possible.

The commercial mixtures that contain a larger amount of the more highly chlorinated compounds are more potent carcinogens. Ito et al. (1974; data not in table) found that the increased incidence of "nodular hyperplasia" was dose-related and was much greater in those groups of male Wistar strain rats fed Kanechlor-500 and Kanechlor-400 for 28 to 52 weeks compared to those fed Kanechlor-300. Incidence in the 100 ppm groups were 12% and 12.5% compared to 4.5%. These findings were confirmed by Schaeffer et al. (1984) using Clophen A 30 and Clophen A 60. Although the incidence of neoplastic nodules in male Wistar strain rats surviving more than 800 days was comparable between the two mixtures, 61% of the rats fed Clophen A 60 developed hepatocellular carcinomas

as opposed to 3% in the rats fed Clophen A 30. Mice exhibited similar sensitivity to the degree of chlorination in Kanechlor mixtures. Nodular hyperplasia and hepatocellular carcinoma were observed only in male dd strain mice fed 500 ppm Kanechlor-500 and not in mice fed Kanechlor-400 or Kanechlor-300 at equivalent doses for 32 weeks (Ito et al., 1973).

It can be concluded from Tables 15 and 16 that various PCB mixtures are carcinogenic in male and female rats. The incidence of hepatocellular carcinoma was statistically significant at 100 ppm Clophen A 60 in male Wistar strain rats (Schaeffer et al., 1984). This result is particularly informative because the PCB mixture was found to be free of dibenzofurans. Unfortunately, the incidence reported for neoplastic nodules and hepatocellular carcinoma add up to a number greater by one over the total number of surviving animals making the use of the number in quantitative risk assessment difficult. Although statistical tests were not conducted by Kimbrough et al. (1975) or Norback and Weltman (1985) calculation of the Chi Square test statistic for comparison of two proportions results in a significant difference between treated and control Sherman strain female rats for hepatocellular carcinoma at 100 ppm (Kimbrough, 1975). A reevaluation of liver lesions by Ward (1985) found a statistically increased incidence of hepatocellular adenoma and carcinoma combined in the 100 ppm group ($p<.05$). Combined hepatocellular carcinoma and adenocarcinoma incidence in male and female Sprague-Dawley rats was also statistically significant (Norback and Weltman, 1985). The effect on cancer incidence from the inclusion of data from hepatectomized rats is not known but may have biased the results in a positive direction.

The NCI study (1978) used the only test design that evaluated multiple PCBs doses. Unfortunately sample sizes were smaller, thereby decreasing the

statistical power of the test to detect a positive increase in hepatocellular carcinoma. A low incidence of hepatocellular carcinoma which increased with increasing dose level occurred in males but was not observed in females. A higher dose-related incidence of neoplastic nodules occurred in both sexes. Ward (1985) found a significant dose related trend for tumor incidence in males when all liver adenomas and carcinomas were combined ($p<.01$). It is plausible that if the experimental period had extended for a longer period, a larger number of hepatocellular carcinomas would have developed. Hepatocellular carcinoma incidence due to Clophen A60 exposure increased from 30% (9/30) in 700 - 800 days to 61% (52/85) in the termination period after 800 days (Schaeffer et al., 1984).

The Environmental Protection Agency's Risk Assessment Forum (1986) concluded after reviewing the scientific literature concerning neoplastic lesions in the rat liver that the use of foci of cellular alteration and neoplastic nodules along with hepatocellular carcinomas in risk assessments on chemicals was appropriate. Although there is general agreement that foci and neoplastic nodules are in some way part of the carcinogenic process, disagreement exists concerning the role of foci and nodules in the development of hepatocellular carcinoma. The following evidence was presented regarding the potential relation of neoplastic nodules to carcinomas.

1. Similar morphologic lesions have been described in other rodents, monkeys and humans.
2. Some scientists do not consider the nodule to be a neoplastic entity while others do.
3. Although foci are relatively common in aged rat livers, neoplastic nodules and hepatocellular carcinomas are not.

4. The incidence of the three lesions is increased by carcinogens but not by noncarcinogens. Agreement exists that an increase in foci and nodules in treated animals is some indication that the liver is at increased risk of cancer formation.

5. Development of the three entities is time-dependent; foci to nodule to carcinomas, although the progression in development has not been established. On the other hand, carcinomas have been observed to arise from neoplastic nodules but not in every instance.

6. Some studies have clearly shown that nodules were persistent and had progressive growth ability, a characteristic of neoplasms. Others have demonstrated regression of neoplastic nodules, particularly early appearing nodules, after removal of a carcinogen. However, even in these studies some nodules persisted. The Forum concluded that "the case is stronger for the progression of some nodules to carcinoma than it is for foci, but in neither case is the evidence conclusive". Nodules are not necessarily obligate precursors of carcinoma.

Therefore, the U.S. EPA takes the position that although the exact contribution of neoplastic nodules to the overall incidence of hepatocellular tumors in the rat is unclear, it is appropriate to combine neoplastic nodules and hepatocellular carcinoma incidence to evaluate statistical significance when merited by the weight of evidence concerning carcinogenicity. Less weight is to be given to responses where only the increase in neoplastic nodules is statistically significant. Quantitative assessment is not considered to be justifiable if there is no human or supporting evidence.

Combining the tumor incidence data from these studies presents some difficulties because of potential double counting. Schaeffer et al. (1984)

and Norback and Weltman (1985) reported results as the highest level of neoplastic lesion observed on an animal.

Strong evidence of carcinogenicity was produced by three studies in rats (Norback and Weltman, 1985, Schaeffer et al., 1984, and Kimbrough et al., 1975). The NCI (1978) results although statistically insignificant, were dose-related. These results, plus the suggestive, but inadequate epidemiological evidence has led the U.S. EPA to place PCBs in Group B2 as a probable human carcinogen.

The International Agency for Research on Cancer (IARC), in its 1987 update of the IARC Monographs, concluded that the degree of evidence for carcinogenicity was limited in humans and sufficient in experimental animals (IARC, 1987). A chemical can be classified as having limited evidence of carcinogenicity according to IARC when "a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence." The IARC Working Group determined that an association between cancer and exposure to PCBs was suggested by the consistent increased risk from hepatobiliary cancer that was found by several researchers. The evidence was considered limited because the numbers were small, dose-response relationships could not be evaluated, and the role of compounds other than PCBs could not be excluded. The evidence for carcinogenicity in animals was sufficient because a causal relationship was established between PCBs exposure and an increased incidence of benign and malignant neoplasms in rats and mice. The overall evaluation of the carcinogenicity of PCBs places this class of compounds into category 2A; probably carcinogenic to humans. IARC concluded that the weight of

evidence in humans is strong enough to place PCBs into the higher Group 2 category.

Table 15. Carcinogenic Response of Male Rats to Chronic Administration of PCBs (percent in parentheses)

	Concentration in Diet (ppm)					
	0	25	50	100	16mo.	100
				50	8mo.	
CLOPHEN A 30 Schaeffer et al, 1984						
NN	2/53 (4)					35/87 (40)
HCx	1/53 (2)					3/87 (3)
AROCLOR-1254 NCI, 1978.						
NN	0	5/24 (20.8)	8/24 (33.3)			12/24 (50)
HCx	0	0/24	1/24 (4.2)			2/24 (8.3)
Ad	0	0/24	0/24			1/24 (4.2)
AROCLOR-1260 Norback & Weltman, 1985.						
NN	0/32			5/46 (11)		
TCx	0/32			2/46 (4)		
AdCx	0/32			0/46		
CLOPHEN A 60 Schaeffer et al, 1984.						
NN	2/53 (4)					34/85 (40)
HCx	1/53 (2)					52/85 (61)

NN: Neoplastic Nodules HCx: Hepatocellular Carcinoma
Ad: Adenoma AdCx: Adenocarcinoma

Norback and Weltman, 1985.

Sprague-Dawley rats, PCB group: 70 male and 70 females per group fed 100 ug/g Aroclor 1260 (in corn oil) in diet for 16 months, then 50 ug/g for 8 months. Control group: 63 males and 63 females fed diet with corn oil for 18 months, basal diet for 5 months. Sacrifice at 24 and 29 months.

NCI, 1978.

Fischer 344 rats, 24 per group, fed 0, 25,50, and 100 ppm Aroclor 1254 in diet for 105 weeks.

Schaeffer et al, 1984.

Male Wistar strain rats fed basic diet (139 rats), basic diet with 100 ppm Clophen A 30 (152 rats), or basic diet with 100 ppm Clophen A 60 (141 rats) for 800 - 832 days.

Table 16. Carcinogenic Response of Female Rats to Chronic Administration of PCBs (percent in parentheses)

		Concentration in Diet (ppm)			
	0	25	50	100 16mo. 50 8mo.	100
AROCLOR-1254					
NCI, 1978.					
NN	0	6/24 (25)	9/22 (40.9)		17/24 (70.8)
HCx	0	0/24	0/22		0/24
Ad	0	0/24	1/22 (4.5)		2/24 (8.3)
AROCLOR-1260					
Norback & Weltman, 1985.					
NN	1/49 (1)			2/47 (4)	
TCx	0/49			19/47 (40)	
AdCx	0/49			24/47 (51)	
Kimbrough et al, 1975.					
NN	0/173				144/184 (78.3)
HCx	1/173 (0.6)				26/184 (14.1)

NN: Neoplastic Nodules HCx: Hepatocellular Carcinoma
Ad: Adenoma AdCx: Adenocarcinoma

Norback and Weltman, 1985.
Sprague-Dawley rats, PCB group: 70 rats/sex/group fed 100 ug/g Aroclor 1260 (in corn oil) in diet for 16 months, then 50 ug/g for 8 months.
Control group: 63 rats/sex/group fed diet with corn oil for 18 months, basal diet for 5 months.
Sacrifice at 24 and 29 months.

NCI, 1978.
Fischer 344 rats, 24 per group, fed 0, 25,50, and 100 ppm Aroclor 1254 in diet for 105 weeks.

Kimbrough et al, 1975.
Sherman strain COBS female rats, 200 per group, fed 0 and 100 ppm Aroclor 1260 for approx. 21 months. Killed at 23 months old.

MECHANISMS OF ACTION

STUDIES OF CANCER PROMOTION

The relative abilities of various PCB mixtures and congeners in the initiation and promotion stages of carcinogenesis has been investigated extensively. Evidence suggests that gene damage is involved in the initiation of normal cells and that the progression to malignancy requires the expansion of the initiated population. It has been postulated that the growth rate of initiated cells is one of the rate-limiting factors in carcinogenesis. Promotion, a process which causes the selective or preferential multiplication of initiated cells, accelerates the growth of initiated cells (Schulte-Hermann. 1987). In the liver, foci of altered hepatocytes have been identified as the progeny of initiated cells and their incidence and size is enhanced by tumor promoters. Schulte-Hermann (1987) has tentatively divided liver tumor promoters into two classes, cytotoxic agents and agents that induce seemingly adaptive responses in the liver.

Aroclor 1254 and Clophen A 50 have promoted diethylnitrosamine-initiated liver tumorigenesis in male Wistar strain rats (Nishizumi, 1976), male F344 rats (Hirose et al., 1981), male Sprague-Dawley non-inbred albino rats (Preston et al., 1981; Pereira et al., 1982). Preston et al. (1981) established that Aroclor 1254, free of polychlorodibenzofuran, is just as potent as the contaminated mixture in promoting diethylnitrosamine (DENA)-initiated hepatocellular carcinoma in male Sprague-Dawley rats. Both mixtures increased the number of rats with carcinoma as well as the number of tumors per tumorous rat compared to the number produced by administration of DENA alone. No liver tumors were produced in the control rats, or the groups fed either the contaminated mixture or the furan-free mixture.

Another significant finding was that by Pereira et al. (1982) who enhanced the rate of appearance of DENA initiated GGTase-positive hepatocellular foci in male Sprague-Dawley rats through only one intraperitoneal injection of 500 mg/kg body weight of Aroclor 1254. An incidence of 2.28 GGTase foci per cm^2 was produced 28 days after an injection of Aroclor 1254 in the DENA initiated partially hepatectomized rats compared to 0.7 GGTase foci per cm^2 in rats treated with DENA alone. A maximum enhancement of 3.47 foci/cm^2 was obtained in 49 days after only two doses of Aroclor 1254. Seventy-one days after an initiating dose of DENA without Aroclor promotion, the number of GGTase foci/cm^2 had increased to 2.07 ± 0.47 which indicates that the time to the first appearance of foci may be shortened, although the final number produced is not increased by the Aroclor. Generally, the requirement of prolonged exposure to a promoting compound has been stated to be characteristic of promoters.

More recent studies have confirmed the dose-related promoting activity of Clophen A 50 at relatively low doses. Female Sprague-Dawley rats, three weeks of age were dosed through oral administration of 8 mg/kg body weight diethylnitrosamine (DEN) (Deml and Oesterle, 1987). One week later Clophen A 50 was given in doses of 0.1, 0.5, 1, 5, and 10 mg/kg body weight to five groups of four rats each. Clophen A 50 was administered three times a week for 11 weeks. Two controls were administered DEN only or olive oil only. Another five groups of four rats each were treated with the same doses of Clophen A 50 without DEN. At 12 weeks livers were screened histochemically for preneoplastic islands showing ATPase-deficient, GGTase positive, or glycogen positive phenotypes. Clophen A 50 treatment alone produced a number of islands that was consistent with control levels (0.3-0.9 per cm^2). The 0.1

and 0.5 mg/kg doses with DEN also did not increase levels above the control. Administration of 1,5, and 10 mg/kg body weight Clophen A 50 with DEN caused a 2- to 7-fold increase in number and a 3- to 15-fold enhancement of total area of ATPase-deficient islands. This increase was dose-related and became significant between 1 and 5 mg/kg. GGTase and glycogen-positive islands were also greatly enhanced in number and area in a dose dependent manner. The 1 mg/kg body weight dose given three times weekly was stated to correspond to 430 ug/kg body weight/day.

These results confirm those of an earlier experiment using higher concentrations of DEN and Clophen A 50 in weanling and adult female Sprague-Dawley rats (Oesterle and Deml, 1984). In this study, Clophen A 50 alone at doses of 2, 10, 25, 50, and 100 mg/kg body weight given p.o. once a week for seven weeks, produced a low number of enzyme-altered and glycogen storing islands in adult rats that was higher than the number found in controls treated with olive oil only (0.2-4.1 per cm^2 vs. 0.06-0.5 per cm^2). A dose-response relationship was not obvious. The promoting effect of Clophen A 50 was dose-dependent however, and a significant increase in the number and area of ATPase-deficient islands with all doses used compared to the effect of DEN alone was reported. A similar effect was observed for GGTase-positive and glycogen storing islands, although the number and areas were lower. The percentage of islands with coincidence of all three markers was also augmented in a dose-related manner. In weanlings, 2 mg/kg body weight did not have a promoting effect over the 12 week period. Promotion of ATPase-deficient islands was significant at the higher doses. Administration of 25, 50 and 100 mg/kg body weight Clophen A 50 alone produced a greater number of ATPase-deficient islands than observed in controls, however the increased incidence was not statistically significant (0.7 - 2.1 vs. 0.3). GGTase positive and

glycogen storing islands were not produced in controls. The authors suggested that Clophen A 50 administered alone promoted previously initiated dormant cells, however it was stated that the possibility of a weak initiating potency of PCBs could not be excluded definitely. The average daily intake of the lowest dose in the experiment was 0.29 mg/kg body weight/day.

Clophen A 50 has also been shown to have co-carcinogenic properties in rats when administered alternating with DEN five days a week for 12 weeks (Deml and Oesterle, 1986). This schedule followed the co-carcinogenesis scheme used in experimental skin carcinogenesis. Concentrations of 0.4 mg/kg/body weight DEN and 1.0 mg/kg body weight Clophen A 50 increased the number and area of islands containing the three previously described markers. The higher dose combination (4.0 mg/kg DEN and 5.0 mg/kg Clophen A 50) enhanced the effect. The two dose levels of Clophen A 50 administered without DEN resulted in a number and area of islands no greater than control levels. The authors concluded that the presence of PCBs enhanced the initiating activity of DEN. These findings are in contrast to other studies which have shown inhibition or no effect on the development of liver tumors or enzyme-altered foci after pretreatment or simultaneous treatment with PCBs or phenobarbitol and the initiator. The results were also stated to be in contrast to other studies which have produced increased numbers of GGTase-positive islands only and not ATPase-deficient markers. It was suggested that the findings may be complicated by differences in dose, dose schedules, and enzyme induction patterns characteristic of PCB mixtures and phenobarbitol.

PCB congeners classified as 3-methylcholanthrene-type enzyme inducers or phenobarbital-type enzyme inducers both promoted DEN initiated hepatic ATPase-deficient islands in female Wistar rats (Buchmann et al., 1986). The PCB

congeners, 3,4,3',4'-tetrachlorobiphenyl (TCBP) and 2,4,5,2',4',5'-hexachlorobiphenyl (HCBP), were administered i.p. at a dose of 150 umol/kg body weight once a week for 8 weeks beginning 10 days after a 10 day treatment with DEN (50 ppm in drinking water). The island number and relative volume of liver occupied by altered tissue was enhanced by both isomers. The effect was similar after 11 weeks, but the effect of TCBP was significantly greater than that of HCBP 19 weeks after the start of the experiment. No significant islet formation was reported for the administration of either isomer alone. These results suggest that cytotoxicity is not the sole mechanism for the promoting activity of PCB mixtures since cytotoxicity has not been demonstrated for the PB-type inducing isomers. The tetrachlorobiphenyl isomers, 2,4,2',4'-TCB and 2,5,2',5'-TCB, also demonstrated promoting ability for GGT-positive islands in rat liver by treatment with diethylnitrosamine (Preston et al.,1985). The activity of 2,4,2',4'-TCB was stronger than 2,5,2',5'-TCB.

2,5,2',5'-TCB and its metabolite, 2,5,2',5'-TCB 3,4 oxide, were also tested in assays for the induction of lung adenomas in A/J mice or skin papillomas in SENCAR mice and did not show initiating ability at the doses administered (Preston et al., 1985). The arene oxide induced a high mortality rate, however, only 51 and 13% of the animals in the two dose groups survived to weaning at 28 weeks compared to 62-70% of the mice given the TCB or solvent only.

Aroclor 1254 promoted N-Nitrosodimethylamine (NDMA) initiated lung and liver tumors in outbred Swiss mice (Anderson et al., 1986). A single dose of Aroclor 1254 (50, 250, or 500 mg/kg) was administered by gastric intubation to infant Swiss mice on post-natal day 8. The male infants had previously been injected i.p. on day 4 with NDMA (5 mg/kg). Controls received saline i.p. and

olive oil by gavage. The mice were killed after 16 or 28 weeks. A clear relationship was found between mean tissue levels of PCBs and dose at both time points. Approximately 80% of the PCBs in the carcasses and livers were identified as 2,4,5,2',4',5'- and 2,3,4,2',4',5'-hexachlorobiphenyls, plus an unidentified pentachlorobiphenyl. Animals given PCBs alone had no lung tumors at 16 weeks and an incidence similar to untreated controls at 40 weeks. The 500 mg PCBs/kg plus NDMA mice had twice the number of lung tumors as the NDMA only mice at both time points. Tumor incidence was dose-dependent at 28 weeks. There was a statistically significant negative relationship between liver content of PCBs and number of lung tumors in the highest dose group at 28 weeks. A nonsignificant positive relationship occurred between carcass content of PCBs and numbers of lung tumors at 28 weeks. In liver, there was a significant increase in the size of proliferative lesions in the 50 mg/kg group at 28 weeks but the range in size of liver tumors was very wide. At 250 mg/kg there was a direct relationship between body content of PCBs and sizes of liver tumors. A statistically significant negative dose-response relationship was reported between PCB exposure and the number of focal hepatocellular lesions at 16 weeks and the number of liver tumors at 28 weeks. A similar negative relationship between carcass content of PCBs and number of focal proliferative lesions was also noted at 28 weeks. The authors concluded that the effects of PCBs on livers after a single dose showed a stimulation of liver tumors. Tumor size increased with increasing body content, however, tumor numbers decreased.

A dose of 100 ug Aroclor 1254, administered twice weekly, did not promote tumors in a two-stage CD-1 mouse skin tumorigenesis assay after 30 weeks (Berry et al., 1978). The authors suggested that if dose levels were

increased to cause epidermal proliferation, toxicity would interfere with the carcinogenic response before the experiment was ended.

Intercellular communication mechanisms have been shown to play important roles in development and well as in control of cellular proliferation and differentiation in multicellular organisms. Several known tumor promoters have exhibited the ability to block metabolic cooperation, a form of cell-to-cell communication, by interfering with gap junction mediated cell transfer (Tsushimoto et al., 1983). An evaluation of cytotoxicity and metabolic cooperation inhibiting abilities of 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) and 3,4,5,3',4',5'-hexachlorobiphenyl was conducted in Chinese hamster cells (Tsushimoto et al., 1983). While 3,4,5-HCB was much more cytotoxic than 2,4,5-HCB, the noncytotoxic congener was able to inhibit intercellular communication. This effect did not occur at concentrations of 2.5 ug/m or lower of the test chemical. The 3,4,5-HCB was unable to inhibit metabolic cooperation at noncytotoxic levels. It was suggested that elimination of cell-cell communication would block the normalizing influence on an initiated cell which could than gain a selective growth advantage allowing the clonal expansion of initiated cells. The multiplication of initiated cells would provide opportunity for additional genetic changes.

GENOTOXICITY

Polychlorinated biphenyls, with one exception, have not demonstrated mutagenic properties in those test systems assayed. Wyndham et al. (1976) reported a positive response in the Ames assay for 4-chlorobiphenyl and to a lesser extent Aroclor 1221 using *Salmonella typhimurium* strain TA1538 and rabbit liver microsomal homogenate. A very weak response was reported for 2,2',5,5'-

tetrachlorobiphenyl, Aroclor 1254, and Aroclor 1268. Mutagenicity increased in reverse relation to the degree of chlorination of the PCB molecules. *In vivo* and *in vitro* metabolism of 4-chloro-4'-deuterobiphenyl resulted in a product consistent with the operation of an arene oxide intermediate. Binding of metabolically activated PCB isomer with endogenous microsomal protein and RNA was demonstrated and it was determined that the tritiated PCB metabolite was associated with the light RNA fraction similar to the type of binding by benzo(a)pyrene.

Aroclor 1254 was evaluated in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 with negative results (Schoeny et al., 1979). The test was run using eight doses of Aroclor 1254 in a range of 0.5 - 500 ug per plate in the presence of PCB-induced and uninduced hepatic S-9 from rats. Mutagenicity was defined as a response of twice the number of spontaneous revertants or greater. The mixture was not mutagenic as defined by the authors and a toxic dose of 500 ug/plate was observed.

Four PCB isomers were subsequently tested in the Ames system by the authors (Schoeny, 1982). The isomers, 4-chlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, 2,4,2',4'-tetrachlorobiphenyl, and 2,4,6,2',4',6'-hexachlorobiphenyl were also not mutagenic in Sprague-Dawley rats or CD-1 rats. Assays incorporating hepatic extracts from rats treated with several hepatic inducing agents were also negative. The authors commented that the tests of 4-chlorobiphenyl at the dose levels used by Wyndham et al. (100 - 200 ug per plate) resulted in many small histidine-requiring colonies, an indication of toxicity. 2,4,2',4'-TCB was toxic at 100 ul/plate while the other isomers were not toxic at the dose levels used in the assays (up to 200 ul/plate).

The investigators noted that the Ames assay is relatively insensitive in the testing of chlorinated hydrocarbons and suggested that the hepatic enzyme preparations are unable to effect dechlorination or other necessary metabolic steps in the production of active agents.

Aroclors 1242 and 1254 did not produce evidence of dominant lethality in Osborne-Mendel rats after acute or multiple (5-day) oral administrations (Green et al., 1975). Negative results were also observed for Aroclor 1254 after 70 days of fedding. Male rats, ten per dose group, were administered single doses of 625, 1250 or 2500 mg/kg/Aroclor 1242 by oral intubation. In addition, male rats, ten per dose group, were given 75, 150, or 300 mg/kg/day Aroclor 1254 for five days. These males were then mated weekly with two females for 10 weeks. In a third study, males were given five daily doses of Aroclor 1242 at 125 or 250 mg/kg/day or of Aroclor 1254 at 75 or 150 mg/kg/day. The males were mated for 11 weeks to ensure three weeks of spermatogonial examination. A significantly greater number of dead implantations/pregnancy were reported for Aroclor 1242 at multiple doses of 75 mg/kg/day (week 5), 150 mg/kg/day (week 1), and 300 mg/kg/day (week 8). It was concluded that these were random increases because dominant lethal effects are rarely restricted to one week increases and usually correlate with specific spermatogenic stages. Moreover, the significant effects were not repeated in the two experiments using identical doses of Aroclor 1254. The positive control compound, TEM, also did not replicate its significant effects in the two five day dosing experiments, however. A significantly greater number of dead implantations per pregnancy were noted on weeks 1, 2, 3, 4, and 5 in one test and on weeks 4, 5, and 11 in the other. All other dose regimens using Aroclor 1242 and 1254 produced negative results. A 70-day feeding study

was conducted to cover the entire spermatogenic cycle. Test groups of 27 and 28 male rats were fed Aroclor 1254 in the diet in concentrations of 25 and 100 ppm. Controls (26 rats) were fed untreated chow. Ten males were each mated with two females for one week to assess dominant lethality. No evidence of dominant lethality was produced.

Cytogenetic anaysis of rat bone marrow and spermatogonia produced no significant mutagenic effects by Aroclors 1242 or 1254 (Green et al., 1975). Single oral doses of Aroclor 1242 (1250, 2500, or 5000 mg/kg) or a multiple dose of 500 mg/kg/day for 4 days were given to Osborne-Mendel rats. Aroclor 1254 was administered at doses of 75, 150, or 300 mg/kg/day for 5 days. One hundred cells per animal were examined for chromosomal abnormalities and 1000 cells per animal for mitotic inhibition. Deaths and/or weight loss was induced in animals at all dose levels except for 1250 mg/kg Aroclor 1242 and 75 mg/kg/day Aroclor 1254 given for 5 days. Mitotic division was not significantly inhibited by Aroclor 1242, however, the two higher doses of Aroclor 1254 decreased the number of bone marrow cells in mitosis. Mitosis in spermatogonial cells was inhibited by Aroclor 1242 at dose levels of 5000 mg/kg and 500 mg/kg/day for 4 days. The significance of this inhibition is uncertain since the reproductive performance of male rats dosed with 250 mg/kg/day Aroclor 1242 for 5 days was not adversely affected (Green et al., 1975). Chromosomal abnormalities were not produced by either chemical at statistically significant levels.

Dose-related changes were also not observed in the incidence of chromosome abnormalities or the number of cells in mitosis from bone marrow or spermatogonial cells by Garthoff et al., (1977). The negative results were

demonstrated using male rats fed either 0, 5, 50, or 500 ppm Aroclor 1254 for 2, 3, or 5 weeks.

In conclusion, experiments conducted to date indicate that PCBs are not mutagenic in bacterial systems and have not produced evidence of dominant lethality or chromosomal abnormalities in bone marrow or spermatogonial cells of Osborne-Mendel rats. Mitotic division was inhibited in bone marrow cells and spermatogonia by PCBs, however, experimental results have not been consistent.

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THE CHARACTERIZATION OF CARCINOGENIC AND OTHER RISKS TO HUMANS EXPOSED TO PCBs

HUMAN EXPOSURE

Polychlorinated biphenyls are a serious environmental and public health problem because they are persistent and bioaccumulative pollutants. PCBs were used widely in industry until the late 1970's when the U.S. EPA banned their manufacture and general use. The thermal stability, resistance to degradation, and high lipid solubility of these compounds has resulted in their accumulation in sediments, aquatic organisms, wildlife and humans. Concentrations of PCBs in the tissues of fish and wildlife are greatest in the predator species which depend on fish as a food source. In humans, concentrations are higher in populations with identified sources of exposure such as through their occupation or via high consumption of contaminated sport fish.

PCB mixtures are composed of various combinations of isomers which vary according to the number and position of chlorine atoms on the biphenyl ring. The more highly chlorinated isomers have a greater lipid solubility and are more resistant to degradation and volatilization. Therefore, it is not surprising that the more highly chlorinated isomers are identified more frequently in human breast milk, blood, and adipose tissue. Several of these isomers, specifically the coplanar isomers, are potent hepatic enzyme inducers, and cause body weight loss, thymus atrophy, dermal disorders, hepatic damage, reproductive toxicity, developmental toxicity, and immunotoxicity in subchronic animal experiments in the same manner as TCDDs and TCDFs (Tanabe, 1988).

The ingestion of PCBs in the diet is the primary environmental exposure for humans and, since 1976, the consumption of fish has been the main food source in the United States (Jelinek and Corneliussen, 1976). Most people in the United States carry a body burden of PCBs (Kutz and Yang, 1976). Jensen (1987) has estimated that the average background level of PCBs in blood plasma is close to 2 ppb. Sport fish eaters have serum PCBs concentrations comparable to the lower range of serum values reported for exposed industrial workers (geometric mean: 21 ppb) (Kreiss, 1985).

Data available from humans from Japan and Taiwan who ingested large amounts of contaminated rice oil (up to 2 grams) indicate that PCBs accumulate in adipose tissue and that concentrations decrease slowly (Masuda et al., 1985). A study of volunteers in Michigan that monitored PCBs serum concentrations after a meal of either lake trout or chinook salmon recorded a sharp rise to a maximum after six hours and then a rapid decrease over the next 18 hours (Humphrey, 1987). Concentrations had decreased to preconsumption levels by 168 hours. The biological effect of periodic high blood concentrations compared to the cumulative toxic effect of PCBs is not known.

The maternal elimination of PCBs through transplacental transfer and through breastfeeding, and the accumulation of PCBs in the developing fetus and nursing infant has been documented in humans (Masuda et al., 1978; Kodama and Ota, 1980; Schwartz et al., 1983; Rogan et al., 1986a). Maternal blood concentrations have been correlated with cord blood and breast milk concentrations. The transfer of PCBs from mother to fetus has also been indicated by the detection of PCBs in adipose tissue, liver, and adrenal glands of 7 - 10 months old still-born fetuses. PCBs concentrations in cord

blood are much lower than those in maternal blood leading to suggestions that a placental barrier exists or that the concentration differences are due to a difference in fat content. However, blood concentrations in nursing infants rise rapidly becoming significantly higher than maternal serum levels. Rogan et al. (1986) showed that median PCBs levels in breast milk declined by 40% after 18 months of lactation. PCBs concentrations were also 12% lower in mothers who had breast fed previously. Thus, excretion through nusing is a major route of elimination of PCBs for women, and breastfeeding is potentially a major source of PCBs exposure in the initial months of life for a developing infant.

In a cohort of Michigan women who had a history of eating Lake Michigan sport fish, 7.5% of the breast milk samples taken contained PCB concentrations that equalled or exceeded the U.S. FDA tolerance limit of 1.5 mg/kg (fat basis) for PCB in cow's milk and dairy products sold commercially (Schwartz et al., 1983). Swain (1988), using various assumptions, estimated the degree of exposure to an infant breast feeding for the first year of life. For a maternal breast milk PCB concentration of 1 ug/kg (fat basis) and a mean lipid content of 4.0 g/100 ml, an infant would have a PCB intake of 6.22 mg over the 12 month period. This represents a daily dose above the U.S. FDA tolerance level for the entire 12 month period, and during the first two months of life exceeds this level by 4-6 times.

HAZARD ASSESSMENT

The absorption, metabolism, distribution, and excretion of PCBs has been studied extensively in animal models. Thus, information is available to support the use of data on rats, mice, and nonhuman primates to predict adverse effects in humans.

Experiments using rats (U.S. EPA, 1987; Jensen and Sundstrom, 1974; Van Miller et al., 1975) and monkeys (Allen et al., 1974; Allen et al., 1975) have shown that greater than 90% of PCB mixtures and various isomers are absorbed from the gastrointestinal tract after intragastric administration.

Rats, mice, monkeys, and dogs have varying abilities to metabolize and excrete PCB isomers. Metabolism is a key factor in determining the rate and extent of excretion. Less than 10% of an administered dose was excreted unmetabolized in rats (Matthews and Anderson, 1975). Isomers with two adjacent unsubstituted carbon atoms on the biphenyl ring are metabolized and excreted more easily than isomers with no adjacent unsubstituted carbons (Matthews and Anderson, 1975; Anderson et al., 1977; Kato et al., 1980). Over 90% of a total i.v. dose of 2,4,5,2',5'-pentachlorobiphenyl was excreted over 42 days, while the excretion of 2,4,5,2',4',5'-hexachlorobiphenyl was estimated to be only 20% over the lifetime of the rat (Matthews and Anderson, 1975). The degree of chlorination may also play a role in the ability of rats to metabolize PCBs. The more highly chlorinated isomers are less easily metabolized (Anderson et al., 1977). Excretion of PCBs by mice is similar to rats (Tuey and Matthews, 1980).

Metabolism of PCBs by nonhuman primates is also dependent upon chlorine position (Sipes et al., 1982a and b). Sixty percent of a dose of 2,3,6,2',3',6'-hexachlorobiphenyl administered to rhesus monkeys was excreted in feces and urine after 15 days, whereas less than 7% of a dose of 2,4,5,2',4',5'-hexachlorobiphenyl was excreted in the same time period. In contrast, dogs excreted 70% of a dose of 236-HCB in three days and 66% of a dose of 245-HCB in fifteen days (Sipes et al., 1982a and b). Thus, the dog is

able to metabolize and excrete isomers which are not metabolized by rats or monkeys.

Transplacental transfer of PCBs to the fetus and the excretion of PCBs through nursing occurs in monkeys (Allen et al., 1974), rats (Shain et al., 1986), and mice (Gallenberg and Voďicnik, 1987) in a manner that is similar to humans. The infant of a rhesus monkey fed 25 ppm Aroclor 1248 for two months had measurable levels of PCBs in its body when it was born eight months after treatment was discontinued, with the highest concentrations in fat and adrenals (Allen et al., 1974). The offspring of mice pretreated with 50 mg/kg 2,4,5,2',4',5'-hexachlorobiphenyl two weeks prior to mating had accumulated only 3% of the mother's dose on the day of birth. However, the dam's entire body burden was subsequently eliminated through lactation by the day of weaning (Gallenberg and Vodicnik, 1987). Shain et al. (1986) found that the same congeners were bioaccumulated in rats during gestation and lactation which indicated that lactation produced a higher effective dose of specific congeners.

The distribution of PCBs in tissues varies little between mammalian species (Van Miller et al., 1975; Allen et al., 1975; Matthews and Anderson, 1975; Matthews and Tuey, 1980; Sipes et al., 1982a and 1982b). The parent compound is rapidly cleared from the blood and is initially distributed to muscle and liver. This is followed by metabolism and biliary excretion or redistribution of the parent compound and lipophilic metabolites into skin and adipose tissue. Distribution is determined by the lipid content of the tissue.

The extent of tissue distribution is determined by the degree to which the compound is metabolized by a species. Therefore, more highly chlorinated

isomers and isomers with no adjacent unsubstituted carbon atoms on the biphenyl ring are more likely to accumulate in the tissues of rats, mice, and nonhuman primates (Matthews and Tuey, 1980; Matthews and Anderson, 1975; Hashimoto et al., 1976; Allen et al., 1976). Again, both the rat and rhesus monkey continued to accumulate 245-HCB in adipose tissue 42 and 90 days respectively after its administration, while the dog appears to able to metabolize this isomer to a significant degree (Sipes et al., 1982b). Although both species accumulated 236-HCB in adipose tissue to a lesser extent, the monkey accumulated a larger proportion of the administered dose of 2,3,6-HCB than did the rat. This indicates that nonhuman primates are able to metabolize isomers with adjacent unsubstituted carbon atoms less easily than the rat causing them to be more susceptible to toxic effects.

The distribution and excretion of poorly metabolized congeners in the tissues of the rat is influenced by its characteristic increase in adipose tissue mass during most of the species adult life (Wyss et al., 1986). The increasing adipose tissue mass in rats fed adlibitum acts as a permanent storage site for PCBs. Concentrations in rats with constant adipose tissue mass (fed at a rate of 50% of ad libitum intake) reached an equilibrium with blood serum levels, and clearance from the blood was slower than in rats fed ad libitum. Thus, a higher effective dose may be delivered to target organs. The behavior of PCBs in the adult human may paralell either the increasing or constant adipose tissue scenarios reported in the rats depending on individual bahavior patterns.

Similar metabolites have been identified in the excreta of all species examined including humans. Several authors have proposed that the formation

of an arene oxide intermediate is a common pathway for metabolism (IARC, 1978).

Metabolism, distribution and elimination do not appear to be affected by the size of dose administered in rats or monkeys. Male Sprague-Dawley rats treated with 0.6 or 6.0 mg 2,4,5,2',5'-pentachlorobiphenyl/kg cleared the material from the blood at equivalent rates (Matthews and Anderson, 1975b). Rhesus monkeys treated with 1.5 or 3.0 g Aroclor 1248/kg body weight by gastric intubation eliminated 5.75 and 5.6% of the total dose in excreta over 14 days (Allen et al, 1974).

Acute and chronic administration appears to have little effect on the pattern of distribution (Allen et al., 1976, Hashimoto et al., 1976). However, metabolism and urinary clearance may increase slightly as duration of exposure is lengthened (Hashimoto, et al., 1976).

Human metabolism appears to be more similar to that of the rat and monkey. The metabolism of 4,4'-di, 2,3,6,2',3',6'-hexa, and 2,4,5,2',4',5'-hexachlorobiphenyl by human, cynomolgus monkey and beagle liver microsomes was compared (Schnellmann and Sipes, 1985). Human and monkey microsomes produced the same metabolites and had similar metabolic rates, while dog microsomes behaved very differently. The relative metabolism rates *in vitro* corresponded to relative metabolic clearance recorded *in vivo* in the monkey. The similarity of human disposition of PCBs to that in rat is suggested by the finding that the fat-to-blood distribution coefficient for 2,4,5-HCB in the rat agrees with the coefficient reported for humans (Anderson et al., 1977).

The value of the ratio used by Lutz et al. (1977) for rats was 400 while the ratio calculated from the adipose tissue and blood concentrations reported by Masuda et al. (1985) for humans was 284. Finally, as described in this report, metabolism and distribution in the rat was similar to that in the monkey.

Systemic Effects in Humans and Experimental Animals

A number of adverse health effects have been reported in humans exposed to PCBs. These include elevated serum triglyceride and cholesterol levels, hypertension, hepatic enzyme induction, liver injury, chloracne, neurologic symptoms, eye and upper respiratory irritation, effects on reproduction and an elevated risk of cancer. Other than a finding of hypertension in an environmentally exposed population, the effects were similar to those reported in experimental animals. A causal conclusion regarding PCBs exposure and health effects in humans is impossible to make because the study populations were exposed to other toxicants, and the degree of exposure was difficult to quantify.

Hepatomegaly and an increase in liver lipids was observed in rodents at dietary PCB concentrations as low as 3.75 ppm - 5 ppm (Bruckner et al., 1974; Koller, 1977). More severe liver lesions occurred with increasing concentration in diet. Hepatotoxicity was also produced in nonhuman primates although the dose levels used also produced severe illness in the animals (Hori, 1982; Allen et al., 1974). The induction of liver enzymes was also observed in rodents fed five ppm PCBs in the diet (Bruckner et al., 1974). Hepatic enzyme induction has been documented in humans (Lawton et al., 1985; Kreiss, 1981; Chase et al., 1982; Alvares et al., 1977).

Biochemical changes have been noted in both experimental animals and humans exposed to PCBs. An increase in serum lipids and serum cholesterol occurred in rodents fed PCBs at a level which also induced liver pathology (30 ppm) (Zinkl, 1977). Concentrations of serum triglyceride, cholesterol, and total lipids decreased in nonhuman primates experiencing severe PCB toxicity (Barsotti and Allen, 1975; Barsotti et al., 1976; Allen and Barsotti, 1976; Allen et al., 1979). An increase in serum lipids and cholesterol was recorded in human populations exposed environmentally and occupationally (Kreiss et al., 1981; Chase et al., 1982). The data in humans is conflicting however, as some studies did not observe this effect. Reductions in hemoglobin were observed in rats fed 5 ppm PCBs (Bruckner et al., 1974). Reductions in hemoglobin have also been reported in rhesus monkeys (Allen et al., 1974) and PCB-poisoned patients (Chang et al., 1980). Further, PCB-induced porphyria has been reported in PCB-poisoned patients (Chang et al., 1980) and rats (Bruckner et al., 1974). Increased urinary coproporphyrin levels were a relatively sensitive indicator of PCB exposure in rats.

Dermatological symptoms noted in humans involved in poisoning incidents in Japan and Taiwan and in workers exposed at higher PCB concentrations (Kimbrough, 1987) are quite similar to those induced at high dietary concentrations in nonhuman primates after exposure to PCB mixtures (Allen et al., 1974; Barsotti and Allen, 1975; Barsotti et al., 1976; Allen and Barsotti, 1976). An effect on the immune system, observed in Yucheng patients (Chang et al., 1982; Chen et al., 1985), has also been produced experimentally in rodents (Safe, 1984; Talcott et al., 1985) and nonhuman primates (Truelove et al., 1982; Hori et al., 1982). Moreover, a decrease in T_4 levels in blood caused by dosing rats with PCBs (Byrne et al., 1987) has been documented in workers exposed to PCBs (Emmett, 1985).

A lowest-observed-effect-level (LOAEL) for thyroid toxicity and alterations in adrenal hormones in rats appears to occur at 1 ppm (0.08 mg/kg body weight/day) in the diet for Aroclor 1254, Aroclor 1242, and Aroclor 1016. These effects were more sensitive indicators of toxicity than increased liver weight which occurred at higher dose levels in the same experiments (Byrne et al., 1987 and Byrne et al., 1988).

Reproductive toxicity produced by low-level PCBs exposure was reported in nonhuman primates and mice. Rhesus monkeys fed 2.5 and 5.0 ppm Aroclor 1248 for six months experienced changes in the length of menstrual cycles and hormone levels (Allen and Barsotti, 1976; Barsotti et al., 1976). The estrus cycle was lengthened in NMRI mice fed 5 ppm (0.8 mg/kg/day) Clophen A 60 in the diet for 62 days (Orberg and Kihlstrom, 1973). In addition, implantation frequency was reduced in NMRI mice exposed to PCBs at the same dose level for 72 - 76 days. For the purposes of this project, the evidence is sufficient to place PCBs in the Probable Positive Reproductive I Weight of Evidence Classification (see Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment and Risk Characterization).

Epidemiologic studies of the children of mothers exposed to PCBs through Lake Michigan fish consumption have revealed physical and behavioral deficits (Jacobson et al., 1983, Fein et al., 1984, Jacobson et al., 1984, and Jacobson et al., 1985). The authors documented an association of lower birthweight, lower gestational age, and smaller head circumference with sport fish consumption and cord blood PCB concentration. When the exposed population was divided into three groups with increasing fish consumption frequency, a dose-response relationship was demonstrated for birthweight, gestational age, head

circumference, and neuromuscular maturity. A decrement was shown in offspring even at a low consumption level of 2 - 3.4 kg/year which is approximately equal to one meal per month or less depending on meal size. It was not reported whether the decrease at this consumption level was significantly different from the controls. Contaminated fish consumption also predicted several abnormal parameters measured by the Brazelton Neonatal Behavioral Assessment Scale (NBAS). Fish consumption predicted abnormal lability of states, motor maturity, amount of startle, and abnormally weak reflexes. Poorer visual recognition memory in seven month old infants was predicted by fish consumption and also by PCB concentrations in cord blood. The authors concluded that these deficits were related to intrauterine exposure. Several potential confounders were carefully evaluated and reported. Unfortunately fish consumption indicators were not correlated with PCBs concentration in cord blood which leaves open the possibility that an additional risk factor may have been responsible for the deficits reported.

The lower visual recognition memory scores noted in the children of fish consumers were correlated with cord serum PCB levels and may signify an irreversible effect due to intrauterine exposure. Altered behavior which may also be irreversible has been observed in adolescent rhesus monkeys exposed pre- and postnatally to Aroclor 1248 (0.5, 1.0, and 2.5 ppm in the maternal diet; Bowman and Heironimus, 1981; Allen et al., 1979).

The developmental effects occurred in a sample with a mean consumption level of 6.7 kg per year of fish species contaminated at levels equivalent to concentrations in lake trout (consumption values ranged from 1.2 - 41.7 kg/yr). The average length of consumption of Lake Michigan fish was 16.1 ± 9.0 years (ranging from 1.0 to 40.0 years). Low birth weight and shorter

gestational age were associated with PCBs in other studies of environmental and occupational exposure and poisoning incidents. These effects were also observed in the offspring of nonhuman primates fed PCB diets (Allen et al., 1979).

A no-observed-adverse-effect-level (NOAEL) of 0.25 ppm for developmental toxicity was reported for Aroclor 1016 fed to rhesus monkeys. One ppm in the diet for seven months prior to and throughout gestation to four months postpartum resulted in significantly lower birth weights (Barsotti and Van Miller, 1984). The offspring of rhesus monkeys exposed to 0.5 and 1.0 ppm Aroclor 1248 in the diet were smaller, gained weight less rapidly than controls, and exhibited behavioral abnormalities (Allen et al., 1979). These PCB concentrations were reported to have no adverse effect on female reproductive capacity. Higher dietary levels produced severe maternal toxicity and adverse effects in exposed offspring.

Fetotoxicity and behavioral teratological effects have also been produced in mice and rats exposed prenatally and through nursing. White-footed mice may be the most sensitive strain among mice tested (Linzey, 1988). Second generation offspring had a depressed body weight gain at four weeks of age and lower uterus and ovary weights at eight and twelve weeks in a three generation continuous feeding experiment. This dose group (10 ppm in diet) also produced a lower number of litters when offspring were paired at twelve weeks of age. Altered behavioral responses occurred in ICR strain mice at a similar dose level (11 ppm during gestation; Storm et al., 1981).

An adverse effect of PCBs exposure on immune response (decreased helper T cell activity) in mice exposed prenatally (Takagi et al., 1987) was also reported

in human patients exposed to PCBs through consumption of rice oil (Chang et al., 1982). The prenatal exposure in mice was concluded to primarily responsible for the immunesuppression in mice. The physical and behavioral deficits in reported in human offspring were also concluded to be due to *in utero* exposure.

Fetotoxicity appears to occur at dietary dose levels of 20 ppm and greater in rats, while a toxic response occurs in the livers and spleens of offspring exposed prenatally and via nursing at lower dose levels (Linder et al., 1974; Overmann et al., 1987). Rats fed 20 ppm (1.5 mg/kg/day) Aroclor 1254 produced a lower number of litters and smaller litter sizes than controls (Linder et al., 1974). Liver damage was observed in the offspring. Dietary levels as low as 1 ppm (0.06 mg/kg/day) increased liver to body weight ratios in second generation 21 day old males. The effect on the liver was present in both sexes in the second and third generation weanlings and in five to seven month old second generation males in the 5 ppm (0.32 mg/kg/day) dose group. A dose level of 20 ppm caused enlarged livers in F_0 adults. No maternal toxicity was reported at lower dose levels. This data indicates that a LOAEL for increased liver weight in rat offspring may occur at 1 ppm in the diet. Enlarged hepatocytes with an indication of lipid accumulation were observed in these offspring as well.

A dose level of 8 mg/kg caused reproductive deficits in male rats exposed postnatally through nursing. It was suggested that sperm motility may be affected (Sager et al., 1987). Low concentrations of three PCB congeners in human sperm were associated with decreased motility in infertile men (Bush et al., 1986).

Severe reproductive toxicity was observed in mink at the lowest dose tested. Complete reproductive failure was produced in eight mink fed 2.0 ppm Aroclor 1254 in the diet for ten months (Aulerich et al., 1977).

The studies of developmental toxicity, particularly those by Linder et al. (1974) in rats, Linzey (1988) in mice, the series of reports on rhesus monkeys (Allen and Barsotti, 1976; Barsotti et al. 1976), and the series of reports on the children of Michigan women who had consumed Lake Michigan sport fish (Jacobson et al., 1983; Fein et al., 1984; Jacobson et al., 1984; Jacobson et al., 1985) are sufficient to place PCBs in the weight of evidence classification of Probable Positive for developmental toxicity as defined by this project.

Carcinogenic Effects

Evidence for carcinogenicity in humans is limited but suggestive. An excess risk of malignant tumors has been reported among Yusho patients with the gastrointestinal tract and lymphatic and hematopoietic tissue most affected (Kuratsune et al., 1987). An investigation of causes of death in this population reported in 1984 confirmed the existence of a statistically significant excess risk of mortality from cancer (SMR = 165) and in particular, an excess risk of mortality due to liver cancer (SMR = 492, OBS = 6). Occupational studies have identified melanoma and pancreatic cancer (Bahn et al., 1976), hepatic and rectal tumors (Brown and Jones, 1982 and Brown, 1987) and cancer of the GI tract and lymphatic tissue (Bertazzi et al, 1987) associated with PCB exposure.

Hepatobiliary cancer is a consistent finding among epidemiological studies of PCBs exposure. However, the epidemiological studies of the association of

occupational PCB exposure with mortality and carcinogenicity do not lead to a definite causal determination. All of the studies were limited by a small number of deaths in the cohort available for analysis, insufficient length of follow-up, and a lack of information regarding potentially confounding variables. Furthermore, it was not possible to obtain a quantitative estimate of the PCB dose received by the workers. Although inconclusive, the risk of mortality from cancer of the liver, gall bladder and biliary tract appears to be elevated among employees exposed to PCBs. This general finding is supported by the finding of an association of PCBs with liver cancer in rodents and lend credibility to the use of rodent studies to infer hypotheses regarding health effects in humans.

Carcinogenic potential has been evaluated in five strains of rats and two strains of mice. Table 1 (also Table 12 in the PCB hazard assessment) . summarizes the study designs used in each investigation. The primary target site for tumorigenesis associated with PCBs is the liver in rats (Ito et al., 1974, Kimbrough et al., 1975, Kimura and Baba, 1973, NCI, 1978, Norback and Weltman, 1985 and Schaeffer et al., 1984) and mice (Ito et al., 1973 and Kimbrough and Linder, 1974). These studies on rats and mice have reported a dose-related occurrence of foci of hepatocellular alteration, hyperplastic nodules, neoplastic nodules, and hepatocellular carcinoma. In addition, adenofibrosis (Ito et al., 1974, Kimbrough et al., 1975, Norback and Weltman, 1985), adenoma (NCI, 1978), and adenocarcinoma (Norback and Weltman, 1985) were reported in rat liver, and hepatoma and adenofibrosis (Kimbrough and Linder, 1974) were reported in mouse liver.

Carcinogenic effects may also be associated with PCB exposure in other organs. NCI (1978) concluded that carcinomas observed in the gastrointestinal tract

may have been related to treatment in male and female rats. Morgan et al. (1981) detected an increased incidence of intestinal metaplasia and adenocarcinoma in the glandular stomach of male and female rats. This is significant because gastointestinal tract cancer was identified in PCB exposed humans (Bertazzi et al., 1987).

The higher chlorinated PCB mixtures are associated with a greater carcinogenic response in both rats (Ito et al., 1974, Schaeffer et al., 1984) and mice (Ito et al., 1973). Limited data suggest that females may be more sensitive to the carcinogenic stimulus than males (Norback and Weltman, 1985).

Table 1. Animal Studies of Carcinogenicity Resulting from Exposure to PCBs.

Species/ Sex/ Reference	PCB[1]	Dose (ppm)	Dose Rate[2] (mg/kg/ day)	Number/ group	Number at Risk	Duration of Study (weeks)
Donyru rats M & F Noritaka & Baba, 1973	K-400	0 38.5- 616	NA	5 10	NR	23 - 80
Wistar rats M Ito et al., 1974	K-500 K-400 K-300	0 100 500 1000	Control 4 20 40	29 29 29 29	K-5 K-4 K-3 18 25 16 22 26 8 19 13 10 15	28 - 52
Sherman rats F Kimbrough et al., 1975	A-1260	0 100	Control 5	200 200	173 184	84

Table 1 continued.

Species/ Sex/ Reference	PCB[1]	Dose (ppm)	Dose Rate[2] (mg/kg/ day)	Number/ group	Number at Risk	Duration of Study
Fisher 344 rats M & F NCI, 1978	A-1254	0	Control	24	NR	105
		25	1.13	24		
		50	2.25	24		
		100	4.5	24		
Sprague-Dawley rats M & F Norback & Weltman, 1985	A-1260	0	Control	140	81	116
		100 for 16 mo., then 50 for 8 mo.	3.75	126	93	
Wistar rats M Schaeffer et al., 1984	C-A30 C-A60	0	Control	139	53	119
		100 C-A30	4	152	87	
		100 C-A60	4	141	85	
dd mice M Ito et al., 1973	K-500 K-400 K-300	0	Control	6	NR	32
		100	16.6	12		
		250	41.6	12		
		500	83.3	12		
BALB/cJ mice M Kimbrough & Linder, 1974	A-1254	0		100	58	44
		300 (6 mo.)	Control 50	50	24	
		300 (11 mo.)	50	50	22	

1. K- Kanechlor A- Aloclor C- Clophen
2. Calculated based on the following assumption of consumption as percent of body weight. Male rats: 4%
   Female rats: 5%
   Male mice: 16.66%
   Female mice: 20%
3. Defined as the number of animals alive at a time designated by the author and considered to be at risk of developing cancer.

The studies which investigated effects on both sexes resulted in contradictory indications of relative sensitivity. Hepatocellular carcinoma was not induced in female rats treated with Aroclor 1254 but was induced in males (NCI, 1978). In contrast, females were found to have a much higher incidence of this cancer (40%) compared to males (4%) after treatment with Aroclor 1260 (Norback and Weltman, 1985). In addition, Kimura and Baba (1973) induced adenomatous nodules in the livers of females which had ingested 1200 - 1500 mg Kanechlor-400 and not in males ingesting equivalent or greater amounts. The average experimental period for these females was longer than for the males.

The commercial mixtures that contain a larger amount of the more highly chlorinated compounds are more potent carcinogens. Ito et al. (1974) found that the increased incidence of "nodular hyperplasia" was dose-related and was much greater in those groups of male Wistar strain rats fed Kanechlor-500 and Kanechlor-400 for 28 to 52 weeks compared to those fed Kanechlor-300. Incidence in the 100 ppm groups were 12% and 12.5% compared to 4.5%. These findings were confirmed by Schaeffer et al. (1984) using Clophen A 30 and Clophen A 60. Although the incidence of neoplastic nodules in male Wistar strain rats surviving more than 800 days was comparable between the two mixtures, 61% of the rats fed Clophen A 60 developed hepatocellular carcinomas as opposed to 3% in the rats fed Clophen A 30. Mice exhibited similar sensitivity to the degree of chlorination in Kanechlor mixtures. Nodular hyperplasia and hepatocellular carcinoma were observed only in male dd strain mice fed 500 ppm Kanechlor-500 and not in mice fed Kanechlor-400 or Kanechlor-300 at equivalent doses for 32 weeks (Ito et al., 1973).

It can be concluded that various PCB mixtures are carcinogenic in male and female rats. The incidence of hepatocellular carcinoma was statistically

significant at 100 ppm Clophen A 60 in male Wistar strain rats (Schaeffer et al., 1984). This result is particularly informative because the PCB mixture was reported to be free of dibenzofurans. Unfortunately, the incidence reported for neoplastic nodules and hepatocellular carcinoma add up to a number greater by one over the total number of surviving animals making the use of the number in quantitative risk assessment difficult (see Table 15 in PCB Hazard Assessment). Although statistical tests were not conducted by Kimbrough et al. (1975) or Norback and Weltman (1985), calculation of the Chi Square test statistic for comparison of two proportions results in a significant difference between treated and control Sherman strain female rats for hepatocellular carcinoma at 100 ppm (Kimbrough, 1975). Combined hepatocellular carcinoma and adenocarcinoma incidence in male and female Sprague-Dawley rats is also statistically significant (Norback and Weltman, 1985).

The Norback and Weltman study (1985) provides the best information for use in a cancer risk model. Although only one dose level was used, the study has some strong attributes including the inclusion of both sexes, a large number of animals per dose group, a follow-up period longer than two years, detailed pathological descriptions, and the use of Sprague-Dawley rats which have a low incidence of spontaneous liver tumors. Hepatectomies were carried out on some of the animals in each dose group at different points in time allowing the collection of data on neoplastic progression. The lesions were classified microscopically as centrolobular cell hypertrophy (first observed at one month), foci (observed at three months), areas of cell alteration (observed at six months), neoplastic nodules (observed at 12 months), trabecular carcinoma (observed at 15 months) and adenocarcinoma (observed at 24 months). The investigators analyzed hepatocytes and the various lesions for gamma glutamyl

transpeptidase (GGT) activity which allows comparisons for this characteristic of malignancy. Hepatocytes of control rats were negative for GGT activity as were areas of centrolobular cell hypertrophy in treated rat hepatocytes. Some of the "foci" were strongyly positive, and neoplastic nodules and hepatocellular carcinomas contained positive cells admixed with negative cells. Adenocarcinoma cells were also positive. The similarity between neoplastic nodules and hepatocellular carcinomas for GGT activity lends support for combining the two lesions in a cancer risk model. The effect on cancer incidence from the inclusion of data from hepatectomized rats is not known but may have biased the results in a positive direction.

The NCI study (1978) was the only test design that evaluated multiple doses. Unfortunately sample sizes were smaller (see Table 6) thereby decreasing the statistical power of the test to detect an increased incidence of hepatocellular carcinoma. A low incidence of hepatocellular carcinoma, which increased with increasing dose level, occurred in the males but was not observed in females. A dose-related incidence of neoplastic nodules was observed in both sexes. Ward (1985) found a significant dose related trend for tumor incidence in males when all liver adenomas and carcinomas were combined ($p<.01$). It is plausible that if the experimental period had extended for a period longer than 105 weeks, a larger number of hepatocellular carcinomas would have developed. Hepatocellular carcinoma incidence due to Clophen A60 exposure increased from 30% (9/30) in 700 - 800 days to 61% (52/85) in the termination period after 800 days (Schaeffer et al., 1984).

The U.S. EPA Risk Assessment Forum (1986) concluded that although the exact contribution of neoplastic nodules to the overall incidence of hepatocellular tumors in the rat is unclear, it is appropriate to combine neoplastic nodules

and hepatocellular carcinoma incidence to evaluate statistical significance when merited by the weight of evidence concerning carcinogenicity. Less weight is to be given to responses where only the increase in neoplastic nodules is statistically significant. Quantitative assessment is not considered to be justifiable if there is no human or supporting evidence.

Combining the tumor incidence data sometimes presents some difficulties because of potential double counting. Schaeffer et al. (1984) and Norback and Weltman (1985) reported results as the highest level of neoplastic lesion observed on an animal, and therefore double counting is not a problem.

Experiments conducted to date indicate that PCBs are not mutagenic in bacterial systems and have not produced evidence of dominant lethality or chromosomal abnormalities in bone marrow or spermatogonial cells of Osborne-Mendel rats. Mitotic division was inhibited in bone marrow cells and spermatogonia by PCBs, however experimental results have not been consistent.

PCB mixtures and specific mono, tetra, and hexachlorinated isomers have been shown by several investigators to bind through covalent and strong noncovalent associations to microsomal proteins, RNA and DNA (Shimada, 1976; Wyndham et al., 1976; Seymour et al., 1976; Morales and Matthews, 1978; Hargraves and Allen, 1979). Some of these results have been obtained after *in vivo* administration. Metabolism is required for binding to occur suggesting that an activated intermediate is responsible. Covalent binding of 236-HCB and 4-DCB metabolites has also been demonstrated in human microsomal protein (Schnellmann and Sipes, 1985). The 236-HCB equivalent was bound to a greater extent than the 4-DCB equivalent. The addition of reduced glutathione to the microsomal incubation resulted in a reduction of covalent binding. This

authors concluded that this was evidence that an arene oxide intermediate was involved.

PCBs have been shown to promote the formation of altered cells and tumors in the liver and lung in several tests. Aroclor 1254 and Clophen A50 have promoted diethylnitrosamine (DENA)-initiated liver tumorigenesis in male Wistar strain rats (Nishizumi, 1976), male F344 rats (Hirose et al., 1981), and male Sprague-Dawley non-inbred albino rats (Preston et al., 1981; Pereira et al., 1982). Preston et al. (1981) established that Aroclor 1254, free of polychlorodibenzofuran, is just as potent as the contaminated mixture in promoting DENA-initiated hepatocellular carcinoma in male Sprague-Dawley rats.

In two studies, PCBs enhanced the tumorigenic effect of the initiator after only one dose. The rate of appearance of DENA initiated GGTase-positive hepatocellular foci in male Sprague-Dawley rats was enhanced by one intraperitoneal injection of 500 mg/kg body weight of Aroclor 1254 (Pereira et al., 1982). Anderson et al. (1986) administered a single dose of Aroclor 1254 (50, 250, or 500 ppm) by gastric intubation to infant Swiss mice on post-natal day 8, four days after an intraperitoneal injection of 5 ppm N-nitrosodimethylamine (NDMA). Mice given 500 ppm PCB plus NDMA had twice the number of lung tumors as the NDMA only group at 16 and 28 weeks, and tumor incidence became dose-dependent at 28 weeks. A significant increase in the size of proliferative lesions in the liver was reported in mice given 50 ppm PCB at 28 weeks. The promotion of tumorigenesis after the administration of only one dose is in contrast to a description of epigenetically active agents by Weisburger and Williams (1980) as active at high sutained doses and demonstrating reversible effects.

Studies by Oesterle and Deml (1984) and Deml and Oesterle (1987) have shown the dose-related promoting activity of Clophen A 50. The number and total area of ATPase-deficient islands was statistically increased in the hepatocytes of female Sprague-Dawley rats treated with the relatively low dose of 5 mg/kg body weight three times a week for 11 weeks after an oral dose of 8 mg/kg body weight DENA (Deml and Oesterle, 1987). GGTase and glycogen-positive islands were also greatly enhanced in number and area in a dose-dependent manner. A dose level of one mg/kg body weight three times weekly (430 ug/kg/day) did not significantly increase these effects. Clophen A 50 also demonstrated co-carcinogenic properties in rats when administered alternating with DEN five days a week for 12 weeks (Deml and Oesterle, 1986). Although the majority of studies of promotion did not observe a tumorigenic effect when the PCB mixture was administered alone, one study by Oesterle and Deml (1984) did report an increase in enzyme alered foci in this group but the number was not significantly greater than that in the control mice.

PCB congeners classified as 3-methylcholanthrene-type inducers or phenobarbital-type inducers both promoted DEN initiated hepatic ATPase-deficient islands in female Wistar rats. The PCB was administered i.p. at a dose of 150 umol/kg body weight once a week for 8 weeks beginning 10 days after a 10 day treatment with DEN (50 ppm in drinking water) (Buchmann et al., 1986). The island number and relative volume of liver occupied by altered tissue was enhanced by both isomers. These results suggest that cytotoxicity is not the sole mechanism for the promoting activity of PCB mixtures since cytotoxicity has not been demonstrated for the PB-type inducing isomers.

The PCB isomer, 2,4,5-HCB, inhibited metabolic cooperation, a form of intercellular communication, in Chinese hamster cells (Tsushimoto et al.,

1983). This isomer is not cytotoxic. A cytotoxic isomer, 2,4,5-HCB, was not able to inhibit metabolic cooperation at noncytotoxic levels. The promoting action of PCBs, therefore, may be due to more than one mechanism.

The studies of promotion usng PCB mixtures indicate that this class of compounds may exert their carcinogenic effect through an epigenetic mechanism. However, one cannot conclude that PCBs are solely promoters because they are able to bind with microsomal protein, DNA, and RNA, and demonstrate some unusual promoting properties. These include the enhancement of tumorigenesis after only one dose, co-carcinogenic action, and possibly more than one type of epigenetic activity. The mechanism of action of PCBs is not clear.

Four chronic studies in rats (Norback and Weltman, 1985, Schaeffer et al., 1984, Kimbrough et al., 1975, and NCI, 1978 as evaluated by Ward, 1985) and one chronic study in mice (Kimbrough and Linder, 1974), provide strong evidence of carcinogenicity. Hepatocellular carcinoma was produced in males and females of various strains (although both sexes were not always studied in each experiment). A dose-related effect on incidence was demonstrated by the NCI study. These results, plus the suggestive, but inadequate epidemiological evidence (as interpreted by U.S. EPA) has led the U.S. EPA to place PCBs in Group B2 as a probable human carcinogen.

The International Agency for Research on Cancer (IARC), in its 1987 update of the IARC Monographs, concluded that the degree of evidence for carcinogenicity was limited in humans and sufficient in experimental animals (IARC, 1987). A chemical can be classified as having limited evidence of carcinogenicity according to IARC when "a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is

considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence." The IARC Working Group determined that an association between cancer and exposure to PCBs was suggested by the consistent increased risk from hepatobiliary cancer that was found by several researchers. The evidence was considered limited because the numbers were small, dose-response relationships could not be evaluated, and the role of compounds other than PCBs (i.e. dibenzofurans) could not be excluded. The evidence for carcinogenicity in animals was sufficient because a causal relationship was established between PCBs exposure and an increased incidence of benign and malignant neoplasms in rats and mice. The overall evaluation of the carcinogenicity of PCBs places this class of compounds into category 2A; probably carcinogenic to humans. IARC concluded that the weight of evidence in humans is strong enough to place PCBs into the higher Group 2 category, whereas the U.S. EPA did not.

DOSE-RESPONSE ASSESSMENT

Systemic Toxicity

Four experimental animal studies were selected to characterize the noncarcinogenic health risk to humans exposed to PCBs. The studies, described in Table 2, found hypothyroidism and adrenal damage in rats (Byrne et al., 1987; Byrne et al., 1988), enzyme induction and liver toxicity in rats (Bruckner et al., 1974), and developmental toxicity in nonhuman primate offspring (Allen et al., 1979). A more detailed presentation of the studies and their results is contained in the hazard assessment section. The study descriptions will be reiterated in the following discussion and the significant data will be presented.

Table 2. Animal Experiments Used to Characterize Noncarcinogenic Risks in Humans Exposed to PCBs.

Reference	PCB Mixture	Species	#/Dose Group	Dose Groups ppm	Dose Groups mg/kg/day	Duration of Dosing	Response
Byrne et al., 1987	Aroclor 1254	Sprague-Dawley rat female	10	0 1 5 10 50	0 0.05 0.25 0.5 2.5	5 months	*Hypothyroidism *Dec. serum T_4 conc. at 1 ppm ($p<.05$)
Byrne et al., 1988	Aroclor 1254, 1242 1016	Sprague-Dawley rat female	10	0 1 5 10	0 0.05 0.25 0.5	5 months	*Dec. Adrenal weight at 1 ppm ($p<.05$) *Dec. DHEA at 1 ppm ($p<.05$) *Dec.Corticosterone, dehydroepiandrosterone, & DHS at 5 ppm ($p<.05$)
Bruckner et al., 1974	Aroclor 1242	Sprague-Dawley rats male	6	0 5 25	0 0.2 1.0	6 months	*Inc. Enzyme Induction, Liver Lipid, Urinary Coproporphyrin Excretion at 5 ppm ($p<.05$)
Allen et al., 1979	Aroclor 1248	Rhesus monkey	8	0 0.5 1.0	0 0.01 0.02	18 months	Dec. birth weight, Inc. behavioral anomalies at 0.5 ppm

Byrne et al., 1987

Female sprague-Dawley rats (10 per dose group) were fed 0, 1, 5, 10, and 50 ppm Aroclor 1254 in the diet for at least five months. Blood samples were taken before treatments began and at intervals during the treatment period. The effect of PCB exposure on serum thyroxine (T_4) and serum triiodothyronine (T_3) is reproduced in Figure 1. Serum thyroxine (T_4) levels were significantly reduced in the 10 and 50 ppm groups by day 14 compared to the

control group and to pretreatment levels. Serum T_4 levels also became significantly decreased in the 1 and 5 ppm groups at day 35. A significant decrease in serum triiodothyronine (T_3) levels occurred in the 10 and 50 ppm groups by day 20 compared to controls and pretreatment levels. The decrease became significantly lower in the 5 ppm group by day 40. Rats in all treatment groups responded to a TSH challenge by increasing T_4 and T_3 levels in serum. The level of response was significantly less than controls in the 10 ppm group.

The disappearance rate of injected doses of L[125I] T_4 was significantly decreased in all treatment groups and its severity was dose related. The T_4 distribution space (TDS) increased with increasing dose and became 8 times the control value in the 50 ppm group. The T_4 production rate (T4PR) was significantly decreased in all treated groups and was dose-related.

The adverse effects were induced by a PCB dose level lower than that which caused liver toxicity. Liver weight was slightly elevated in the 10 and 50 ppm dose groups, however thyroid weight was not significantly increased. Food consumption was not affected by treatment and body weights were not significantly different.

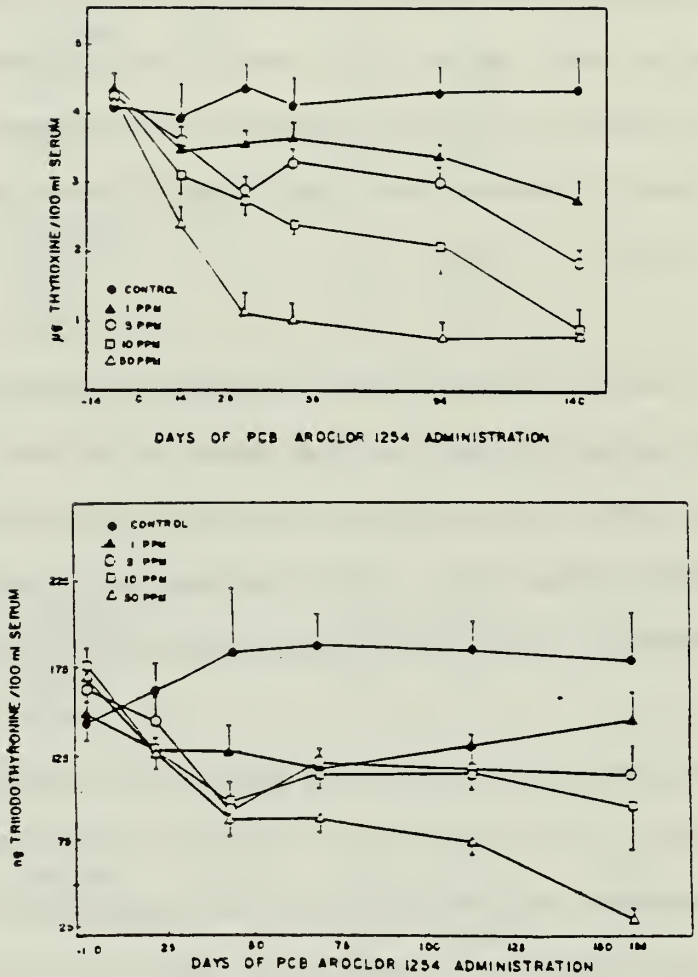
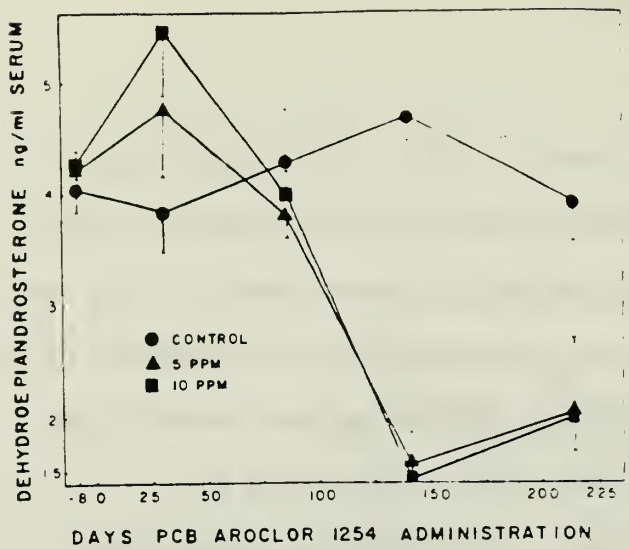


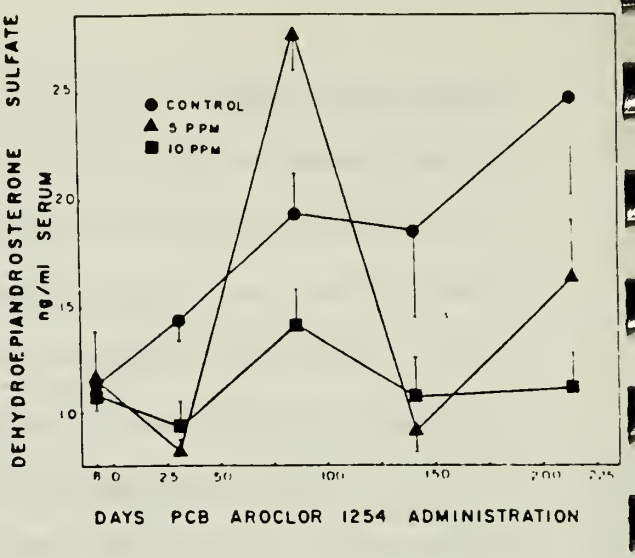
Figure 1. Effect of Chronic PCB Treatment on Serum T4 and Serum T3 Levels in Rats. Source: Byrne et al., 1987.

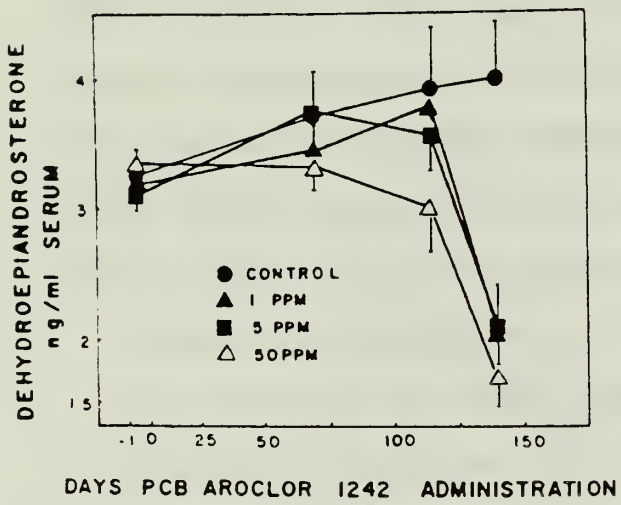
Byrne et al., 1988

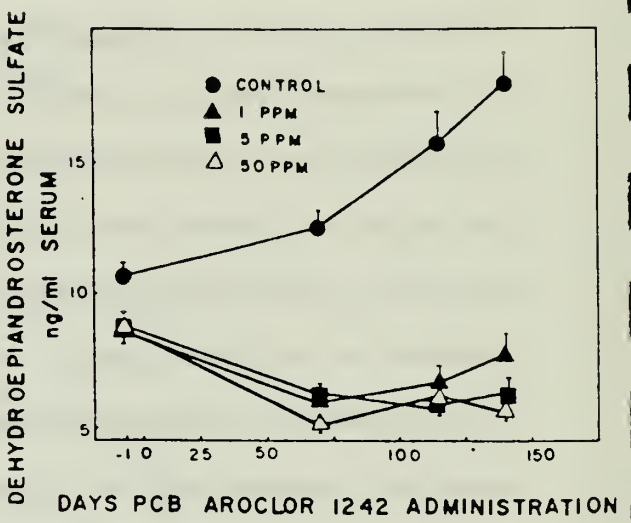
Concentrations of 1, 5, 10, or 50 ppm of Aroclor 1254, Aroclor 1242, or Aroclor 1016 were fed to female Sprague-Dawley rats for five months. Figure 2 presents the dose related effect of PCB treatment on serum hormone levels over time. Aroclor 1254 significantly decreased corticosterone (B) levels in serum compared to control and pretreatment levels in all dose groups except 1 ppm. B levels in the 50 ppm group were five times lower than control and pretreatment levels by the end of the study. Serum dehydroepiandrosterone (DHEA) levels were significantly decreased in rats fed 5 and 10 ppm after a transitory increase during the first month in rats treated with Aroclor 1254. All treatment levels of Aroclor 1242 and Aroclor 1016 significantly decreased serum DHEA levels by the end of the five months, although the depression did not begin until much later. Levels of dehydroepiandrosterone sulfate (DHS) were also significantly decreased by chronic treatment of all three Aroclor mixtures at all dose levels. As the animals aged, control DHS increased steadily while it did not in the treated groups with the exception of an anomalous spike on day 80. Hormone levels were inhibited to the greatest extent in Aroclor 1254 treated rats.

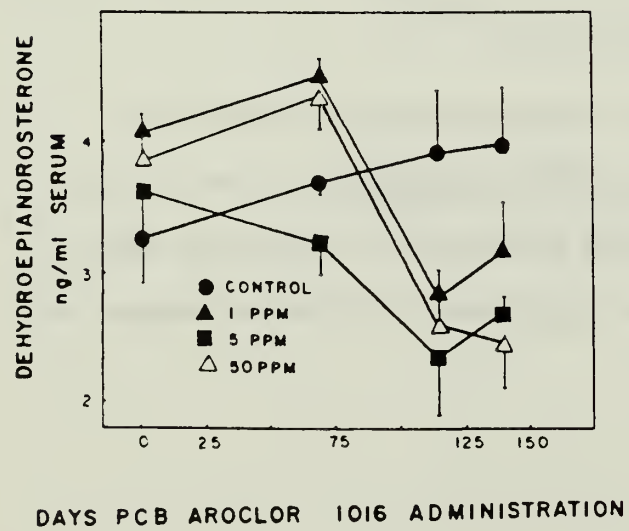
Adrenal weights/100 grams body weight were significantly lower than the controls at all dose levels in the Aroclor 1254 treated groups however liver weights were not affected. Table 3 presents the effect of increasing dose on adrenal weights. Food consumption was not significantly different between and among control and treatment grops.











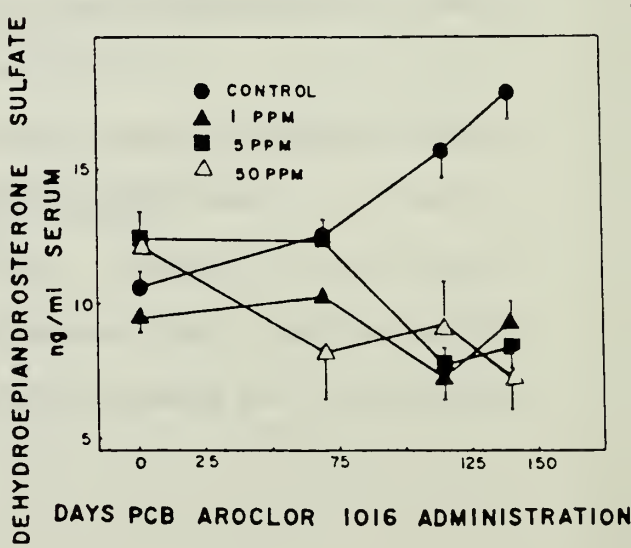


Figure 2. Effect of Chronic PCB Treatment on Serum Hormone Levels in Rats.
Source: Byrne et al., 1988.

Table 3. Adrenal Weights (mg/100 grams b.w.) of Rats Treated for Five or More Months with Aroclor 1254.

Dose (ppm)	Adrenal Weight[a] (mg/100 g body wt)
0	28.9 ± 1.5[b]
1	23.1 ± 2.1*
5	24.4 ± 0.9*
10	20.8 ± 0.8*
50	19.8 ± 0.9*

[a] Combined weight of left and right adrenal.
[b] Mean ± SEM.
* Significantly different ($p<.05$) from control group (student t test).

Bruckner et al., 1974

Groups of six Sprague-Dawley rats were fed 0, 5, or 25 ppm Aroclor 1242 for 2, 4, or 6 months. Liver weight expressed as a percent of body weight was elevated in the 25 ppm group after four months. Table 4 shows the effect of PCB treatment on liver lipid and urinary coproporphyrin levels over time. Liver lipid (mg/g wet weight) was elevated after only two months and remained elevated in the 25 ppm and 5 ppm groups after six months. Urinary coproporphyrin excretion was significantly increased by 5 ppm in the diet for 6 months, and some animals exhibited high urinary coproporphyrin levels at 2 and 4 months. Histopathological changes involving increased lipid deposition, lipid vacuolation, and smooth endoplasmic reticulum proliferation were reported. Table 5 shows the dose-related increase in hepatic enzyme levels in PCB treated rats. Mixed function oxidase activity was induced at a level that was statistically significant after two months in the 25 ppm group and after four months in the five ppm group. The increased activity was dose-dependent. N-demethylase activity was significantly induced by 25 ppm Aroclor 1242 after four months.

Table 4. Urinary Coproporphyrin levels and Total Lipids in the Livers of PCB treated Rats.

Months	Dose (ppm)	Urinary Coproporphyrin (ug/24 hrs)	Total Lipids (mg/g liver wet wt)
2	0	4.7 ± 0.3	31.2 ± 0.7
	5	5.8 ± 0.4 (p<0.1)	35.3 ± 1.1 (p<0.01)
	25	9.9 ± 0.6 (p<0.001)	38.2 ± 1.0 (p<0.001)
4	0	5.9 ± 0.9	33.4 ± 2.0
	5	8.5 ± 1.4 (0.01)	40.0 ± 2.1 (p<0.05)
	25	16.6 ± 4.4 (p<0.05)	41.5 ± 2.0 (p<0.01)
6	0	3.9 ± 0.7	31.9 ± 0.7
	5	7.9 ± 1.4 (p<0.05)	34.1 ± 1.3 (p<0.1)
	25	6.3 ± 0.9 (p<0.1)	36.0 ± 1.1 (p<0.01)

Table 5. Hepatic Enzyme Induction in Rats Fed PCBs for 6 Months.

Months	Dose (ppm)	Hydroxylase Activity N-acetyl-p-aminophenol Formed (ug/mg protein/20 min.)
2	0	0.14 ± 0.01
	5	0.19 ± 0.02 (p<0.1)
	25	0.53 ± 0.04 (p<0.01)
4	0	0.31 ± 0.03
	5	0.51 ± 0.03 (p<0.01)
	25	1.06 ± 0.03 (p<0.001)
6	0	0.34 ± 0.08
	5	0.51 ± 0.03 (p<0.05)
	25	1.38 ± 0.06 (p<0.001)

Allen et al., 1979

Rhesus monkeys were fed diets with 0.5 and 1.0 ppm Aroclor 1248 three times per week for approximately 18 months. Females were bred to control males after a seven month exposure period. No effect was noted on menstruation, hormone levels, or conception in these animals. Six out of eight females in the 0.5 ppm group and seven out of eight of the females in the 1.0 ppm group

gave birth to live infants. The infants were reported to be "somewhat smaller" than controls and gained weight less rapidly. Birth weights were 463 grams and 466 grams in the 0.5 ppm and 1.0 ppm dose groups respectively. Birth weights were not reported for the control offspring but may be compared to 507 ± 59 grams reported for the control group in a previous study using higher dose levels. Focal areas of skin hyperpigmentation developed during nursing. The offspring were weaned at four months. These infants also showed behavioral patterns similar to the infants exposed to higher levels of PCBs.

A summary of this study was reported in a review of several studies conducted by the author. Therefore methods and data are not well reported. However, it is important to consider these results because they report a sensitive indicator of developmental toxicity (behavior) at a very low dose rate in a species biologically similar to humans.

Calculation of the Reference Dose (RfD)

Table 6 presents the NOAELs or LOAELs, uncertainty factors (UF), and modifying factors (MF) for each study and the reference doses upon which they are based. Two reference doses were calculated for each study. The RfD(total) represents the "allowable" PCB dose from all exposure sources. The RfD(fish) represents the "allowable" PCB dose from fish obtained commercially and by sport fishing. A factor of 0.9 was applied to the RfD(total) to obtain the RfD(fish). An assumption was made that 90% of human exposure to PCBs occurs as a result of fish consumption. Uncertainty remains in the RfD because the animals in these studies were not studied for a lifetime.

Table 6. Parameters Used to Calculate Reference Doses for Systemic Effects in Humans Exposed to PCBs.

Reference	Target Organ	Dose (mg/kg/day)	End-point	Study Length (months)	UF[2]	MF[3]	Reference Dose (mg/kg/day) Total	Fish
Byrne et al., 1987 & 1988	Thyroid Adrenal	0.05 mg/kg/day	LOAEL[1]	5 months	10H 10A 10L	1	5 (10^{-5})	4.5 (10^{-5})
Bruckner et al., 1974	Liver	0.2 mg/kg/day	LOAEL	6 months	10H 10A 10L	1	2 (10^{-4})	1.8 (10^{-4})
Allen et al, 1979	Off-spring	0.01 mg/kg/day	LOAEL	18 months	10H 10A 10L	1	1 (10^{-5})	0.9 (10-5)

[1]LOAEL = Lowest-adverse-effect-level.
[2]UF = Uncertainty factor. Uncertainty factors are used to calculate the reference dose. The no-adverse-effect-level (NOAEL) is divided by factors of 10 to account for variation in sensitivity among the members of the human population, 10H; uncertainty in extrapolating from animal data to humans, 10A; and uncertainty in extrapolating from a LOAEL instead of a NOAEL, 10L. See Table 5, Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment, and Risk Characterization for details.
[3]MF = Modifying factor. An additional uncertainty factor, greater than zero and less than or equal to 10, to further adjust for scientific uncertainties of the study.

Calculation of the potency factor (q^*)

Potency factors (95% upperbound) were calculated using the methods described in this document for risk characterization and the specific parameters and assumptions presented in Tables 7 and 8. The potency factor, based on the study by Norback and Weltman, is believed to more accurately represent the potency of PCBs due to various aspects of its study design which have already been discussed. A potency factor using the results of the Kimbrough study was also calculated for comparative purposes.

Table 7. Parameters, assumptions, and resulting potency factors based on Norback and Weltman (1985).

	Scenario I	Scenario II
Species	Sprague-Dawley rats	Sprague-Dawley rats
Sex	Female	Female
Material	Aroclor 1260	Aroclor 1260
Dietary Conc.	100 ppm (16 months) 50 ppm (8 months)	100 ppm (16 months) 50 ppm (8 months)
% Food Rate	5%	5%
TWA Dose Rate	3.45 mg/kg/day	3.45 mg/kg/day
Absorption	100%	100%
Length of Exposure	24 months	24 months
Duration of Study	29 months	29 months
Incidence	43/47 treated 0/49 control	45/47 treated 1/49 control
Potency	5.74 $mg/kg/day^{-1}$	7.2 $mg/kg/day^{-1}$
Comments	Carcinomas Only	Carcinomas and nodules combined

Table 8. Parameters, assumptions, and resulting potency factors based on Kimbrough et al., 1975.

	Scenario I	Scenario II
Species	Sherman strain rats	Sherman strain rats
Sex	Female	Female
Material	Aroclor 1260	Aroclor 1260
Dietary conc.	88.4 ppm (70 - 107 ppm)	88.4 ppm (70 - 107 ppm)
% Food Rate	5%	5%
TWA Dose Rate	4.42 mg/kg/day	4.42 mg/kg/day
% Absorption	100%	100%
Length of Exposure	625 days	625 days
Duration of Study	670 days	670 days
Incidence	144/184 treated 1/173 controls	170/184 treated 1/173 controls
Potency	2.49 mg/kg/day^{-1}	4.27 mg/kg/day^{-1}
Comments	Carcinomas Only	Carcinomas and nodules combined

RISK CHARACTERIZATION

Tables in the Appendix to this chapter present the upper 95% limit on cancer risk and hazard indices for each sport fish species using alternative values for meal size and meal frequency, and the average, minimum, and maximum tissue concentration in fish samples collected in 1985 and 1986. The tissue concentration data for both years were combined when they were available, or data from either year were used when a species was sampled in only one of those years (see exposure assessment section).

The estimates of cancer risk and other health hazards are for a 70 kg adult. It is important to note that children and lighter adults will have a higher PCB dose rate per meal, and are more susceptible to health problems than these estimates indicate. For example, a 13.7 kg (30 lb) toddler eating 12 quarter pound meals of brown trout (all sizes) per year, will have a PCB intake of 0.00048 mg/kg/day. This dose rate is five times higher than the dose for a 70 kg adult eating the same amount.

Hazard Indices and Contribution to Uncertainty

Table 9 shows the hazard index for each species for a meal size of 0.114 kg (0.25 lb) and a frequency of one meal per month. Hazard indices are shown based on three targets for toxicity; thyroid, liver and offspring. At this consumption rate, the hazard indices based on thyroid effects and mean tissue concentration are one or less for 10-20 inch chinook salmon, coho salmon (all sizes), and perch. The hazard index for 10-20 inch lake trout is slightly greater than one. The hazard indices based on liver toxicity are one or less for all species and sizes except for lake trout above 30 inches. The hazard indices based on effects in offspring are greater than one for all species at

this consumption level. The HI for perch is only slightly greater than one. The range in tissue concentration results in a variation in hazard indices of one to two orders of magnitude. The variability is greater for 20-30 inch lake trout, walleye, and chiook salmon larger than 30 inches.

Upper 95% Limits on Cancer Risk Estimates and Contributions to Uncertainty

An upper 95% confidence limit for potency of 7.6 mg/kg/day-1 was estimated from the Norback and Weltman study using a combined incidence of carcinomas and neoplastic nodules. The potency factor (q^*) for carcinomas alone of 5.74 mg/kg/day-1 indicates that the incidence of nodules increased the potency by 24%. A potency factor of 4.27 mg/kg/day-1 was estimated from the results of Kimbrough et al. The potency decreases by 42% to 2.49 mg/kg/day-1 when only carcinomas are considered. The 44% increase in the estimate of potency derived from the Norback and Weltman study compared to the Kimbrough et al. study in large part may be due to the longer follow-up period used in the former experiment. This was also probably a factor in the lesser contribution made by nodules to the potency estimate by the Norback and Weltman results.

The risk specific dose based on the maximum likelihood estimate (MLE), using the Norback and Weltman data for carcinomas and nodules combined, was 1.37 times higher than the risk specific dose based on the upper 95% confidence interval. The risk specific dose based on the maximum likelihood estimate, using the data for carcinomas alone, was 1.45 times higher than the risk specific dose based on the upper 95% confidence interval. The close agreement between the MLE and upper-bound estimate of potency indicates that the risk estimate is not distorted by using the upper-bound estimate. This risk estimate applies to malignant tumors reported in the animals since nearly all of the rats in the Norback and Weltman study developed carcinomas.

The potency estimates were calculated using an assumed interspecies dose equivalency based on surface area. These estimates are about six times higher than potency factors calculated based on an assumed interspecies dose equivalency based on body weight. The use of surface area is supported by the evidence suggesting that an arene oxide metabolite may be a factor in the carcinogenic ability of PCB mixtures.

The use of only one dose group by Norback and Weltman and Kimbrough et al. does not allow the evaluation of risk by mathematical models other than the linearized multistage model. The Weibull and gamma multihit models become linear when the number of stages of hits is one.

It is important to note that an additional source of uncertainty derives from the different chemico-physical characteristics of the PCB isomers present in a particular mixture. Since the relative concentrations of PCB isomers present in a fish meal will differ from the composition of isomers present in the original mixture (e.g. Aroclor 1260) and tested in animal studies, the carcinogenic potency (and general toxicity as well) of the ingested mixture may be greater or less than the potency estimate calculated in this chapter. The data on health effects and tissue concentration for specific isomers are not extensive enough to allow the quantification of this source of uncertainty.

The upper 95% limit on cancer risk resulting from the consumption of a 0.114 kg (0.25 lb) meal once a month or once a year are summarized for all sport fish species in Table 10 and 11. The upper limit for cancer using average tissue concentration for a species (all size classes) ranges from 7.7×10^{-5}

in perch to 1.3×10^{-3} in lake trout when eaten once an month for a lifetime. The upper limit for cancer ranges from 6.1×10^{-6} in perch to 1×10^{-4} in lake trout when eaten once a year for a lifetime. Fish species fall into the following rank order from lowest to highest risk; perch, walleye, coho salmon, chinook salmon, brown trout, and lake trout.

Tissue concentration data for certain species showed a rising trend in tissue concentration with increasing length. Therefore, it was possible for those species to evaluate the change in risk for different size classes. The cancer risk from lake trout consumption increases approximately an order of magnitude between 10-20 inch fish and fish longer than 30 inches. The 95% upper-bound on cancer risk for lake trout eaten once a month (Table 10) increases by 278% between 10-20 inches and 20-30 inches, and 143% between 20-30 inches and >30 inches. Brown trout show a 56% increase in risk between 10-20 inches and 20-30 inches.

The range in tissue concentration within a species causes the risk estimates to vary by approximately one order of magnitude for brown trout (all sizes). Risk estimates for walleye (all sizes) vary by approximately two orders of magnitude. Coho (all sizes) vary by three orders of magnitude with a two order magnitude difference within the 20-30 inch size class. Lake trout (all sizes) risk estimates also vary by three orders of magnitude with a variation of one and two orders of magnitude within the 10-20 inch and 20-30 inch size classes. Risk estimates for perch and chinook salmon range by four orders of magnitude. Most of the variation in risk estimates for chinook salmon due to tissue concentration variability is found in the 10-20 inch size class.

Figure 3 presents the effect of meal frequency and meal size on cancer risk estimates using brown trout as an example. For all meal sizes, the risk decreases by approximately one order of magnitude as meal frequency decreases from 12 meals per year to one meal per year. As meal frequency is decreased by one-half, risk decreases by one-half. For a constant meal frequency, risk decreases by 30% when meal size decreases from 0.34 kg to 0.228 kg, and by 50% when meal size decreases from 0.228 kg to 0.114 kg.

Tables 12, 13, and 14 present the number of meals per year that would result in an upper 95% cancer risk of 1×10^{-6}, 1×10^{-5}, and 1×10^{-4}. An upper limit risk of 1×10^{-6} is associated with the consumption of less than one meal per year of any species. An upper limit risk of 1×10^{-5} is associated with the consumption of one 0.114 kg meal of perch per year. An upper limit risk of 1×10^{-4} is associated with the consumption of one to several meals of 10-20 inch lake trout, 10-20 inch brown trout, chinook salmon less than 30 inches, walleye, coho salmon, and perch depending on the size of the meal eaten and the species selected.

FIGURE 3. Brown Trout (All Sizes)

Mean Conc. for All Fish, 1.76 ppm

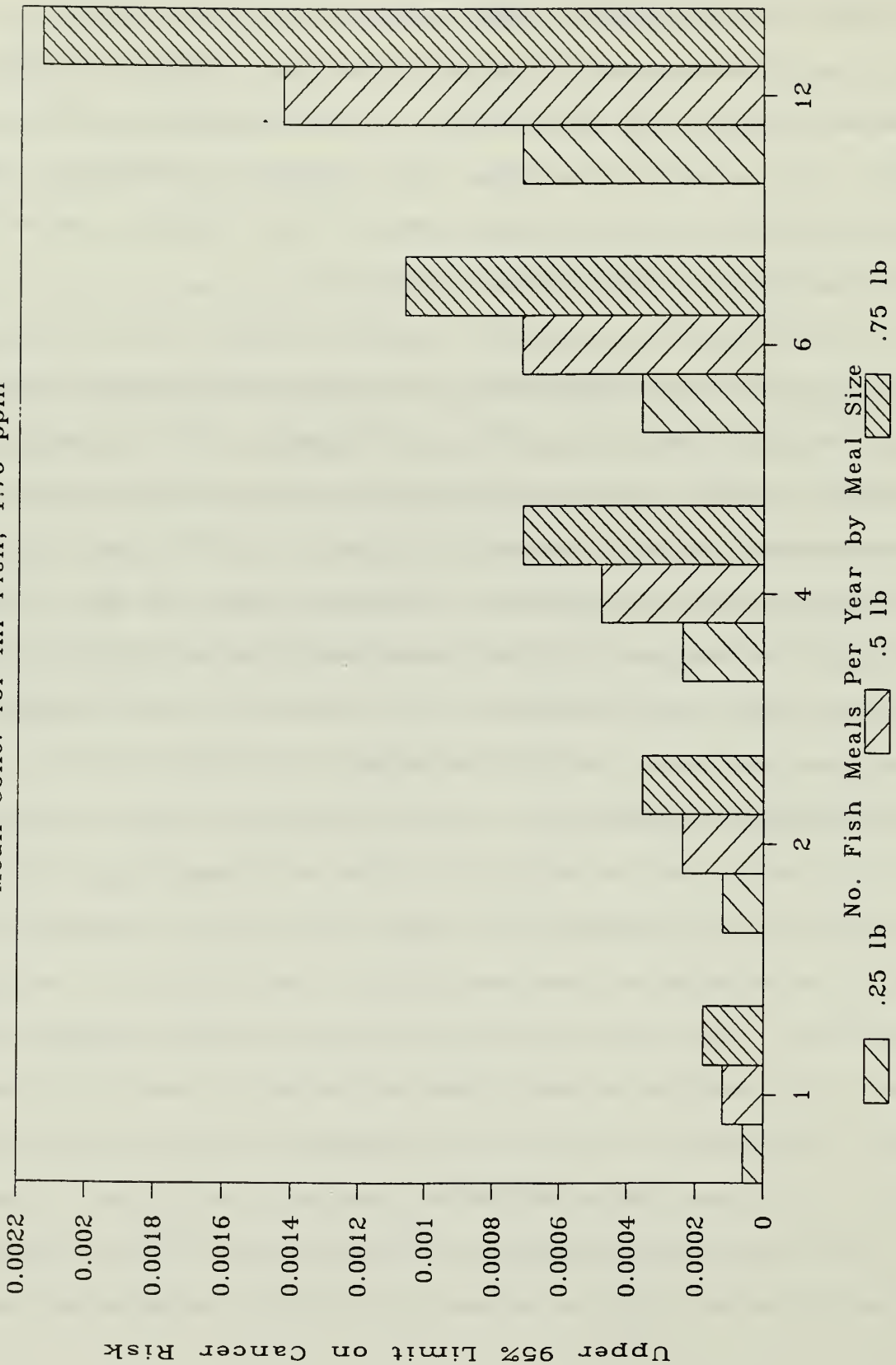


Table 9.
Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Once a Month with Meal Size 0.114 Kg (0.25 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

	HI1 Tissue Concentration		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	3.8	42.6	0.5
Lake Trout (10-20 inches)	1.1	3.3	0.5
Lake Trout (20-30 inches)	4.0	42.6	0.6
Lake Trout (>30 inches)	9.9	20.2	5.4
Brown Trout (All Sizes)	2.1	7.3	0.4
Brown Trout (10-20 inches)	1.6	4.2	0.8
Broun Trout (20-30 inches)	2.5	7.3	1.3
Chinook (All Sizes)	1.5	2.5	0.0
Chinook (10-20 inches)	0.5	1.3	0.0
Chinook (20-30 inches)	1.4	3.2	0.3
Chinook (>30 inches)	2.9	13.1	0.7
Coho (All Sizes)	1.0	3.5	0.0
Coho (20-30 inches)	0.7	3.5	0.1
Walleye (All Sizes)	0.8	14.3	0.1
Perch (All Sizes)	0.2	0.5	0.0

Table 9 continued.

HI2 Tissue Concentration			HI3 Tissue Concentration		
Mean	Max.	Min.	Mean	Max.	Min.
0.9	10.6	0.1	18.9	212.9	2.6
0.3	0.8	0.1	5.4	16.7	2.6
1.0	10.6	0.2	20.2	212.9	3.0
2.5	5.1	1.3	49.4	101.1	26.8
0.5	1.8	0.1	10.5	36.3	2.0
0.4	1.0	0.2	8.0	20.8	3.9
0.6	1.8	0.3	12.6	36.3	6.3
0.4	0.6	0.0	7.3	12.6	0.0
0.1	0.3	0.0	2.3	6.5	0.0
0.3	0.8	0.1	7.0	16.1	1.5
0.7	3.3	0.2	14.5	65.4	3.4
0.2	0.9	0.0	4.9	17.3	0.0
0.2	0.9	0.0	3.3	17.3	0.7
0.2	3.6	0.0	4.0	71.4	0.4
0.1	0.1	0.0	1.1	2.4	0.0

Table 10.
Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Month with Meal Size 0.114 Kg (0.25 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

Species	Size Class	Upper Limit (95%) on Risk — Tissue Concentration: Mean	Max.	Min.
Lake Trout	(all sizes)	1.3E-03	1.5E-02	7.3E-05
Lake Trout	(10-20 in)	3.7E-04	1.1E-03	1.7E-04
Lake Trout	(20-30 in)	1.4E-03	1.5E-02	2.1E-04
Lake Trout	(>30 inches)	3.4E-03	6.9E-03	1.8E-03
Brown Trout	(All Sizes)	7.2E-04	2.5E-03	1.4E-04
Brown Trout	(10-20 in)	5.5E-04	1.4E-03	2.6E-04
Brown Trout	(20-30 in)	8.6E-04	2.5E-03	4.3E-04
Chinook	(All Sizes)	5.0E-04	8.6E-04	0.0E+00
Chinook	(10-20 inches)	1.6E-04	4.5E-04	0.0E+00
Chinook	(20-30 inches)	4.8E-04	1.1E-03	1.1E-04
Chinook	(>30 inches)	9.9E-04	4.5E-03	2.3E-04
Coho	(All Sizes)	3.4E-04	1.2E-03	0.0E+00
Coho	(20-30 inches)	2.3E-04	1.2E-03	4.9E-05
Walleye	(All Sizes)	2.7E-04	4.9E-03	2.4E-05
Perch	(All Sizes)	7.7E-05	1.6E-04	0.0E+00

Table 11.
Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Year With Meal Size 0.114 Kg (0.25 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk		
	Tissue Concentration		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	1.0E-04	1.1E-03	5.8E-06
Lake Trout (10-20 inches)	2.9E-05	9.0E-05	1.4E-05
Lake Trout (20-30 inches)	1.1E-04	1.1E-03	1.6E-05
Lake Trout (>30 inches)	2.7E-04	5.5E-04	1.4E-04
Brown Trout (All Sizes)	5.7E-05	2.0E-04	1.1E-05
Brown Trout (10-20 inches)	4.3E-05	1.1E-04	2.1E-05
Brown Trout (20-30 inches)	4.3E-05	1.1E-04	2.1E-05
Chinook (All Sizes)	4.0E-05	6.8E-05	0.0E+00
Chinook (10-20 inches)	1.3E-05	3.5E-05	0.0E+00
Chinook (20-30 inches)	3.8E-05	8.7E-05	8.4E-06
Chinook (>30 inches)	7.8E-05	3.5E-04	1.8E-05
Coho (All Sizes)	2.7E-05	9.3E-05	0.0E+00
Coho (20-30 inches)	1.8E-05	9.3E-05	3.9E-06
Walleye (All Sizes)	2.3E-05	3.9E-04	1.9E-06
Perch (All Sizes)	6.1E-06	1.3E-05	0.0E+00

Table 12.
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-6, 1E-5, or 1E-4 For a Meal Size of 0.114 kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

	Meal Frequency (/Yr)		
Species (Size Class)	Based on 1.0E-06 Risk	Based on 1.0E-05 Risk	Based on 1.0E-04 Risk
Lake Trout (All Sizes)	0.01	0.09	0.93
Lake Trout (10-20 in)	0.03	0.32	3.22
Lake Trout (20-30 in)	0.01	0.09	0.87
Lake Trout (>30 in)	0.00	0.04	0.36
Brown Trout (All Sizes)	0.02	0.17	1.68
Brown Trout (10-20 in)	0.02	0.22	2.19
Brown Trout (20-30 in)	0.01	0.14	1.39
Chinook (All Sizes)	0.02	0.24	2.40
Chinook (10-20 in)	0.08	0.76	7.57
Chinook (20-30 in)	0.03	0.25	2.52
Chinook (>30 in)	0.01	0.12	1.22
Coho (All Sizes)	0.04	0.36	3.56
Coho (20-30 in)	0.05	0.53	5.27
Walleye (All Sizes)	0.04	0.44	4.41
Perch (All Sizes)	0.16	1.55	15.55

Table 13.
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-6, 1E-5, or 1E-4 For a Meal Size of 0.228 Kg For Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

	Meal Frequency (/Yr)		
Species (Size Class)	Based on 1.0E-06 Risk	Based on 1.0E-05 Risk	Based on 1.0E-04 Risk
Lake Trout (All Sizes)	0.00	0.05	0.47
Lake Trout (10-20 in)	0.02	0.16	1.61
Lake Trout (20-30 in)	0.00	0.04	0.43
Lake Trout (>30 in)	0.00	0.02	0.18
Brown Trout (All Sizes)	0.01	0.08	0.84
Brown Trout (10-20 in)	0.01	0.11	1.09
Brown Trout (20-30 in)	0.01	0.07	0.70
Chinook (All Sizes)	0.01	0.12	1.20
Chinook (10-20 in)	0.04	0.38	3.79
Chinook (20-30 in)	0.01	0.13	1.26
Chinook (>30 in)	0.01	0.06	0.61
Coho (All Sizes)	0.02	0.18	1.78
Coho (20-30 in)	0.03	0.26	2.64
Walleye (All Sizes)	0.02	0.22	2.20
Perch (All Sizes)	0.08	0.78	7.77

Table 14 .
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-6, 1E-5, or 1E-4 For a Meal Size of 0.34 Kg For Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr) Based on 1.0E-06 Risk	Based on 1.0E-05 Risk	Based on 1.0E-04 Risk
Lake Trout (All Sizes)	0.00	0.03	0.31
Lake Trout (10-20 in)	0.01	0.11	1.08
Lake Trout (20-30 in)	0.00	0.03	0.29
Lake Trout (>30 in)	0.00	0.01	0.12
Brown Trout (All Sizes)	0.01	0.06	0.56
Brown Trout (10-20 in)	0.01	0.07	0.73
Brown Trout (20-30 in)	0.00	0.05	0.47
Chinook (All Sizes)	0.01	0.08	0.81
Chinook (10-20 in)	0.03	0.25	2.54
Chinook (20-30 in)	0.01	0.08	0.85
Chinook (>30 in)	0.00	0.04	0.41
Coho (All Sizes)	0.01	0.12	1.19
Coho (20-30 in)	0.02	0.18	1.77
Walleye (All Sizes)	0.01	0.15	1.48
Perch (All Sizes)	0.05	0.52	5.21

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CHAPTER FOUR APPENDIX

Brown Trout (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	1.76
Max.	6.1
Min.	0.34
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000008	0.000027	0.000002
0.114	2	0.000625	0.000016	0.000054	0.000003
0.114	4	0.001249	0.000031	0.000109	0.000006
0.114	6	0.001874	0.000047	0.000163	0.000009
0.114	12	0.003748	0.000094	0.000327	0.000018
0.114	24	0.007496	0.000188	0.000653	0.000036
0.114	52	0.016241	0.000408	0.001415	0.000079
0.114	104	0.032482	0.000817	0.002831	0.000158
0.114	156	0.048723	0.001225	0.004246	0.000237
0.228	1	0.000625	0.000016	0.000054	0.000003
0.228	2	0.001249	0.000031	0.000109	0.000006
0.228	4	0.002499	0.000063	0.000218	0.000012
0.228	6	0.003748	0.000094	0.000327	0.000018
0.228	12	0.007496	0.000188	0.000653	0.000036
0.228	24	0.014992	0.000377	0.001306	0.000073
0.228	52	0.032482	0.000817	0.002831	0.000158
0.228	104	0.064964	0.001633	0.005661	0.000316
0.228	156	0.097447	0.002450	0.008492	0.000473
0.34	1	0.000932	0.000023	0.000081	0.000005
0.34	2	0.001863	0.000047	0.000162	0.000009
0.34	4	0.003726	0.000094	0.000325	0.000018
0.34	6	0.005589	0.000141	0.000487	0.000027
0.34	12	0.011178	0.000281	0.000974	0.000054
0.34	24	0.022356	0.000562	0.001948	0.000109
0.34	52	0.048438	0.001218	0.004221	0.000235
0.34	104	0.096877	0.002436	0.008442	0.000471
0.34	156	0.145315	0.003654	0.012663	0.000706

Brown Trout (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
6.0E-05	2.1E-04	1.2E-05	3.4E-05	1.2E-04	6.5E-06
1.2E-04	4.1E-04	2.3E-05	6.7E-05	2.3E-04	1.3E-05
2.4E-04	8.3E-04	4.6E-05	1.3E-04	4.6E-04	2.6E-05
3.6E-04	1.2E-03	6.9E-05	2.0E-04	7.0E-04	3.9E-05
7.2E-04	2.5E-03	1.4E-04	4.0E-04	1.4E-03	7.8E-05
1.4E-03	5.0E-03	2.8E-04	8.0E-04	2.8E-03	1.6E-04
3.1E-03	1.1E-02	6.0E-04	1.7E-03	6.0E-03	3.4E-04
6.2E-03	2.2E-02	1.2E-03	3.5E-03	1.2E-02	6.7E-04
9.3E-03	3.2E-02	1.8E-03	5.2E-03	1.8E-02	1.0E-03
1.2E-04	4.1E-04	2.3E-05	6.7E-05	2.3E-04	1.3E-05
2.4E-04	8.3E-04	4.6E-05	1.3E-04	4.6E-04	2.6E-05
4.8E-04	1.7E-03	9.2E-05	2.7E-04	9.3E-04	5.2E-05
7.2E-04	2.5E-03	1.4E-04	4.0E-04	1.4E-03	7.8E-05
1.4E-03	5.0E-03	2.8E-04	8.0E-04	2.8E-03	1.6E-04
2.9E-03	9.9E-03	5.5E-04	1.6E-03	5.6E-03	3.1E-04
6.2E-03	2.2E-02	1.2E-03	3.5E-03	1.2E-02	6.7E-04
1.2E-02	4.3E-02	2.4E-03	7.0E-03	2.4E-02	1.3E-03
1.9E-02	6.5E-02	3.6E-03	1.0E-02	3.6E-02	2.0E-03
1.8E-04	6.2E-04	3.4E-05	1.0E-04	3.5E-04	1.9E-05
3.6E-04	1.2E-03	6.9E-05	2.0E-04	6.9E-04	3.9E-05
7.1E-04	2.5E-03	1.4E-04	4.0E-04	1.4E-03	7.7E-05
1.1E-03	3.7E-03	2.1E-04	6.0E-04	2.1E-03	1.2E-04
2.1E-03	7.4E-03	4.1E-04	1.2E-03	4.2E-03	2.3E-04
4.3E-03	1.5E-02	8.3E-04	2.4E-03	8.3E-03	4.6E-04
9.3E-03	3.2E-02	1.8E-03	5.2E-03	1.8E-02	1.0E-03
1.9E-02	6.4E-02	3.6E-03	1.0E-02	3.6E-02	2.0E-03
2.8E-02	9.6E-02	5.4E-03	1.6E-02	5.4E-02	3.0E-03

Brown Trout (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.2	0.6	0.0	0.0	0.2	0.0
0.3	1.2	0.1	0.1	0.3	0.0
0.7	2.4	0.1	0.2	0.6	0.0
1.0	3.6	0.2	0.3	0.9	0.1
2.1	7.3	0.4	0.5	1.8	0.1
4.2	14.5	0.8	1.0	3.6	0.2
9.1	31.5	1.8	2.3	7.9	0.4
18.1	62.9	3.5	4.5	15.7	0.9
27.2	94.4	5.3	6.8	23.6	1.3
0.3	1.2	0.1	0.1	0.3	0.0
0.7	2.4	0.1	0.2	0.6	0.0
1.4	4.8	0.3	0.3	1.2	0.1
2.1	7.3	0.4	0.5	1.8	0.1
4.2	14.5	0.8	1.0	3.6	0.2
8.4	29.0	1.6	2.1	7.3	0.4
18.1	62.9	3.5	4.5	15.7	0.9
36.3	125.8	7.0	9.1	31.5	1.8
54.4	188.7	10.5	13.6	47.2	2.6
0.5	1.8	0.1	0.1	0.5	0.0
1.0	3.6	0.2	0.3	0.9	0.1
2.1	7.2	0.4	0.5	1.8	0.1
3.1	10.8	0.6	0.8	2.7	0.2
6.2	21.6	1.2	1.6	5.4	0.3
12.5	43.3	2.4	3.1	10.8	0.6
27.1	93.8	5.2	6.8	23.5	1.3
54.1	187.6	10.5	13.5	46.9	2.6
81.2	281.4	15.7	20.3	70.4	3.9

Brown Trout (All Sizes)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.9	3.0	0.2
1.7	6.0	0.3
3.5	12.1	0.7
5.2	18.1	1.0
10.5	36.3	2.0
20.9	72.6	4.0
45.4	157.3	8.8
90.7	314.5	17.5
136.1	471.8	26.3
1.7	6.0	0.3
3.5	12.1	0.7
7.0	24.2	1.3
10.5	36.3	2.0
20.9	72.6	4.0
41.9	145.2	8.1
90.7	314.5	17.5
181.5	629.0	35.1
272.2	943.5	52.6
2.6	9.0	0.5
5.2	18.0	1.0
10.4	36.1	2.0
15.6	54.1	3.0
31.2	108.2	6.0
62.5	216.5	12.1
135.3	469.0	26.1
270.6	938.0	52.3
406.0	1407.0	78.4

Brown Trout (10-20 inches)

Tissue Concentration (85 + 86)	ppm
Mean	1.35
Max.	3.5
Min.	0.65
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000006	0.000016	0.000003
0.114	2	0.000625	0.000012	0.000031	0.000006
0.114	4	0.001249	0.000024	0.000062	0.000012
0.114	6	0.001874	0.000036	0.000094	0.000017
0.114	12	0.003748	0.000072	0.000187	0.000035
0.114	24	0.007496	0.000145	0.000375	0.000070
0.114	52	0.016241	0.000313	0.000812	0.000151
0.114	104	0.032482	0.000626	0.001624	0.000302
0.114	156	0.048723	0.000940	0.002436	0.000452
0.228	1	0.000625	0.000012	0.000031	0.000006
0.228	2	0.001249	0.000024	0.000062	0.000012
0.228	4	0.002499	0.000048	0.000125	0.000023
0.228	6	0.003748	0.000072	0.000187	0.000035
0.228	12	0.007496	0.000145	0.000375	0.000070
0.228	24	0.014992	0.000289	0.000750	0.000139
0.228	52	0.032482	0.000626	0.001624	0.000302
0.228	104	0.064964	0.001253	0.003248	0.000603
0.228	156	0.097447	0.001879	0.004872	0.000905
0.34	1	0.000932	0.000018	0.000047	0.000009
0.34	2	0.001863	0.000036	0.000093	0.000017
0.34	4	0.003726	0.000072	0.000186	0.000035
0.34	6	0.005589	0.000108	0.000279	0.000052
0.34	12	0.011178	0.000216	0.000559	0.000104
0.34	24	0.022356	0.000431	0.001118	0.000208
0.34	52	0.048438	0.000934	0.002422	0.000450
0.34	104	0.096877	0.001868	0.004844	0.000900
0.34	156	0.145315	0.002803	0.007266	0.001349

Brown Trout (10-20 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
4.6E-05	1.2E-04	2.2E-05	2.6E-05	6.7E-05	1.2E-05
9.2E-05	2.4E-04	4.4E-05	5.1E-05	1.3E-04	2.5E-05
1.8E-04	4.7E-04	8.8E-05	1.0E-04	2.7E-04	5.0E-05
2.7E-04	7.1E-04	1.3E-04	1.5E-04	4.0E-04	7.4E-05
5.5E-04	1.4E-03	2.6E-04	3.1E-04	8.0E-04	1.5E-04
1.1E-03	2.8E-03	5.3E-04	6.2E-04	1.6E-03	3.0E-04
2.4E-03	6.2E-03	1.1E-03	1.3E-03	3.5E-03	6.4E-04
4.8E-03	1.2E-02	2.3E-03	2.7E-03	6.9E-03	1.3E-03
7.1E-03	1.9E-02	3.4E-03	4.0E-03	1.0E-02	1.9E-03
9.2E-05	2.4E-04	4.4E-05	5.1E-05	1.3E-04	2.5E-05
1.8E-04	4.7E-04	8.8E-05	1.0E-04	2.7E-04	5.0E-05
3.7E-04	9.5E-04	1.8E-04	2.1E-04	5.3E-04	9.9E-05
5.5E-04	1.4E-03	2.6E-04	3.1E-04	8.0E-04	1.5E-04
1.1E-03	2.8E-03	5.3E-04	6.2E-04	1.6E-03	3.0E-04
2.2E-03	5.7E-03	1.1E-03	1.2E-03	3.2E-03	5.9E-04
4.8E-03	1.2E-02	2.3E-03	2.7E-03	6.9E-03	1.3E-03
9.5E-03	2.5E-02	4.6E-03	5.3E-03	1.4E-02	2.6E-03
1.4E-02	3.7E-02	6.9E-03	8.0E-03	2.1E-02	3.9E-03
1.4E-04	3.5E-04	6.6E-05	7.7E-05	2.0E-04	3.7E-05
2.7E-04	7.1E-04	1.3E-04	1.5E-04	4.0E-04	7.4E-05
5.5E-04	1.4E-03	2.6E-04	3.1E-04	8.0E-04	1.5E-04
8.2E-04	2.1E-03	3.9E-04	4.6E-04	1.2E-03	2.2E-04
1.6E-03	4.2E-03	7.9E-04	9.2E-04	2.4E-03	4.4E-04
3.3E-03	8.5E-03	1.6E-03	1.8E-03	4.8E-03	8.9E-04
7.1E-03	1.8E-02	3.4E-03	4.0E-03	1.0E-02	1.9E-03
1.4E-02	3.7E-02	6.8E-03	8.0E-03	2.1E-02	3.8E-03
2.1E-02	5.5E-02	1.0E-02	1.2E-02	3.1E-02	5.8E-03

Brown Trout (10-20 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.13	0.35	0.06	0.03	0.09	0.02
0.27	0.69	0.13	0.07	0.17	0.03
0.54	1.39	0.26	0.13	0.35	0.06
0.80	2.08	0.39	0.20	0.52	0.10
1.61	4.16	0.77	0.40	1.04	0.19
3.21	8.33	1.55	0.80	2.08	0.39
6.96	18.05	3.35	1.74	4.51	0.84
13.92	36.09	6.70	3.48	9.02	1.68
20.88	54.14	10.05	5.22	13.53	2.51
0.27	0.69	0.13	0.07	0.17	0.03
0.54	1.39	0.26	0.13	0.35	0.06
1.07	2.78	0.52	0.27	0.69	0.13
1.61	4.16	0.77	0.40	1.04	0.19
3.21	8.33	1.55	0.80	2.08	0.39
6.43	16.66	3.09	1.61	4.16	0.77
13.92	36.09	6.70	3.48	9.02	1.68
27.84	72.18	13.41	6.96	18.05	3.35
41.76	108.27	20.11	10.44	27.07	5.03
0.40	1.04	0.19	0.10	0.26	0.05
0.80	2.07	0.38	0.20	0.52	0.10
1.60	4.14	0.77	0.40	1.04	0.19
2.40	6.21	1.15	0.60	1.55	0.29
4.79	12.42	2.31	1.20	3.11	0.58
9.58	24.84	4.61	2.40	6.21	1.15
20.76	53.82	10.00	5.19	13.46	2.50
41.52	107.64	19.99	10.38	26.91	5.00
62.28	161.46	29.99	15.57	40.37	7.50

Brown Trout (10-20 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.67	1.74	0.32
1.34	3.47	0.64
2.68	6.94	1.29
4.02	10.41	1.93
8.03	20.82	3.87
16.06	41.64	7.73
34.80	90.23	16.76
69.60	180.46	33.51
104.41	270.68	50.27
1.34	3.47	0.64
2.68	6.94	1.29
5.35	13.88	2.58
8.03	20.82	3.87
16.06	41.64	7.73
32.13	83.29	15.47
69.60	180.46	33.51
139.21	360.91	67.03
208.81	541.37	100.54
2.00	5.18	0.96
3.99	10.35	1.92
7.98	20.70	3.84
11.98	31.05	5.77
23.95	62.10	11.53
47.91	124.20	23.07
103.80	269.10	49.98
207.59	538.20	99.95
311.39	807.31	149.93

Brown Trout (20-30 inches)

Tissue Concentration (85 + 86)	ppm
Mean	2.12
Max.	6.1
Min.	1.06
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000009	0.000027	0.000005
0.114	2	0.000625	0.000019	0.000054	0.000009
0.114	4	0.001249	0.000038	0.000109	0.000019
0.114	6	0.001874	0.000057	0.000163	0.000028
0.114	12	0.003748	0.000114	0.000327	0.000057
0.114	24	0.007496	0.000227	0.000653	0.000114
0.114	52	0.016241	0.000492	0.001415	0.000246
0.114	104	0.032482	0.000984	0.002831	0.000492
0.114	156	0.048723	0.001476	0.004246	0.000738
0.228	1	0.000625	0.000019	0.000054	0.000009
0.228	2	0.001249	0.000038	0.000109	0.000019
0.228	4	0.002499	0.000076	0.000218	0.000038
0.228	6	0.003748	0.000114	0.000327	0.000057
0.228	12	0.007496	0.000227	0.000653	0.000114
0.228	24	0.014992	0.000454	0.001306	0.000227
0.228	52	0.032482	0.000984	0.002831	0.000492
0.228	104	0.064964	0.001967	0.005661	0.000984
0.228	156	0.097447	0.002951	0.008492	0.001476
0.34	1	0.000932	0.000028	0.000081	0.000014
0.34	2	0.001863	0.000056	0.000162	0.000028
0.34	4	0.003726	0.000113	0.000325	0.000056
0.34	6	0.005589	0.000169	0.000487	0.000085
0.34	12	0.011178	0.000339	0.000974	0.000169
0.34	24	0.022356	0.000677	0.001948	0.000339
0.34	52	0.048438	0.001467	0.004221	0.000733
0.34	104	0.096877	0.002934	0.008442	0.001467
0.34	156	0.145315	0.004401	0.012663	0.002200

Brown Trout (20-30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
7.2E-05	2.1E-04	3.6E-05	4.0E-05	1.2E-04	2.0E-05
1.4E-04	4.1E-04	7.2E-05	8.1E-05	2.3E-04	4.0E-05
2.9E-04	8.3E-04	1.4E-04	1.6E-04	4.6E-04	8.1E-05
4.3E-04	1.2E-03	2.2E-04	2.4E-04	7.0E-04	1.2E-04
8.6E-04	2.5E-03	4.3E-04	4.8E-04	1.4E-03	2.4E-04
1.7E-03	5.0E-03	8.6E-04	9.7E-04	2.8E-03	4.8E-04
3.7E-03	1.1E-02	1.9E-03	2.1E-03	6.0E-03	1.1E-03
7.5E-03	2.2E-02	3.7E-03	4.2E-03	1.2E-02	2.1E-03
1.1E-02	3.2E-02	5.6E-03	6.3E-03	1.8E-02	3.2E-03
1.4E-04	4.1E-04	7.2E-05	8.1E-05	2.3E-04	4.0E-05
2.9E-04	8.3E-04	1.4E-04	1.6E-04	4.6E-04	8.1E-05
5.8E-04	1.7E-03	2.9E-04	3.2E-04	9.3E-04	1.6E-04
8.6E-04	2.5E-03	4.3E-04	4.8E-04	1.4E-03	2.4E-04
1.7E-03	5.0E-03	8.6E-04	9.7E-04	2.8E-03	4.8E-04
3.5E-03	9.9E-03	1.7E-03	1.9E-03	5.6E-03	9.7E-04
7.5E-03	2.2E-02	3.7E-03	4.2E-03	1.2E-02	2.1E-03
1.5E-02	4.3E-02	7.5E-03	8.4E-03	2.4E-02	4.2E-03
2.2E-02	6.5E-02	1.1E-02	1.3E-02	3.6E-02	6.3E-03
2.1E-04	6.2E-04	1.1E-04	1.2E-04	3.5E-04	6.0E-05
4.3E-04	1.2E-03	2.1E-04	2.4E-04	6.9E-04	1.2E-04
8.6E-04	2.5E-03	4.3E-04	4.8E-04	1.4E-03	2.4E-04
1.3E-03	3.7E-03	6.4E-04	7.2E-04	2.1E-03	3.6E-04
2.6E-03	7.4E-03	1.3E-03	1.4E-03	4.2E-03	7.2E-04
5.1E-03	1.5E-02	2.6E-03	2.9E-03	8.3E-03	1.4E-03
1.1E-02	3.2E-02	5.6E-03	6.3E-03	1.8E-02	3.1E-03
2.2E-02	6.4E-02	1.1E-02	1.3E-02	3.6E-02	6.3E-03
3.3E-02	9.6E-02	1.7E-02	1.9E-02	5.4E-02	9.4E-03

Brown Trout (20-30 inches)

Hazard Index RfD = 0.000045 Thyroid			Hazard Index RfD = 0.00018 Liver		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
0.21	0.60	0.11	0.05	0.15	0.03
0.42	1.21	0.21	0.11	0.30	0.05
0.84	2.42	0.42	0.21	0.60	0.11
1.26	3.63	0.63	0.32	0.91	0.16
2.52	7.26	1.26	0.63	1.81	0.32
5.04	14.52	2.52	1.26	3.63	0.63
10.93	31.45	5.47	2.73	7.86	1.37
21.86	62.90	10.93	5.47	15.73	2.73
32.79	94.35	16.40	8.20	23.59	4.10
0.42	1.21	0.21	0.11	0.30	0.05
0.84	2.42	0.42	0.21	0.60	0.11
1.68	4.84	0.84	0.42	1.21	0.21
2.52	7.26	1.26	0.63	1.81	0.32
5.04	14.52	2.52	1.26	3.63	0.63
10.09	29.03	5.04	2.52	7.26	1.26
21.86	62.90	10.93	5.47	15.73	2.73
43.72	125.80	21.86	10.93	31.45	5.47
65.58	188.71	32.79	16.40	47.18	8.20
0.63	1.80	0.31	0.16	0.45	0.08
1.25	3.61	0.63	0.31	0.90	0.16
2.51	7.22	1.25	0.63	1.80	0.31
3.76	10.82	1.88	0.94	2.71	0.47
7.52	21.65	3.76	1.88	5.41	0.94
15.05	43.29	7.52	3.76	10.82	1.88
32.60	93.80	16.30	8.15	23.45	4.07
65.20	187.60	32.60	16.30	46.90	8.15
97.80	281.40	48.90	24.45	70.35	12.22

Brown Trout (20-30 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
1.05	3.02	0.53
2.10	6.05	1.05
4.20	12.10	2.10
6.31	18.14	3.15
12.61	36.29	6.31
25.22	72.58	12.61
54.65	157.26	27.33
109.31	314.51	54.65
163.96	471.77	81.98
2.10	6.05	1.05
4.20	12.10	2.10
8.41	24.19	4.20
12.61	36.29	6.31
25.22	72.58	12.61
50.45	145.16	25.22
109.31	314.51	54.65
218.61	629.02	109.31
327.92	943.53	163.96
3.13	9.02	1.57
6.27	18.04	3.13
12.54	36.08	6.27
18.81	54.12	9.40
37.62	108.23	18.81
75.23	216.46	37.62
163.00	469.01	81.50
326.00	938.01	163.00
489.00	1407.02	244.50

Chinook (All Sizes)

Tissue Concentration (85 + 86)	ppm
Mean	1.23
Max.	11
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000005	0.000049	0.000000
0.114	2	0.000625	0.000011	0.000019	0.000000
0.114	4	0.001249	0.000022	0.000038	0.000000
0.114	6	0.001874	0.000033	0.000056	0.000000
0.114	12	0.003748	0.000066	0.000113	0.000000
0.114	24	0.007496	0.000132	0.000226	0.000000
0.114	52	0.016241	0.000285	0.000489	0.000000
0.114	104	0.032482	0.000571	0.000979	0.000000
0.114	156	0.048723	0.000856	0.001468	0.000000
0.228	1	0.000625	0.000011	0.000019	0.000000
0.228	2	0.001249	0.000022	0.000038	0.000000
0.228	4	0.002499	0.000044	0.000075	0.000000
0.228	6	0.003748	0.000066	0.000113	0.000000
0.228	12	0.007496	0.000132	0.000226	0.000000
0.228	24	0.014992	0.000263	0.000452	0.000000
0.228	52	0.032482	0.000571	0.000979	0.000000
0.228	104	0.064964	0.001142	0.001958	0.000000
0.228	156	0.097447	0.001712	0.002937	0.000000
0.34	1	0.000932	0.000016	0.000028	0.000000
0.34	2	0.001863	0.000033	0.000056	0.000000
0.34	4	0.003726	0.000065	0.000112	0.000000
0.34	6	0.005589	0.000098	0.000168	0.000000
0.34	12	0.011178	0.000196	0.000337	0.000000
0.34	24	0.022356	0.000393	0.000674	0.000000
0.34	52	0.048438	0.000851	0.001460	0.000000
0.34	104	0.096877	0.001702	0.002920	0.000000
0.34	156	0.145315	0.002553	0.004379	0.000000

Chinook (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.2E-05	3.7E-04	0.0E+00	2.3E-05	2.1E-04	0.0E+00
8.3E-05	1.4E-04	0.0E+00	4.7E-05	8.0E-05	0.0E+00
1.7E-04	2.9E-04	0.0E+00	9.4E-05	1.6E-04	0.0E+00
2.5E-04	4.3E-04	0.0E+00	1.4E-04	2.4E-04	0.0E+00
5.0E-04	8.6E-04	0.0E+00	2.8E-04	4.8E-04	0.0E+00
1.0E-03	1.7E-03	0.0E+00	5.6E-04	9.6E-04	0.0E+00
2.2E-03	3.7E-03	0.0E+00	1.2E-03	2.1E-03	0.0E+00
4.3E-03	7.4E-03	0.0E+00	2.4E-03	4.2E-03	0.0E+00
6.5E-03	1.1E-02	0.0E+00	3.7E-03	6.3E-03	0.0E+00
8.3E-05	1.4E-04	0.0E+00	4.7E-05	8.0E-05	0.0E+00
1.7E-04	2.9E-04	0.0E+00	9.4E-05	1.6E-04	0.0E+00
3.3E-04	5.7E-04	0.0E+00	1.9E-04	3.2E-04	0.0E+00
5.0E-04	8.6E-04	0.0E+00	2.8E-04	4.8E-04	0.0E+00
1.0E-03	1.7E-03	0.0E+00	5.6E-04	9.6E-04	0.0E+00
2.0E-03	3.4E-03	0.0E+00	1.1E-03	1.9E-03	0.0E+00
4.3E-03	7.4E-03	0.0E+00	2.4E-03	4.2E-03	0.0E+00
8.7E-03	1.5E-02	0.0E+00	4.9E-03	8.4E-03	0.0E+00
1.3E-02	2.2E-02	0.0E+00	7.3E-03	1.3E-02	0.0E+00
1.2E-04	2.1E-04	0.0E+00	7.0E-05	1.2E-04	0.0E+00
2.5E-04	4.3E-04	0.0E+00	1.4E-04	2.4E-04	0.0E+00
5.0E-04	8.5E-04	0.0E+00	2.8E-04	4.8E-04	0.0E+00
7.5E-04	1.3E-03	0.0E+00	4.2E-04	7.2E-04	0.0E+00
1.5E-03	2.6E-03	0.0E+00	8.4E-04	1.4E-03	0.0E+00
3.0E-03	5.1E-03	0.0E+00	1.7E-03	2.9E-03	0.0E+00
6.5E-03	1.1E-02	0.0E+00	3.6E-03	6.2E-03	0.0E+00
1.3E-02	2.2E-02	0.0E+00	7.3E-03	1.2E-02	0.0E+00
1.9E-02	3.3E-02	0.0E+00	1.1E-02	1.9E-02	0.0E+00

Chinook (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.12	1.09	0.00	0.03	0.27	0.00
0.24	0.42	0.00	0.06	0.10	0.00
0.49	0.84	0.00	0.12	0.21	0.00
0.73	1.26	0.00	0.18	0.31	0.00
1.46	2.51	0.00	0.37	0.63	0.00
2.93	5.02	0.00	0.73	1.26	0.00
6.34	10.88	0.00	1.59	2.72	0.00
12.68	21.75	0.00	3.17	5.44	0.00
19.03	32.63	0.00	4.76	8.16	0.00
0.24	0.42	0.00	0.06	0.10	0.00
0.49	0.84	0.00	0.12	0.21	0.00
0.98	1.67	0.00	0.24	0.42	0.00
1.46	2.51	0.00	0.37	0.63	0.00
2.93	5.02	0.00	0.73	1.26	0.00
5.85	10.04	0.00	1.46	2.51	0.00
12.68	21.75	0.00	3.17	5.44	0.00
25.37	43.51	0.00	6.34	10.88	0.00
38.05	65.26	0.00	9.51	16.32	0.00
0.36	0.62	0.00	0.09	0.16	0.00
0.73	1.25	0.00	0.18	0.31	0.00
1.45	2.50	0.00	0.36	0.62	0.00
2.18	3.74	0.00	0.55	0.94	0.00
4.36	7.49	0.00	1.09	1.87	0.00
8.73	14.97	0.00	2.18	3.74	0.00
18.91	32.44	0.00	4.73	8.11	0.00
37.83	64.88	0.00	9.46	16.22	0.00
56.74	97.32	0.00	14.19	24.33	0.00

Chinook (All Sizes)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.61	5.45	0.00
1.22	2.09	0.00
2.44	4.18	0.00
3.66	6.28	0.00
7.32	12.55	0.00
14.63	25.10	0.00
31.71	54.38	0.00
63.42	108.77	0.00
95.13	163.15	0.00
1.22	2.09	0.00
2.44	4.18	0.00
4.88	8.37	0.00
7.32	12.55	0.00
14.63	25.10	0.00
29.27	50.20	0.00
63.42	108.77	0.00
126.84	217.54	0.00
190.25	326.31	0.00
1.82	3.12	0.00
3.64	6.24	0.00
7.27	12.48	0.00
10.91	18.72	0.00
21.82	37.43	0.00
43.65	74.86	0.00
94.57	162.20	0.00
189.14	324.40	0.00
283.71	486.60	0.00

Chinook (10-20 inches)

Tissue Concentration (85 + 86)	ppm
Mean	0.39
Max.	1.1
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000002	0.000005	0.000000
0.114	2	0.000625	0.000003	0.000010	0.000000
0.114	4	0.001249	0.000007	0.000020	0.000000
0.114	6	0.001874	0.000010	0.000029	0.000000
0.114	12	0.003748	0.000021	0.000059	0.000000
0.114	24	0.007496	0.000042	0.000118	0.000000
0.114	52	0.016241	0.000090	0.000255	0.000000
0.114	104	0.032482	0.000181	0.000510	0.000000
0.114	156	0.048723	0.000271	0.000766	0.000000
0.228	1	0.000625	0.000003	0.000010	0.000000
0.228	2	0.001249	0.000007	0.000020	0.000000
0.228	4	0.002499	0.000014	0.000039	0.000000
0.228	6	0.003748	0.000021	0.000059	0.000000
0.228	12	0.007496	0.000042	0.000118	0.000000
0.228	24	0.014992	0.000084	0.000236	0.000000
0.228	52	0.032482	0.000181	0.000510	0.000000
0.228	104	0.064964	0.000362	0.001021	0.000000
0.228	156	0.097447	0.000543	0.001531	0.000000
0.34	1	0.000932	0.000005	0.000015	0.000000
0.34	2	0.001863	0.000010	0.000029	0.000000
0.34	4	0.003726	0.000021	0.000059	0.000000
0.34	6	0.005589	0.000031	0.000088	0.000000
0.34	12	0.011178	0.000062	0.000176	0.000000
0.34	24	0.022356	0.000125	0.000351	0.000000
0.34	52	0.048438	0.000270	0.000761	0.000000
0.34	104	0.096877	0.000540	0.001522	0.000000
0.34	156	0.145315	0.000810	0.002284	0.000000

Chinook (10-20 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
1.3E-05	3.7E-05	0.0E+00	7.4E-06	2.1E-05	0.0E+00
2.6E-05	7.5E-05	0.0E+00	1.5E-05	4.2E-05	0.0E+00
5.3E-05	1.5E-04	0.0E+00	3.0E-05	8.4E-05	0.0E+00
7.9E-05	2.2E-04	0.0E+00	4.5E-05	1.3E-04	0.0E+00
1.6E-04	4.5E-04	0.0E+00	8.9E-05	2.5E-04	0.0E+00
3.2E-04	9.0E-04	0.0E+00	1.8E-04	5.0E-04	0.0E+00
6.9E-04	1.9E-03	0.0E+00	3.9E-04	1.1E-03	0.0E+00
1.4E-03	3.9E-03	0.0E+00	7.7E-04	2.2E-03	0.0E+00
2.1E-03	5.8E-03	0.0E+00	1.2E-03	3.3E-03	0.0E+00
2.6E-05	7.5E-05	0.0E+00	1.5E-05	4.2E-05	0.0E+00
5.3E-05	1.5E-04	0.0E+00	3.0E-05	8.4E-05	0.0E+00
1.1E-04	3.0E-04	0.0E+00	5.9E-05	1.7E-04	0.0E+00
1.6E-04	4.5E-04	0.0E+00	8.9E-05	2.5E-04	0.0E+00
3.2E-04	9.0E-04	0.0E+00	1.8E-04	5.0E-04	0.0E+00
6.3E-04	1.8E-03	0.0E+00	3.6E-04	1.0E-03	0.0E+00
1.4E-03	3.9E-03	0.0E+00	7.7E-04	2.2E-03	0.0E+00
2.8E-03	7.8E-03	0.0E+00	1.5E-03	4.4E-03	0.0E+00
4.1E-03	1.2E-02	0.0E+00	2.3E-03	6.5E-03	0.0E+00
3.9E-05	1.1E-04	0.0E+00	2.2E-05	6.3E-05	0.0E+00
7.9E-05	2.2E-04	0.0E+00	4.4E-05	1.3E-04	0.0E+00
1.6E-04	4.4E-04	0.0E+00	8.9E-05	2.5E-04	0.0E+00
2.4E-04	6.7E-04	0.0E+00	1.3E-04	3.8E-04	0.0E+00
4.7E-04	1.3E-03	0.0E+00	2.7E-04	7.5E-04	0.0E+00
9.5E-04	2.7E-03	0.0E+00	5.3E-04	1.5E-03	0.0E+00
2.1E-03	5.8E-03	0.0E+00	1.2E-03	3.3E-03	0.0E+00
4.1E-03	1.2E-02	0.0E+00	2.3E-03	6.5E-03	0.0E+00
6.2E-03	1.7E-02	0.0E+00	3.5E-03	9.8E-03	0.0E+00

Chinook (10-20 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.04	0.11	0.00	0.01	0.03	0.00
0.08	0.22	0.00	0.02	0.05	0.00
0.15	0.44	0.00	0.04	0.11	0.00
0.23	0.65	0.00	0.06	0.16	0.00
0.46	1.31	0.00	0.12	0.33	0.00
0.93	2.62	0.00	0.23	0.65	0.00
2.01	5.67	0.00	0.50	1.42	0.00
4.02	11.34	0.00	1.01	2.84	0.00
6.03	17.01	0.00	1.51	4.25	0.00
0.08	0.22	0.00	0.02	0.05	0.00
0.15	0.44	0.00	0.04	0.11	0.00
0.31	0.87	0.00	0.08	0.22	0.00
0.46	1.31	0.00	0.12	0.33	0.00
0.93	2.62	0.00	0.23	0.65	0.00
1.86	5.24	0.00	0.46	1.31	0.00
4.02	11.34	0.00	1.01	2.84	0.00
8.04	22.69	0.00	2.01	5.67	0.00
12.06	34.03	0.00	3.02	8.51	0.00
0.12	0.33	0.00	0.03	0.08	0.00
0.23	0.65	0.00	0.06	0.16	0.00
0.46	1.30	0.00	0.12	0.33	0.00
0.69	1.95	0.00	0.17	0.49	0.00
1.38	3.90	0.00	0.35	0.98	0.00
2.77	7.81	0.00	0.69	1.95	0.00
6.00	16.91	0.00	1.50	4.23	0.00
11.99	33.83	0.00	3.00	8.46	0.00
17.99	50.74	0.00	4.50	12.69	0.00

Chinook (10-20 inches)

Hazard Index RfD = 0.000009 Mean Conc.	Max. Conc.	Offspring Min. Conc.
0.19	0.55	0.00
0.39	1.09	0.00
0.77	2.18	0.00
1.16	3.27	0.00
2.32	6.54	0.00
4.64	13.09	0.00
10.05	28.36	0.00
20.11	56.71	0.00
30.16	85.07	0.00
0.39	1.09	0.00
0.77	2.18	0.00
1.55	4.36	0.00
2.32	6.54	0.00
4.64	13.09	0.00
9.28	26.18	0.00
20.11	56.71	0.00
40.22	113.43	0.00
60.32	170.14	0.00
0.58	1.63	0.00
1.15	3.25	0.00
2.31	6.51	0.00
3.46	9.76	0.00
6.92	19.52	0.00
13.84	39.03	0.00
29.99	84.57	0.00
59.97	169.15	0.00
89.96	253.72	0.00

Chinook (20-30 inches)

Tissue Concentration (85 + 86)	ppm
Mean	1.17
Max.	2.7
Min.	0.26
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000005	0.000012	0.000001
0.114	2	0.000625	0.000010	0.000024	0.000002
0.114	4	0.001249	0.000021	0.000048	0.000005
0.114	6	0.001874	0.000031	0.000072	0.000007
0.114	12	0.003748	0.000063	0.000145	0.000014
0.114	24	0.007496	0.000125	0.000289	0.000028
0.114	52	0.016241	0.000271	0.000626	0.000060
0.114	104	0.032482	0.000543	0.001253	0.000121
0.114	156	0.048723	0.000814	0.001879	0.000181
0.228	1	0.000625	0.000010	0.000024	0.000002
0.228	2	0.001249	0.000021	0.000048	0.000005
0.228	4	0.002499	0.000042	0.000096	0.000009
0.228	6	0.003748	0.000063	0.000145	0.000014
0.228	12	0.007496	0.000125	0.000289	0.000028
0.228	24	0.014992	0.000251	0.000578	0.000056
0.228	52	0.032482	0.000543	0.001253	0.000121
0.228	104	0.064964	0.001086	0.002506	0.000241
0.228	156	0.097447	0.001629	0.003759	0.000362
0.34	1	0.000932	0.000016	0.000036	0.000003
0.34	2	0.001863	0.000031	0.000072	0.000007
0.34	4	0.003726	0.000062	0.000144	0.000014
0.34	6	0.005589	0.000093	0.000216	0.000021
0.34	12	0.011178	0.000187	0.000431	0.000042
0.34	24	0.022356	0.000374	0.000862	0.000083
0.34	52	0.048438	0.000810	0.001868	0.000180
0.34	104	0.096877	0.001619	0.003737	0.000360
0.34	156	0.145315	0.002429	0.005605	0.000540

Chinook (20-30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
4.0E-05	9.2E-05	8.8E-06	2.2E-05	5.1E-05	5.0E-06
7.9E-05	1.8E-04	1.8E-05	4.5E-05	1.0E-04	9.9E-06
1.6E-04	3.7E-04	3.5E-05	8.9E-05	2.1E-04	2.0E-05
2.4E-04	5.5E-04	5.3E-05	1.3E-04	3.1E-04	3.0E-05
4.8E-04	1.1E-03	1.1E-04	2.7E-04	6.2E-04	5.9E-05
9.5E-04	2.2E-03	2.1E-04	5.3E-04	1.2E-03	1.2E-04
2.1E-03	4.8E-03	4.6E-04	1.2E-03	2.7E-03	2.6E-04
4.1E-03	9.5E-03	9.2E-04	2.3E-03	5.3E-03	5.2E-04
6.2E-03	1.4E-02	1.4E-03	3.5E-03	8.0E-03	7.7E-04
7.9E-05	1.8E-04	1.8E-05	4.5E-05	1.0E-04	9.9E-06
1.6E-04	3.7E-04	3.5E-05	8.9E-05	2.1E-04	2.0E-05
3.2E-04	7.3E-04	7.1E-05	1.8E-04	4.1E-04	4.0E-05
4.8E-04	1.1E-03	1.1E-04	2.7E-04	6.2E-04	5.9E-05
9.5E-04	2.2E-03	2.1E-04	5.3E-04	1.2E-03	1.2E-04
1.9E-03	4.4E-03	4.2E-04	1.1E-03	2.5E-03	2.4E-04
4.1E-03	9.5E-03	9.2E-04	2.3E-03	5.3E-03	5.2E-04
8.3E-03	1.9E-02	1.8E-03	4.6E-03	1.1E-02	1.0E-03
1.2E-02	2.9E-02	2.8E-03	7.0E-03	1.6E-02	1.5E-03
1.2E-04	2.7E-04	2.6E-05	6.6E-05	1.5E-04	1.5E-05
2.4E-04	5.5E-04	5.3E-05	1.3E-04	3.1E-04	3.0E-05
4.7E-04	1.1E-03	1.1E-04	2.7E-04	6.1E-04	5.9E-05
7.1E-04	1.6E-03	1.6E-04	4.0E-04	9.2E-04	8.9E-05
1.4E-03	3.3E-03	3.2E-04	8.0E-04	1.8E-03	1.8E-04
2.8E-03	6.6E-03	6.3E-04	1.6E-03	3.7E-03	3.5E-04
6.2E-03	1.4E-02	1.4E-03	3.5E-03	8.0E-03	7.7E-04
1.2E-02	2.8E-02	2.7E-03	6.9E-03	1.6E-02	1.5E-03
1.8E-02	4.3E-02	4.1E-03	1.0E-02	2.4E-02	2.3E-03

Chinook (20-30 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.12	0.27	0.03	0.03	0.07	0.01
0.23	0.54	0.05	0.06	0.13	0.01
0.46	1.07	0.10	0.12	0.27	0.03
0.70	1.61	0.15	0.17	0.40	0.04
1.39	3.21	0.31	0.35	0.80	0.08
2.78	6.43	0.62	0.70	1.61	0.15
6.03	13.92	1.34	1.51	3.48	0.34
12.06	27.84	2.68	3.02	6.96	0.67
18.10	41.76	4.02	4.52	10.44	1.01
0.23	0.54	0.05	0.06	0.13	0.01
0.46	1.07	0.10	0.12	0.27	0.03
0.93	2.14	0.21	0.23	0.54	0.05
1.39	3.21	0.31	0.35	0.80	0.08
2.78	6.43	0.62	0.70	1.61	0.15
5.57	12.85	1.24	1.39	3.21	0.31
12.06	27.84	2.68	3.02	6.96	0.67
24.13	55.68	5.36	6.03	13.92	1.34
36.19	83.53	8.04	9.05	20.88	2.01
0.35	0.80	0.08	0.09	0.20	0.02
0.69	1.60	0.15	0.17	0.40	0.04
1.38	3.19	0.31	0.35	0.80	0.08
2.08	4.79	0.46	0.52	1.20	0.12
4.15	9.58	0.92	1.04	2.40	0.23
8.30	19.16	1.85	2.08	4.79	0.46
17.99	41.52	4.00	4.50	10.38	1.00
35.98	83.04	8.00	9.00	20.76	2.00
53.97	124.56	11.99	13.49	31.14	3.00

Chinook (20-30 inches)

Hazard Index RfD = 0.000009 Mean Conc.	Max. Conc.	Offspring Min. Conc.
0.58	1.34	0.13
1.16	2.68	0.26
2.32	5.35	0.52
3.48	8.03	0.77
6.96	16.06	1.55
13.92	32.13	3.09
30.16	69.60	6.70
60.32	139.21	13.41
90.49	208.81	20.11
1.16	2.68	0.26
2.32	5.35	0.52
4.64	10.71	1.03
6.96	16.06	1.55
13.92	32.13	3.09
27.84	64.25	6.19
60.32	139.21	13.41
120.65	278.42	26.81
180.97	417.63	40.22
1.73	3.99	0.38
3.46	7.98	0.77
6.92	15.97	1.54
10.38	23.95	2.31
20.76	47.91	4.61
41.52	95.81	9.23
89.96	207.59	19.99
179.91	415.19	39.98
269.87	622.78	59.97

Chinook (>30 inches)

Tissue Concentration (85 + 86)	ppm
Mean	2.43
Max.	11
Min.	0.57
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000011	0.000049	0.000003
0.114	2	0.000625	0.000022	0.000098	0.000005
0.114	4	0.001249	0.000043	0.000196	0.000010
0.114	6	0.001874	0.000065	0.000294	0.000015
0.114	12	0.003748	0.000130	0.000589	0.000031
0.114	24	0.007496	0.000260	0.001178	0.000061
0.114	52	0.016241	0.000564	0.002552	0.000132
0.114	104	0.032482	0.001128	0.005104	0.000264
0.114	156	0.048723	0.001691	0.007657	0.000397
0.228	1	0.000625	0.000022	0.000098	0.000005
0.228	2	0.001249	0.000043	0.000196	0.000010
0.228	4	0.002499	0.000087	0.000393	0.000020
0.228	6	0.003748	0.000130	0.000589	0.000031
0.228	12	0.007496	0.000260	0.001178	0.000061
0.228	24	0.014992	0.000520	0.002356	0.000122
0.228	52	0.032482	0.001128	0.005104	0.000264
0.228	104	0.064964	0.002255	0.010209	0.000529
0.228	156	0.097447	0.003383	0.015313	0.000793
0.34	1	0.000932	0.000032	0.000146	0.000008
0.34	2	0.001863	0.000065	0.000293	0.000015
0.34	4	0.003726	0.000129	0.000586	0.000030
0.34	6	0.005589	0.000194	0.000878	0.000046
0.34	12	0.011178	0.000388	0.001757	0.000091
0.34	24	0.022356	0.000776	0.003513	0.000182
0.34	52	0.048438	0.001682	0.007612	0.000394
0.34	104	0.096877	0.003363	0.015223	0.000789
0.34	156	0.145315	0.005045	0.022835	0.001183

Chinook (>30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
8.2E-05	3.7E-04	1.9E-05	4.6E-05	2.1E-04	1.1E-05
1.6E-04	7.5E-04	3.9E-05	9.3E-05	4.2E-04	2.2E-05
3.3E-04	1.5E-03	7.7E-05	1.9E-04	8.4E-04	4.3E-05
4.9E-04	2.2E-03	1.2E-04	2.8E-04	1.3E-03	6.5E-05
9.9E-04	4.5E-03	2.3E-04	5.6E-04	2.5E-03	1.3E-04
2.0E-03	9.0E-03	4.6E-04	1.1E-03	5.0E-03	2.6E-04
4.3E-03	1.9E-02	1.0E-03	2.4E-03	1.1E-02	5.6E-04
8.6E-03	3.9E-02	2.0E-03	4.8E-03	2.2E-02	1.1E-03
1.3E-02	5.8E-02	3.0E-03	7.2E-03	3.3E-02	1.7E-03
1.6E-04	7.5E-04	3.9E-05	9.3E-05	4.2E-04	2.2E-05
3.3E-04	1.5E-03	7.7E-05	1.9E-04	8.4E-04	4.3E-05
6.6E-04	3.0E-03	1.5E-04	3.7E-04	1.7E-03	8.7E-05
9.9E-04	4.5E-03	2.3E-04	5.6E-04	2.5E-03	1.3E-04
2.0E-03	9.0E-03	4.6E-04	1.1E-03	5.0E-03	2.6E-04
4.0E-03	1.8E-02	9.3E-04	2.2E-03	1.0E-02	5.2E-04
8.6E-03	3.9E-02	2.0E-03	4.8E-03	2.2E-02	1.1E-03
1.7E-02	7.8E-02	4.0E-03	9.6E-03	4.4E-02	2.3E-03
2.6E-02	1.2E-01	6.0E-03	1.4E-02	6.5E-02	3.4E-03
2.5E-04	1.1E-03	5.8E-05	1.4E-04	6.3E-04	3.2E-05
4.9E-04	2.2E-03	1.2E-04	2.8E-04	1.3E-03	6.5E-05
9.8E-04	4.4E-03	2.3E-04	5.5E-04	2.5E-03	1.3E-04
1.5E-03	6.7E-03	3.5E-04	8.3E-04	3.8E-03	1.9E-04
2.9E-03	1.3E-02	6.9E-04	1.7E-03	7.5E-03	3.9E-04
5.9E-03	2.7E-02	1.4E-03	3.3E-03	1.5E-02	7.8E-04
1.3E-02	5.8E-02	3.0E-03	7.2E-03	3.3E-02	1.7E-03
2.6E-02	1.2E-01	6.0E-03	1.4E-02	6.5E-02	3.4E-03
3.8E-02	1.7E-01	9.0E-03	2.2E-02	9.8E-02	5.1E-03

Chinook (>30 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.24	1.09	0.06	0.06	0.27	0.01
0.48	2.18	0.00	0.12	0.55	0.03
0.96	4.36	0.00	0.24	1.09	0.06
1.45	6.54	0.00	0.36	1.64	0.08
2.89	13.09	0.00	0.72	3.27	0.17
5.78	26.18	0.00	1.45	6.54	0.34
12.53	56.71	0.00	3.13	14.18	0.73
25.06	113.43	0.01	6.26	28.36	1.47
37.59	170.14	0.01	9.40	42.54	2.20
0.48	2.18	0.00	0.12	0.55	0.03
0.96	4.36	0.00	0.24	1.09	0.06
1.93	8.73	0.00	0.48	2.18	0.11
2.89	13.09	0.00	0.72	3.27	0.17
5.78	26.18	0.00	1.45	6.54	0.34
11.57	52.35	0.00	2.89	13.09	0.68
25.06	113.43	0.01	6.26	28.36	1.47
50.12	226.86	0.01	12.53	56.71	2.94
75.17	340.29	0.02	18.79	85.07	4.41
0.72	3.25	0.00	0.18	0.81	0.04
1.44	6.51	0.00	0.36	1.63	0.08
2.87	13.01	0.00	0.72	3.25	0.17
4.31	19.52	0.00	1.08	4.88	0.25
8.62	39.03	0.00	2.16	9.76	0.51
17.25	78.07	0.00	4.31	19.52	1.01
37.37	169.15	0.01	9.34	42.29	2.19
74.73	338.30	0.02	18.68	84.57	4.38
112.10	507.45	0.03	28.03	126.86	6.57

Chinook (>30 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
1.20	5.45	0.28
2.41	10.91	0.57
4.82	21.81	1.13
7.23	32.72	1.70
14.46	65.44	3.39
28.91	130.88	6.78
62.64	283.57	14.69
125.29	567.15	29.39
187.93	850.72	44.08
2.41	10.91	0.57
4.82	21.81	1.13
9.64	43.63	2.26
14.46	65.44	3.39
28.91	130.88	6.78
57.83	261.76	13.56
125.29	567.15	29.39
250.58	1134.30	58.78
375.87	1701.45	88.17
3.59	16.26	0.84
7.19	32.53	1.69
14.37	65.06	3.37
21.56	97.59	5.06
43.12	195.17	10.11
86.23	390.35	20.23
186.83	845.75	43.83
373.67	1691.50	87.65
560.50	2537.25	131.48

Coho (All Sizes)

Tissue Concentration (85 + 86)	ppm
Mean	0.828
Max.	2.9
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000004	0.000013	0.000000
0.114	2	0.000625	0.000007	0.000026	0.000000
0.114	4	0.001249	0.000015	0.000052	0.000000
0.114	6	0.001874	0.000022	0.000078	0.000000
0.114	12	0.003748	0.000044	0.000155	0.000000
0.114	24	0.007496	0.000089	0.000311	0.000000
0.114	52	0.016241	0.000192	0.000673	0.000000
0.114	104	0.032482	0.000384	0.001346	0.000000
0.114	156	0.048723	0.000576	0.002019	0.000000
0.228	1	0.000625	0.000007	0.000026	0.000000
0.228	2	0.001249	0.000015	0.000052	0.000000
0.228	4	0.002499	0.000030	0.000104	0.000000
0.228	6	0.003748	0.000044	0.000155	0.000000
0.228	12	0.007496	0.000089	0.000311	0.000000
0.228	24	0.014992	0.000177	0.000621	0.000000
0.228	52	0.032482	0.000384	0.001346	0.000000
0.228	104	0.064964	0.000768	0.002691	0.000000
0.228	156	0.097447	0.001153	0.004037	0.000000
0.34	1	0.000932	0.000011	0.000039	0.000000
0.34	2	0.001863	0.000022	0.000077	0.000000
0.34	4	0.003726	0.000044	0.000154	0.000000
0.34	6	0.005589	0.000066	0.000232	0.000000
0.34	12	0.011178	0.000132	0.000463	0.000000
0.34	24	0.022356	0.000264	0.000926	0.000000
0.34	52	0.048438	0.000573	0.002007	0.000000
0.34	104	0.096877	0.001146	0.004013	0.000000
0.34	156	0.145315	0.001719	0.006020	0.000000

Coho (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
2.8E-05	9.8E-05	0.0E+00	1.6E-05	5.5E-05	0.0E+00
5.6E-05	2.0E-04	0.0E+00	3.2E-05	1.1E-04	0.0E+00
1.1E-04	3.9E-04	0.0E+00	6.3E-05	2.2E-04	0.0E+00
1.7E-04	5.9E-04	0.0E+00	9.5E-05	3.3E-04	0.0E+00
3.4E-04	1.2E-03	0.0E+00	1.9E-04	6.6E-04	0.0E+00
6.7E-04	2.4E-03	0.0E+00	3.8E-04	1.3E-03	0.0E+00
1.5E-03	5.1E-03	0.0E+00	8.2E-04	2.9E-03	0.0E+00
2.9E-03	1.0E-02	0.0E+00	1.6E-03	5.7E-03	0.0E+00
4.4E-03	1.5E-02	0.0E+00	2.5E-03	8.6E-03	0.0E+00
5.6E-05	2.0E-04	0.0E+00	3.2E-05	1.1E-04	0.0E+00
1.1E-04	3.9E-04	0.0E+00	6.3E-05	2.2E-04	0.0E+00
2.2E-04	7.9E-04	0.0E+00	1.3E-04	4.4E-04	0.0E+00
3.4E-04	1.2E-03	0.0E+00	1.9E-04	6.6E-04	0.0E+00
6.7E-04	2.4E-03	0.0E+00	3.8E-04	1.3E-03	0.0E+00
1.3E-03	4.7E-03	0.0E+00	7.6E-04	2.7E-03	0.0E+00
2.9E-03	1.0E-02	0.0E+00	1.6E-03	5.7E-03	0.0E+00
5.8E-03	2.0E-02	0.0E+00	3.3E-03	1.1E-02	0.0E+00
8.8E-03	3.1E-02	0.0E+00	4.9E-03	1.7E-02	0.0E+00
8.4E-05	2.9E-04	0.0E+00	4.7E-05	1.6E-04	0.0E+00
1.7E-04	5.9E-04	0.0E+00	9.4E-05	3.3E-04	0.0E+00
3.3E-04	1.2E-03	0.0E+00	1.9E-04	6.6E-04	0.0E+00
5.0E-04	1.8E-03	0.0E+00	2.8E-04	9.9E-04	0.0E+00
1.0E-03	3.5E-03	0.0E+00	5.6E-04	2.0E-03	0.0E+00
2.0E-03	7.0E-03	0.0E+00	1.1E-03	4.0E-03	0.0E+00
4.4E-03	1.5E-02	0.0E+00	2.4E-03	8.6E-03	0.0E+00
8.7E-03	3.1E-02	0.0E+00	4.9E-03	1.7E-02	0.0E+00
1.3E-02	4.6E-02	0.0E+00	7.3E-03	2.6E-02	0.0E+00

Coho (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.08	0.29	0.00	0.02	0.07	0.00
0.16	0.58	0.00	0.04	0.14	0.00
0.33	1.15	0.00	0.08	0.29	0.00
0.49	1.73	0.00	0.12	0.43	0.00
0.99	3.45	0.00	0.25	0.86	0.00
1.97	6.90	0.00	0.49	1.73	0.00
4.27	14.95	0.00	1.07	3.74	0.00
8.54	29.90	0.00	2.13	7.48	0.00
12.81	44.86	0.00	3.20	11.21	0.00
0.16	0.58	0.00	0.04	0.14	0.00
0.33	1.15	0.00	0.08	0.29	0.00
0.66	2.30	0.00	0.16	0.58	0.00
0.99	3.45	0.00	0.25	0.86	0.00
1.97	6.90	0.00	0.49	1.73	0.00
3.94	13.80	0.00	0.99	3.45	0.00
8.54	29.90	0.00	2.13	7.48	0.00
17.08	59.81	0.00	4.27	14.95	0.00
25.61	89.71	0.00	6.40	22.43	0.00
0.24	0.86	0.00	0.06	0.21	0.00
0.49	1.72	0.00	0.12	0.43	0.00
0.98	3.43	0.00	0.24	0.86	0.00
1.47	5.15	0.00	0.37	1.29	0.00
2.94	10.29	0.00	0.73	2.57	0.00
5.88	20.58	0.00	1.47	5.15	0.00
12.73	44.59	0.00	3.18	11.15	0.00
25.46	89.19	0.00	6.37	22.30	0.00
38.20	133.78	0.00	9.55	33.45	0.00

Coho (All Sizes)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.41	1.44	0.00
0.82	2.88	0.00
1.64	5.75	0.00
2.46	8.63	0.00
4.93	17.25	0.00
9.85	34.50	0.00
21.35	74.76	0.00
42.69	149.52	0.00
64.04	224.28	0.00
0.82	2.88	0.00
1.64	5.75	0.00
3.28	11.50	0.00
4.93	17.25	0.00
9.85	34.50	0.00
19.70	69.01	0.00
42.69	149.52	0.00
85.38	299.04	0.00
128.07	448.56	0.00
1.22	4.29	0.00
2.45	8.58	0.00
4.90	17.15	0.00
7.35	25.73	0.00
14.69	51.45	0.00
29.38	102.91	0.00
63.66	222.97	0.00
127.32	445.94	0.00
190.99	668.91	0.00

Coho (20-30 inches)

Tissue Concentration (85 + 86)	
Mean	0.56
Max.	2.9
Min.	0.12
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000002	0.000013	0.000001
0.114	2	0.000625	0.000005	0.000026	0.000001
0.114	4	0.001249	0.000010	0.000052	0.000002
0.114	6	0.001874	0.000015	0.000078	0.000003
0.114	12	0.003748	0.000030	0.000155	0.000006
0.114	24	0.007496	0.000060	0.000311	0.000013
0.114	52	0.016241	0.000130	0.000673	0.000028
0.114	104	0.032482	0.000260	0.001346	0.000056
0.114	156	0.048723	0.000390	0.002019	0.000084
0.228	1	0.000625	0.000005	0.000026	0.000001
0.228	2	0.001249	0.000010	0.000052	0.000002
0.228	4	0.002499	0.000020	0.000104	0.000004
0.228	6	0.003748	0.000030	0.000155	0.000006
0.228	12	0.007496	0.000060	0.000311	0.000013
0.228	24	0.014992	0.000120	0.000621	0.000026
0.228	52	0.032482	0.000260	0.001346	0.000056
0.228	104	0.064964	0.000520	0.002691	0.000111
0.228	156	0.097447	0.000780	0.004037	0.000167
0.34	1	0.000932	0.000007	0.000039	0.000002
0.34	2	0.001863	0.000015	0.000077	0.000003
0.34	4	0.003726	0.000030	0.000154	0.000006
0.34	6	0.005589	0.000045	0.000232	0.000010
0.34	12	0.011178	0.000089	0.000463	0.000019
0.34	24	0.022356	0.000179	0.000926	0.000038
0.34	52	0.048438	0.000388	0.002007	0.000083
0.34	104	0.096877	0.000775	0.004013	0.000166
0.34	156	0.145315	0.001163	0.006020	0.000249

Coho (20-30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
1.9E-05	9.8E-05	4.1E-06	1.1E-05	5.5E-05	2.3E-06
3.8E-05	2.0E-04	8.1E-06	2.1E-05	1.1E-04	4.6E-06
7.6E-05	3.9E-04	1.6E-05	4.3E-05	2.2E-04	9.1E-06
1.1E-04	5.9E-04	2.4E-05	6.4E-05	3.3E-04	1.4E-05
2.3E-04	1.2E-03	4.9E-05	1.3E-04	6.6E-04	2.7E-05
4.6E-04	2.4E-03	9.8E-05	2.6E-04	1.3E-03	5.5E-05
9.9E-04	5.1E-03	2.1E-04	5.5E-04	2.9E-03	1.2E-04
2.0E-03	1.0E-02	4.2E-04	1.1E-03	5.7E-03	2.4E-04
3.0E-03	1.5E-02	6.3E-04	1.7E-03	8.6E-03	3.6E-04
3.8E-05	2.0E-04	8.1E-06	2.1E-05	1.1E-04	4.6E-06
7.6E-05	3.9E-04	1.6E-05	4.3E-05	2.2E-04	9.1E-06
1.5E-04	7.9E-04	3.3E-05	8.5E-05	4.4E-04	1.8E-05
2.3E-04	1.2E-03	4.9E-05	1.3E-04	6.6E-04	2.7E-05
4.6E-04	2.4E-03	9.8E-05	2.6E-04	1.3E-03	5.5E-05
9.1E-04	4.7E-03	2.0E-04	5.1E-04	2.7E-03	1.1E-04
2.0E-03	1.0E-02	4.2E-04	1.1E-03	5.7E-03	2.4E-04
3.9E-03	2.0E-02	8.5E-04	2.2E-03	1.1E-02	4.8E-04
5.9E-03	3.1E-02	1.3E-03	3.3E-03	1.7E-02	7.1E-04
5.7E-05	2.9E-04	1.2E-05	3.2E-05	1.6E-04	6.8E-06
1.1E-04	5.9E-04	2.4E-05	6.4E-05	3.3E-04	1.4E-05
2.3E-04	1.2E-03	4.9E-05	1.3E-04	6.6E-04	2.7E-05
3.4E-04	1.8E-03	7.3E-05	1.9E-04	9.9E-04	4.1E-05
6.8E-04	3.5E-03	1.5E-04	3.8E-04	2.0E-03	8.2E-05
1.4E-03	7.0E-03	2.9E-04	7.6E-04	4.0E-03	1.6E-04
2.9E-03	1.5E-02	6.3E-04	1.7E-03	8.6E-03	3.5E-04
5.9E-03	3.1E-02	1.3E-03	3.3E-03	1.7E-02	7.1E-04
8.8E-03	4.6E-02	1.9E-03	5.0E-03	2.6E-02	1.1E-03

Coho (20-30 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.06	0.29	0.01	0.01	0.07	0.00
0.11	0.58	0.02	0.03	0.14	0.01
0.22	1.15	0.05	0.06	0.29	0.01
0.33	1.73	0.07	0.08	0.43	0.02
0.67	3.45	0.14	0.17	0.86	0.04
1.33	6.90	0.29	0.33	1.73	0.07
2.89	14.95	0.62	0.72	3.74	0.15
5.77	29.90	1.24	1.44	7.48	0.31
8.66	44.86	1.86	2.17	11.21	0.46
0.11	0.58	0.02	0.03	0.14	0.01
0.22	1.15	0.05	0.06	0.29	0.01
0.44	2.30	0.10	0.11	0.58	0.02
0.67	3.45	0.14	0.17	0.86	0.04
1.33	6.90	0.29	0.33	1.73	0.07
2.67	13.80	0.57	0.67	3.45	0.14
5.77	29.90	1.24	1.44	7.48	0.31
11.55	59.81	2.47	2.89	14.95	0.62
17.32	89.71	3.71	4.33	22.43	0.93
0.17	0.86	0.04	0.04	0.21	0.01
0.33	1.72	0.07	0.08	0.43	0.02
0.66	3.43	0.14	0.17	0.86	0.04
0.99	5.15	0.21	0.25	1.29	0.05
1.99	10.29	0.43	0.50	2.57	0.11
3.97	20.58	0.85	0.99	5.15	0.21
8.61	44.59	1.85	2.15	11.15	0.46
17.22	89.19	3.69	4.31	22.30	0.92
25.83	133.78	5.54	6.46	33.45	1.38

Coho (20-30 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.28	1.44	0.06
0.56	2.88	0.12
1.11	5.75	0.24
1.67	8.63	0.36
3.33	17.25	0.71
6.66	34.50	1.43
14.44	74.76	3.09
28.87	149.52	6.19
43.31	224.28	9.28
0.56	2.88	0.12
1.11	5.75	0.24
2.22	11.50	0.48
3.33	17.25	0.71
6.66	34.50	1.43
13.33	69.01	2.86
28.87	149.52	6.19
57.75	299.04	12.37
86.62	448.56	18.56
0.83	4.29	0.18
1.66	8.58	0.35
3.31	17.15	0.71
4.97	25.73	1.06
9.94	51.45	2.13
19.87	102.91	4.26
43.06	222.97	9.23
86.11	445.94	18.45
129.17	668.91	27.68

Lake Trout (All Sizes)

Tissue Conc.	ppm
Mean	3.17
Max.	35.78
Min.	0.18
Adult Body Weight (kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000014	0.000160	0.000001
0.114	2	0.000625	0.000028	0.000319	0.000002
0.114	4	0.001249	0.000057	0.000639	0.000003
0.114	6	0.001874	0.000085	0.000958	0.000005
0.114	12	0.003748	0.000170	0.001916	0.000010
0.114	24	0.007496	0.000339	0.003831	0.000019
0.114	52	0.016241	0.000735	0.008302	0.000042
0.114	104	0.032482	0.001471	0.016603	0.000084
0.114	156	0.048723	0.002206	0.024905	0.000125
0.228	1	0.000625	0.000028	0.000319	0.000002
0.228	2	0.001249	0.000057	0.000639	0.000003
0.228	4	0.002499	0.000113	0.001277	0.000006
0.228	6	0.003748	0.000170	0.001916	0.000010
0.228	12	0.007496	0.000339	0.003831	0.000019
0.228	24	0.014992	0.000679	0.007663	0.000039
0.228	52	0.032482	0.001471	0.016603	0.000084
0.228	104	0.064964	0.002942	0.033206	0.000167
0.228	156	0.097447	0.004413	0.049809	0.000251
0.34	1	0.000932	0.000042	0.000476	0.000002
0.34	2	0.001863	0.000084	0.000952	0.000005
0.34	4	0.003726	0.000169	0.001905	0.000010
0.34	6	0.005589	0.000253	0.002857	0.000014
0.34	12	0.011178	0.000506	0.005714	0.000029
0.34	24	0.022356	0.001012	0.011427	0.000057
0.34	52	0.048438	0.002194	0.024759	0.000125
0.34	104	0.096877	0.004387	0.049518	0.000249
0.34	156	0.145315	0.006581	0.074277	0.000374

Lake Trout (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.1E-04	1.2E-03	6.1E-06	6.0E-05	6.8E-04	3.4E-06
2.1E-04	2.4E-03	1.2E-05	1.2E-04	1.4E-03	6.9E-06
4.3E-04	4.9E-03	2.4E-05	2.4E-04	2.7E-03	1.4E-05
6.4E-04	7.3E-03	3.7E-05	3.6E-04	4.1E-03	2.1E-05
1.3E-03	1.5E-02	7.3E-05	7.2E-04	8.2E-03	4.1E-05
2.6E-03	2.9E-02	1.5E-04	1.4E-03	1.6E-02	8.2E-05
5.6E-03	6.3E-02	3.2E-04	3.1E-03	3.5E-02	1.8E-04
1.1E-02	1.3E-01	6.3E-04	6.3E-03	7.1E-02	3.6E-04
1.7E-02	1.9E-01	9.5E-04	9.4E-03	1.1E-01	5.3E-04
2.1E-04	2.4E-03	1.2E-05	1.2E-04	1.4E-03	6.9E-06
4.3E-04	4.9E-03	2.4E-05	2.4E-04	2.7E-03	1.4E-05
8.6E-04	9.7E-03	4.9E-05	4.8E-04	5.5E-03	2.7E-05
1.3E-03	1.5E-02	7.3E-05	7.2E-04	8.2E-03	4.1E-05
2.6E-03	2.9E-02	1.5E-04	1.4E-03	1.6E-02	8.2E-05
5.2E-03	5.8E-02	2.9E-04	2.9E-03	3.3E-02	1.6E-04
1.1E-02	1.3E-01	6.3E-04	6.3E-03	7.1E-02	3.6E-04
2.2E-02	2.5E-01	1.3E-03	1.3E-02	1.4E-01	7.1E-04
3.4E-02	3.8E-01	1.9E-03	1.9E-02	2.1E-01	1.1E-03
3.2E-04	3.6E-03	1.8E-05	1.8E-04	2.0E-03	1.0E-05
6.4E-04	7.2E-03	3.6E-05	3.6E-04	4.1E-03	2.0E-05
1.3E-03	1.4E-02	7.3E-05	7.2E-04	8.1E-03	4.1E-05
1.9E-03	2.2E-02	1.1E-04	1.1E-03	1.2E-02	6.1E-05
3.8E-03	4.3E-02	2.2E-04	2.2E-03	2.4E-02	1.2E-04
7.7E-03	8.7E-02	4.4E-04	4.3E-03	4.9E-02	2.5E-04
1.7E-02	1.9E-01	9.5E-04	9.4E-03	1.1E-01	5.3E-04
3.3E-02	3.8E-01	1.9E-03	1.9E-02	2.1E-01	1.1E-03
5.0E-02	5.6E-01	2.8E-03	2.8E-02	3.2E-01	1.6E-03

Lake Trout (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.31	3.55	0.02	0.08	0.89	0.00
0.63	7.10	0.04	0.16	1.77	0.01
1.26	14.19	0.07	0.31	3.55	0.02
1.89	21.29	0.11	0.47	5.32	0.03
3.77	42.57	0.21	0.94	10.64	0.05
7.54	85.14	0.43	1.89	21.29	0.11
16.34	184.48	0.93	4.09	46.12	0.23
32.69	368.96	1.86	8.17	92.24	0.46
49.03	553.43	2.78	12.26	138.36	0.70
0.63	7.10	0.04	0.16	1.77	0.01
1.26	14.19	0.07	0.31	3.55	0.02
2.51	28.38	0.14	0.63	7.10	0.04
3.77	42.57	0.21	0.94	10.64	0.05
7.54	85.14	0.43	1.89	21.29	0.11
15.09	170.29	0.86	3.77	42.57	0.21
32.69	368.96	1.86	8.17	92.24	0.46
65.38	737.91	3.71	16.34	184.48	0.93
98.07	1106.87	5.57	24.52	276.72	1.39
0.94	10.58	0.05	0.23	2.65	0.01
1.87	21.16	0.11	0.47	5.29	0.03
3.75	42.32	0.21	0.94	10.58	0.05
5.62	63.48	0.32	1.41	15.87	0.08
11.25	126.97	0.64	2.81	31.74	0.16
22.50	253.94	1.28	5.62	63.48	0.32
48.75	550.20	2.77	12.19	137.55	0.69
97.49	1100.40	5.54	24.37	275.10	1.38
146.24	1650.59	8.30	36.56	412.65	2.08

Lake Trout (All Sizes)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
1.57	17.74	0.09
3.14	35.48	0.18
6.29	70.95	0.36
9.43	106.43	0.54
18.86	212.86	1.07
37.72	425.72	2.14
81.72	922.39	4.64
163.44	1844.78	9.28
245.16	2767.17	13.92
3.14	35.48	0.18
6.29	70.95	0.36
12.57	141.91	0.71
18.86	212.86	1.07
37.72	425.72	2.14
75.43	851.44	4.28
163.44	1844.78	9.28
326.88	3689.56	18.56
490.33	5534.35	27.84
4.69	52.90	0.27
9.37	105.81	0.53
18.75	211.61	1.06
28.12	317.42	1.60
56.25	634.84	3.19
112.49	1269.69	6.39
243.73	2750.99	13.84
487.46	5501.98	27.68
731.19	8252.97	41.52

Lake Trout (10-20 inches)

Tissue Concentration (85 + 86)	ppm
Mean	0.916
Max.	2.8
Min.	0.43
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000004	0.000012	0.000002
0.114	2	0.000625	0.000008	0.000025	0.000004
0.114	4	0.001249	0.000016	0.000050	0.000008
0.114	6	0.001874	0.000025	0.000075	0.000012
0.114	12	0.003748	0.000049	0.000150	0.000023
0.114	24	0.007496	0.000098	0.000300	0.000046
0.114	52	0.016241	0.000213	0.000650	0.000100
0.114	104	0.032482	0.000425	0.001299	0.000200
0.114	156	0.048723	0.000638	0.001949	0.000299
0.228	1	0.000625	0.000008	0.000025	0.000004
0.228	2	0.001249	0.000016	0.000050	0.000008
0.228	4	0.002499	0.000033	0.000100	0.000015
0.228	6	0.003748	0.000049	0.000150	0.000023
0.228	12	0.007496	0.000098	0.000300	0.000046
0.228	24	0.014992	0.000196	0.000600	0.000092
0.228	52	0.032482	0.000425	0.001299	0.000200
0.228	104	0.064964	0.000850	0.002599	0.000399
0.228	156	0.097447	0.001275	0.003898	0.000599
0.34	1	0.000932	0.000012	0.000037	0.000006
0.34	2	0.001863	0.000024	0.000075	0.000011
0.34	4	0.003726	0.000049	0.000149	0.000023
0.34	6	0.005589	0.000073	0.000224	0.000034
0.34	12	0.011178	0.000146	0.000447	0.000069
0.34	24	0.022356	0.000293	0.000894	0.000137
0.34	52	0.048438	0.000634	0.001938	0.000298
0.34	104	0.096877	0.001268	0.003875	0.000595
0.34	156	0.145315	0.001902	0.005813	0.000893

Lake Trout (10-20 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
3.1E-05	9.5E-05	1.5E-05	1.7E-05	5.3E-05	8.2E-06
6.2E-05	1.9E-04	2.9E-05	3.5E-05	1.1E-04	1.6E-05
1.2E-04	3.8E-04	5.8E-05	7.0E-05	2.1E-04	3.3E-05
1.9E-04	5.7E-04	8.7E-05	1.0E-04	3.2E-04	4.9E-05
3.7E-04	1.1E-03	1.7E-04	2.1E-04	6.4E-04	9.8E-05
7.5E-04	2.3E-03	3.5E-04	4.2E-04	1.3E-03	2.0E-04
1.6E-03	4.9E-03	7.6E-04	9.1E-04	2.8E-03	4.3E-04
3.2E-03	9.9E-03	1.5E-03	1.8E-03	5.5E-03	8.5E-04
4.8E-03	1.5E-02	2.3E-03	2.7E-03	8.3E-03	1.3E-03
6.2E-05	1.9E-04	2.9E-05	3.5E-05	1.1E-04	1.6E-05
1.2E-04	3.8E-04	5.8E-05	7.0E-05	2.1E-04	3.3E-05
2.5E-04	7.6E-04	1.2E-04	1.4E-04	4.3E-04	6.6E-05
3.7E-04	1.1E-03	1.7E-04	2.1E-04	6.4E-04	9.8E-05
7.5E-04	2.3E-03	3.5E-04	4.2E-04	1.3E-03	2.0E-04
1.5E-03	4.6E-03	7.0E-04	8.4E-04	2.6E-03	3.9E-04
3.2E-03	9.9E-03	1.5E-03	1.8E-03	5.5E-03	8.5E-04
6.5E-03	2.0E-02	3.0E-03	3.6E-03	1.1E-02	1.7E-03
9.7E-03	3.0E-02	4.5E-03	5.4E-03	1.7E-02	2.6E-03
9.3E-05	2.8E-04	4.3E-05	5.2E-05	1.6E-04	2.4E-05
1.9E-04	5.7E-04	8.7E-05	1.0E-04	3.2E-04	4.9E-05
3.7E-04	1.1E-03	1.7E-04	2.1E-04	6.4E-04	9.8E-05
5.6E-04	1.7E-03	2.6E-04	3.1E-04	9.5E-04	1.5E-04
1.1E-03	3.4E-03	5.2E-04	6.2E-04	1.9E-03	2.9E-04
2.2E-03	6.8E-03	1.0E-03	1.2E-03	3.8E-03	5.9E-04
4.8E-03	1.5E-02	2.3E-03	2.7E-03	8.3E-03	1.3E-03
9.6E-03	2.9E-02	4.5E-03	5.4E-03	1.7E-02	2.5E-03
1.4E-02	4.4E-02	6.8E-03	8.1E-03	2.5E-02	3.8E-03

Lake Trout (10-20 inches)

Hazard Index RfD = 0.000045 Thyroid			Hazard Index RfD = 0.00018 Liver		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
0.09	0.28	0.04	0.02	0.07	0.01
0.18	0.56	0.09	0.05	0.14	0.02
0.36	1.11	0.17	0.09	0.28	0.04
0.54	1.67	0.26	0.14	0.42	0.06
1.09	3.33	0.51	0.27	0.83	0.13
2.18	6.66	1.02	0.54	1.67	0.26
4.72	14.44	2.22	1.18	3.61	0.55
9.45	28.87	4.43	2.36	7.22	1.11
14.17	43.31	6.65	3.54	10.83	1.66
0.18	0.56	0.09	0.05	0.14	0.02
0.36	1.11	0.17	0.09	0.28	0.04
0.73	2.22	0.34	0.18	0.56	0.09
1.09	3.33	0.51	0.27	0.83	0.13
2.18	6.66	1.02	0.54	1.67	0.26
4.36	13.33	2.05	1.09	3.33	0.51
9.45	28.87	4.43	2.36	7.22	1.11
18.89	57.75	8.87	4.72	14.44	2.22
28.34	86.62	13.30	7.08	21.65	3.33
0.27	0.83	0.13	0.07	0.21	0.03
0.54	1.66	0.25	0.14	0.41	0.06
1.08	3.31	0.51	0.27	0.83	0.13
1.63	4.97	0.76	0.41	1.24	0.19
3.25	9.94	1.53	0.81	2.48	0.38
6.50	19.87	3.05	1.63	4.97	0.76
14.09	43.06	6.61	3.52	10.76	1.65
28.17	86.11	13.22	7.04	21.53	3.31
42.26	129.17	19.84	10.56	32.29	4.96

Lake Trout (10-20 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.45	1.39	0.21
0.91	2.78	0.43
1.82	5.55	0.85
2.72	8.33	1.28
5.45	16.66	2.56
10.90	33.32	5.12
23.61	72.18	11.09
47.23	144.37	22.17
70.84	216.55	33.26
0.91	2.78	0.43
1.82	5.55	0.85
3.63	11.11	1.71
5.45	16.66	2.56
10.90	33.32	5.12
21.80	66.63	10.23
47.23	144.37	22.17
94.46	288.73	44.34
141.68	433.10	66.51
1.35	4.14	0.64
2.71	8.28	1.27
5.42	16.56	2.54
8.13	24.84	3.81
16.25	49.68	7.63
32.51	99.36	15.26
70.43	215.28	33.06
140.86	430.56	66.12
211.28	645.84	99.18

Lake Trout (20-30 inches)

Tissue Concentration (85 + 86)	
Mean	3.44
Max.	35.78
Min.	0.51
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000015	0.000160	0.000002
0.114	2	0.000625	0.000031	0.000319	0.000005
0.114	4	0.001249	0.000061	0.000639	0.000009
0.114	6	0.001874	0.000092	0.000958	0.000014
0.114	12	0.003748	0.000184	0.001916	0.000027
0.114	24	0.007496	0.000368	0.003831	0.000055
0.114	52	0.016241	0.000798	0.008302	0.000118
0.114	104	0.032482	0.001596	0.016603	0.000237
0.114	156	0.048723	0.002394	0.024905	0.000355
0.228	1	0.000625	0.000031	0.000319	0.000005
0.228	2	0.001249	0.000061	0.000639	0.000009
0.228	4	0.002499	0.000123	0.001277	0.000018
0.228	6	0.003748	0.000184	0.001916	0.000027
0.228	12	0.007496	0.000368	0.003831	0.000055
0.228	24	0.014992	0.000737	0.007663	0.000109
0.228	52	0.032482	0.001596	0.016603	0.000237
0.228	104	0.064964	0.003193	0.033206	0.000473
0.228	156	0.097447	0.004789	0.049809	0.000710
0.34	1	0.000932	0.000046	0.000476	0.000007
0.34	2	0.001863	0.000092	0.000952	0.000014
0.34	4	0.003726	0.000183	0.001905	0.000027
0.34	6	0.005589	0.000275	0.002857	0.000041
0.34	12	0.011178	0.000549	0.005714	0.000081
0.34	24	0.022356	0.001099	0.011427	0.000163
0.34	52	0.048438	0.002380	0.024759	0.000353
0.34	104	0.096877	0.004761	0.049518	0.000706
0.34	156	0.145315	0.007141	0.074277	0.001059

Lake Trout (20-30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
1.2E-04	1.2E-03	1.7E-05	6.6E-05	6.8E-04	9.7E-06
2.3E-04	2.4E-03	3.5E-05	1.3E-04	1.4E-03	1.9E-05
4.7E-04	4.9E-03	6.9E-05	2.6E-04	2.7E-03	3.9E-05
7.0E-04	7.3E-03	1.0E-04	3.9E-04	4.1E-03	5.8E-05
1.4E-03	1.5E-02	2.1E-04	7.9E-04	8.2E-03	1.2E-04
2.8E-03	2.9E-02	4.2E-04	1.6E-03	1.6E-02	2.3E-04
6.1E-03	6.3E-02	9.0E-04	3.4E-03	3.5E-02	5.1E-04
1.2E-02	1.3E-01	1.8E-03	6.8E-03	7.1E-02	1.0E-03
1.8E-02	1.9E-01	2.7E-03	1.0E-02	1.1E-01	1.5E-03
2.3E-04	2.4E-03	3.5E-05	1.3E-04	1.4E-03	1.9E-05
4.7E-04	4.9E-03	6.9E-05	2.6E-04	2.7E-03	3.9E-05
9.3E-04	9.7E-03	1.4E-04	5.2E-04	5.5E-03	7.8E-05
1.4E-03	1.5E-02	2.1E-04	7.9E-04	8.2E-03	1.2E-04
2.8E-03	2.9E-02	4.2E-04	1.6E-03	1.6E-02	2.3E-04
5.6E-03	5.8E-02	8.3E-04	3.1E-03	3.3E-02	4.7E-04
1.2E-02	1.3E-01	1.8E-03	6.8E-03	7.1E-02	1.0E-03
2.4E-02	2.5E-01	3.6E-03	1.4E-02	1.4E-01	2.0E-03
3.6E-02	3.8E-01	5.4E-03	2.0E-02	2.1E-01	3.0E-03
3.5E-04	3.6E-03	5.2E-05	2.0E-04	2.0E-03	2.9E-05
7.0E-04	7.2E-03	1.0E-04	3.9E-04	4.1E-03	5.8E-05
1.4E-03	1.4E-02	2.1E-04	7.8E-04	8.1E-03	1.2E-04
2.1E-03	2.2E-02	3.1E-04	1.2E-03	1.2E-02	1.7E-04
4.2E-03	4.3E-02	6.2E-04	2.3E-03	2.4E-02	3.5E-04
8.3E-03	8.7E-02	1.2E-03	4.7E-03	4.9E-02	7.0E-04
1.8E-02	1.9E-01	2.7E-03	1.0E-02	1.1E-01	1.5E-03
3.6E-02	3.8E-01	5.4E-03	2.0E-02	2.1E-01	3.0E-03
5.4E-02	5.6E-01	8.0E-03	3.0E-02	3.2E-01	4.5E-03

Lake Trout (20-30 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.34	3.55	0.05	0.09	0.89	0.01
0.68	7.10	0.10	0.17	1.77	0.03
1.36	14.19	0.20	0.34	3.55	0.05
2.05	21.29	0.30	0.51	5.32	0.08
4.09	42.57	0.61	1.02	10.64	0.15
8.19	85.14	1.21	2.05	21.29	0.30
17.74	184.48	2.63	4.43	46.12	0.66
35.47	368.96	5.26	8.87	92.24	1.31
53.21	553.43	7.89	13.30	138.36	1.97
0.68	7.10	0.10	0.17	1.77	0.03
1.36	14.19	0.20	0.34	3.55	0.05
2.73	28.38	0.40	0.68	7.10	0.10
4.09	42.57	0.61	1.02	10.64	0.15
8.19	85.14	1.21	2.05	21.29	0.30
16.37	170.29	2.43	4.09	42.57	0.61
35.47	368.96	5.26	8.87	92.24	1.31
70.95	737.91	10.52	17.74	184.48	2.63
106.42	1106.87	15.78	26.60	276.72	3.94
1.02	10.58	0.15	0.25	2.65	0.04
2.03	21.16	0.30	0.51	5.29	0.08
4.07	42.32	0.60	1.02	10.58	0.15
6.10	63.48	0.90	1.53	15.87	0.23
12.21	126.97	1.81	3.05	31.74	0.45
24.41	253.94	3.62	6.10	63.48	0.90
52.90	550.20	7.84	13.22	137.55	1.96
105.80	1100.40	15.68	26.45	275.10	3.92
158.69	1650.59	23.53	39.67	412.65	5.88

Lake Trout (20-30 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
1.71	17.74	0.25
3.41	35.48	0.51
6.82	70.95	1.01
10.23	106.43	1.52
20.46	212.86	3.03
40.93	425.72	6.07
88.68	922.39	13.15
177.36	1844.78	26.30
266.04	2767.17	39.44
3.41	35.48	0.51
6.82	70.95	1.01
13.64	141.91	2.02
20.46	212.86	3.03
40.93	425.72	6.07
81.86	851.44	12.14
177.36	1844.78	26.30
354.73	3689.56	52.59
532.09	5534.35	78.89
5.09	52.90	0.75
10.17	105.81	1.51
20.35	211.61	3.02
30.52	317.42	4.52
61.04	634.84	9.05
122.07	1269.69	18.10
264.49	2750.99	39.21
528.98	5501.98	78.42
793.47	8252.97	117.64

Lake Trout (>30 inches)

Tissue Concentration (85 + 86)	ppm
Mean	8.3
Max.	17
Min.	4.5
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc
0.114	1	0.000312	0.000037	0.000076	0.000020
0.114	2	0.000625	0.000074	0.000152	0.000040
0.114	4	0.001249	0.000148	0.000303	0.000080
0.114	6	0.001874	0.000222	0.000455	0.000120
0.114	12	0.003748	0.000444	0.000910	0.000241
0.114	24	0.007496	0.000889	0.001820	0.000482
0.114	52	0.016241	0.001926	0.003944	0.001044
0.114	104	0.032482	0.003851	0.007889	0.002088
0.114	156	0.048723	0.005777	0.011833	0.003132
0.228	1	0.000625	0.000074	0.000152	0.000040
0.228	2	0.001249	0.000148	0.000303	0.000080
0.228	4	0.002499	0.000296	0.000607	0.000161
0.228	6	0.003748	0.000444	0.000910	0.000241
0.228	12	0.007496	0.000889	0.001820	0.000482
0.228	24	0.014992	0.001778	0.003641	0.000964
0.228	52	0.032482	0.003851	0.007889	0.002088
0.228	104	0.064964	0.007703	0.015777	0.004176
0.228	156	0.097447	0.011554	0.023666	0.006264
0.34	1	0.000932	0.000110	0.000226	0.000060
0.34	2	0.001863	0.000221	0.000452	0.000120
0.34	4	0.003726	0.000442	0.000905	0.000240
0.34	6	0.005589	0.000663	0.001357	0.000359
0.34	12	0.011178	0.001325	0.002715	0.000719
0.34	24	0.022356	0.002651	0.005429	0.001437
0.34	52	0.048438	0.005743	0.011764	0.003114
0.34	104	0.096877	0.011487	0.023527	0.006228
0.34	156	0.145315	0.017230	0.035291	0.009342

Lake Trout (>30 inches)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
2.8E-04	5.8E-04	1.5E-04	1.6E-04	3.2E-04	8.6E-05
5.6E-04	1.2E-03	3.1E-04	3.2E-04	6.5E-04	1.7E-04
1.1E-03	2.3E-03	6.1E-04	6.3E-04	1.3E-03	3.4E-04
1.7E-03	3.5E-03	9.2E-04	9.5E-04	1.9E-03	5.1E-04
3.4E-03	6.9E-03	1.8E-03	1.9E-03	3.9E-03	1.0E-03
6.8E-03	1.4E-02	3.7E-03	3.8E-03	7.8E-03	2.1E-03
1.5E-02	3.0E-02	7.9E-03	8.2E-03	1.7E-02	4.5E-03
2.9E-02	6.0E-02	1.6E-02	1.6E-02	3.4E-02	8.9E-03
4.4E-02	9.0E-02	2.4E-02	2.5E-02	5.1E-02	1.3E-02
5.6E-04	1.2E-03	3.1E-04	3.2E-04	6.5E-04	1.7E-04
1.1E-03	2.3E-03	6.1E-04	6.3E-04	1.3E-03	3.4E-04
2.3E-03	4.6E-03	1.2E-03	1.3E-03	2.6E-03	6.9E-04
3.4E-03	6.9E-03	1.8E-03	1.9E-03	3.9E-03	1.0E-03
6.8E-03	1.4E-02	3.7E-03	3.8E-03	7.8E-03	2.1E-03
1.4E-02	2.8E-02	7.3E-03	7.6E-03	1.6E-02	4.1E-03
2.9E-02	6.0E-02	1.6E-02	1.6E-02	3.4E-02	8.9E-03
5.9E-02	1.2E-01	3.2E-02	3.3E-02	6.7E-02	1.8E-02
8.8E-02	1.8E-01	4.8E-02	4.9E-02	1.0E-01	2.7E-02
8.4E-04	1.7E-03	4.6E-04	4.7E-04	9.7E-04	2.6E-04
1.7E-03	3.4E-03	9.1E-04	9.4E-04	1.9E-03	5.1E-04
3.4E-03	6.9E-03	1.8E-03	1.9E-03	3.9E-03	1.0E-03
5.0E-03	1.0E-02	2.7E-03	2.8E-03	5.8E-03	1.5E-03
1.0E-02	2.1E-02	5.5E-03	5.7E-03	1.2E-02	3.1E-03
2.0E-02	4.1E-02	1.1E-02	1.1E-02	2.3E-02	6.1E-03
4.4E-02	8.9E-02	2.4E-02	2.5E-02	5.0E-02	1.3E-02
8.7E-02	1.8E-01	4.7E-02	4.9E-02	1.0E-01	2.7E-02
1.3E-01	2.7E-01	7.1E-02	7.4E-02	1.5E-01	4.0E-02

Lake Trout (>30 inches)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.8	1.7	0.4	0.2	0.4	0.1
1.6	3.4	0.9	0.4	0.8	0.2
3.3	6.7	1.8	0.8	1.7	0.4
4.9	10.1	2.7	1.2	2.5	0.7
9.9	20.2	5.4	2.5	5.1	1.3
19.8	40.5	10.7	4.9	10.1	2.7
42.8	87.7	23.2	10.7	21.9	5.8
85.6	175.3	46.4	21.4	43.8	11.6
128.4	263.0	69.6	32.1	65.7	17.4
1.6	3.4	0.9	0.4	0.8	0.2
3.3	6.7	1.8	0.8	1.7	0.4
6.6	13.5	3.6	1.6	3.4	0.9
9.9	20.2	5.4	2.5	5.1	1.3
19.8	40.5	10.7	4.9	10.1	2.7
39.5	80.9	21.4	9.9	20.2	5.4
85.6	175.3	46.4	21.4	43.8	11.6
171.2	350.6	92.8	42.8	87.7	23.2
256.8	525.9	139.2	64.2	131.5	34.8
2.5	5.0	1.3	0.6	1.3	0.3
4.9	10.1	2.7	1.2	2.5	0.7
9.8	20.1	5.3	2.5	5.0	1.3
14.7	30.2	8.0	3.7	7.5	2.0
29.5	60.3	16.0	7.4	15.1	4.0
58.9	120.7	31.9	14.7	30.2	8.0
127.6	261.4	69.2	31.9	65.4	17.3
255.3	522.8	138.4	63.8	130.7	34.6
382.9	784.2	207.6	95.7	196.1	51.9

Lake Trout (>30 inches)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
4.1	8.4	2.2
8.2	16.9	4.5
16.5	33.7	8.9
24.7	50.6	13.4
49.4	101.1	26.8
98.8	202.3	53.5
214.0	438.3	116.0
427.9	876.5	232.0
641.9	1314.8	348.0
8.2	16.9	4.5
16.5	33.7	8.9
32.9	67.4	17.8
49.4	101.1	26.8
98.8	202.3	53.5
197.5	404.5	107.1
427.9	876.5	232.0
855.9	1753.0	464.0
1283.8	2629.5	696.0
12.3	25.1	6.7
24.5	50.3	13.3
49.1	100.5	26.6
73.6	150.8	39.9
147.3	301.6	79.8
294.5	603.3	159.7
638.2	1307.1	346.0
1276.3	2614.1	692.0
1914.5	3921.2	1038.0

Perch (All Sizes)

Tissue Concentration (85 + 86)	ppm
Mean	0.19
Max.	0.4
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000002	0.000000
0.114	2	0.000625	0.000002	0.000004	0.000000
0.114	4	0.001249	0.000003	0.000007	0.000000
0.114	6	0.001874	0.000005	0.000011	0.000000
0.114	12	0.003748	0.000010	0.000021	0.000000
0.114	24	0.007496	0.000020	0.000043	0.000000
0.114	52	0.016241	0.000044	0.000093	0.000000
0.114	104	0.032482	0.000088	0.000186	0.000000
0.114	156	0.048723	0.000132	0.000278	0.000000
0.228	1	0.000625	0.000002	0.000004	0.000000
0.228	2	0.001249	0.000003	0.000007	0.000000
0.228	4	0.002499	0.000007	0.000014	0.000000
0.228	6	0.003748	0.000010	0.000021	0.000000
0.228	12	0.007496	0.000020	0.000043	0.000000
0.228	24	0.014992	0.000041	0.000086	0.000000
0.228	52	0.032482	0.000088	0.000186	0.000000
0.228	104	0.064964	0.000176	0.000371	0.000000
0.228	156	0.097447	0.000264	0.000557	0.000000
0.34	1	0.000932	0.000003	0.000005	0.000000
0.34	2	0.001863	0.000005	0.000011	0.000000
0.34	4	0.003726	0.000010	0.000021	0.000000
0.34	6	0.005589	0.000015	0.000032	0.000000
0.34	12	0.011178	0.000030	0.000064	0.000000
0.34	24	0.022356	0.000061	0.000128	0.000000
0.34	52	0.048438	0.000131	0.000277	0.000000
0.34	104	0.096877	0.000263	0.000554	0.000000
0.34	156	0.145315	0.000394	0.000830	0.000000

Perch (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
6.4E-06	1.4E-05	0.0E+00	3.6E-06	7.6E-06	0.0E+00
1.3E-05	2.7E-05	0.0E+00	7.2E-06	1.5E-05	0.0E+00
2.6E-05	5.4E-05	0.0E+00	1.4E-05	3.0E-05	0.0E+00
3.9E-05	8.1E-05	0.0E+00	2.2E-05	4.6E-05	0.0E+00
7.7E-05	1.6E-04	0.0E+00	4.3E-05	9.1E-05	0.0E+00
1.5E-04	3.3E-04	0.0E+00	8.7E-05	1.8E-04	0.0E+00
3.4E-04	7.1E-04	0.0E+00	1.9E-04	4.0E-04	0.0E+00
6.7E-04	1.4E-03	0.0E+00	3.8E-04	7.9E-04	0.0E+00
1.0E-03	2.1E-03	0.0E+00	5.6E-04	1.2E-03	0.0E+00
1.3E-05	2.7E-05	0.0E+00	7.2E-06	1.5E-05	0.0E+00
2.6E-05	5.4E-05	0.0E+00	1.4E-05	3.0E-05	0.0E+00
5.2E-05	1.1E-04	0.0E+00	2.9E-05	6.1E-05	0.0E+00
7.7E-05	1.6E-04	0.0E+00	4.3E-05	9.1E-05	0.0E+00
1.5E-04	3.3E-04	0.0E+00	8.7E-05	1.8E-04	0.0E+00
3.1E-04	6.5E-04	0.0E+00	1.7E-04	3.7E-04	0.0E+00
6.7E-04	1.4E-03	0.0E+00	3.8E-04	7.9E-04	0.0E+00
1.3E-03	2.8E-03	0.0E+00	7.5E-04	1.6E-03	0.0E+00
2.0E-03	4.2E-03	0.0E+00	1.1E-03	2.4E-03	0.0E+00
1.9E-05	4.0E-05	0.0E+00	1.1E-05	2.3E-05	0.0E+00
3.8E-05	8.1E-05	0.0E+00	2.2E-05	4.5E-05	0.0E+00
7.7E-05	1.6E-04	0.0E+00	4.3E-05	9.1E-05	0.0E+00
1.2E-04	2.4E-04	0.0E+00	6.5E-05	1.4E-04	0.0E+00
2.3E-04	4.9E-04	0.0E+00	1.3E-04	2.7E-04	0.0E+00
4.6E-04	9.7E-04	0.0E+00	2.6E-04	5.5E-04	0.0E+00
1.0E-03	2.1E-03	0.0E+00	5.6E-04	1.2E-03	0.0E+00
2.0E-03	4.2E-03	0.0E+00	1.1E-03	2.4E-03	0.0E+00
3.0E-03	6.3E-03	0.0E+00	1.7E-03	3.5E-03	0.0E+00

Perch (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.1	0.0	0.0	0.0	0.0
0.1	0.2	0.0	0.0	0.0	0.0
0.1	0.2	0.0	0.0	0.1	0.0
0.2	0.5	0.0	0.1	0.1	0.0
0.5	1.0	0.0	0.1	0.2	0.0
1.0	2.1	0.0	0.2	0.5	0.0
2.0	4.1	0.0	0.5	1.0	0.0
2.9	6.2	0.0	0.7	1.5	0.0
0.0	0.1	0.0	0.0	0.0	0.0
0.1	0.2	0.0	0.0	0.0	0.0
0.2	0.3	0.0	0.0	0.1	0.0
0.2	0.5	0.0	0.1	0.1	0.0
0.5	1.0	0.0	0.1	0.2	0.0
0.9	1.9	0.0	0.2	0.5	0.0
2.0	4.1	0.0	0.5	1.0	0.0
3.9	8.2	0.0	1.0	2.1	0.0
5.9	12.4	0.0	1.5	3.1	0.0
0.1	0.1	0.0	0.0	0.0	0.0
0.1	0.2	0.0	0.0	0.1	0.0
0.2	0.5	0.0	0.1	0.1	0.0
0.3	0.7	0.0	0.1	0.2	0.0
0.7	1.4	0.0	0.2	0.4	0.0
1.3	2.8	0.0	0.3	0.7	0.0
2.9	6.2	0.0	0.7	1.5	0.0
5.8	12.3	0.0	1.5	3.1	0.0
8.8	18.5	0.0	2.2	4.6	0.0

Perch (All Sizes)

Hazard Index RfD = 0.000009 Mean Conc.	Max. Conc.	Offspring Min. Conc.
0.1	0.2	0.0
0.2	0.4	0.0
0.4	0.8	0.0
0.6	1.2	0.0
1.1	2.4	0.0
2.3	4.8	0.0
4.9	10.3	0.0
9.8	20.6	0.0
14.7	30.9	0.0
0.2	0.4	0.0
0.4	0.8	0.0
0.8	1.6	0.0
1.1	2.4	0.0
2.3	4.8	0.0
4.5	9.5	0.0
9.8	20.6	0.0
19.6	41.2	0.0
29.4	61.9	0.0
0.3	0.6	0.0
0.6	1.2	0.0
1.1	2.4	0.0
1.7	3.5	0.0
3.4	7.1	0.0
6.7	14.2	0.0
14.6	30.8	0.0
29.2	61.5	0.0
43.8	92.3	0.0

Walleye (All Sizes)

Tissue Concentration (85 + 86)	ppm
Mean	0.67
Max.	3.55
Min.	0.06
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000003	0.000016	0.000000
0.114	2	0.000625	0.000006	0.000032	0.000001
0.114	4	0.001249	0.000012	0.000063	0.000001
0.114	6	0.001874	0.000018	0.000095	0.000002
0.114	12	0.003748	0.000036	0.000190	0.000003
0.114	24	0.007496	0.000072	0.000380	0.000006
0.114	52	0.016241	0.000155	0.000824	0.000014
0.114	104	0.032482	0.000311	0.001647	0.000028
0.114	156	0.048723	0.000466	0.002471	0.000042
0.228	1	0.000625	0.000006	0.000032	0.000001
0.228	2	0.001249	0.000012	0.000063	0.000001
0.228	4	0.002499	0.000024	0.000127	0.000002
0.228	6	0.003748	0.000036	0.000190	0.000003
0.228	12	0.007496	0.000072	0.000380	0.000006
0.228	24	0.014992	0.000143	0.000760	0.000013
0.228	52	0.032482	0.000311	0.001647	0.000028
0.228	104	0.064964	0.000622	0.003295	0.000056
0.228	156	0.097447	0.000933	0.004942	0.000084
0.34	1	0.000932	0.000009	0.000047	0.000001
0.34	2	0.001863	0.000018	0.000094	0.000002
0.34	4	0.003726	0.000036	0.000189	0.000003
0.34	6	0.005589	0.000053	0.000283	0.000005
0.34	12	0.011178	0.000107	0.000567	0.000010
0.34	24	0.022356	0.000214	0.001134	0.000019
0.34	52	0.048438	0.000464	0.002457	0.000042
0.34	104	0.096877	0.000927	0.004913	0.000083
0.34	156	0.145315	0.001391	0.007370	0.000125

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
2.3E-05	1.2E-04	2.0E-06	1.3E-05	6.8E-05	1.1E-06
4.5E-05	2.4E-04	4.1E-06	2.6E-05	1.4E-04	2.3E-06
9.1E-05	4.8E-04	8.1E-06	5.1E-05	2.7E-04	4.6E-06
1.4E-04	7.2E-04	1.2E-05	7.7E-05	4.1E-04	6.9E-06
2.7E-04	1.4E-03	2.4E-05	1.5E-04	8.1E-04	1.4E-05
5.5E-04	2.9E-03	4.9E-05	3.1E-04	1.6E-03	2.7E-05
1.2E-03	6.3E-03	1.1E-04	6.6E-04	3.5E-03	5.9E-05
2.4E-03	1.3E-02	2.1E-04	1.3E-03	7.0E-03	1.2E-04
3.5E-03	1.9E-02	3.2E-04	2.0E-03	1.1E-02	1.8E-04
4.5E-05	2.4E-04	4.1E-06	2.6E-05	1.4E-04	2.3E-06
9.1E-05	4.8E-04	8.1E-06	5.1E-05	2.7E-04	4.6E-06
1.8E-04	9.6E-04	1.6E-05	1.0E-04	5.4E-04	9.1E-06
2.7E-04	1.4E-03	2.4E-05	1.5E-04	8.1E-04	1.4E-05
5.5E-04	2.9E-03	4.9E-05	3.1E-04	1.6E-03	2.7E-05
1.1E-03	5.8E-03	9.8E-05	6.1E-04	3.2E-03	5.5E-05
2.4E-03	1.3E-02	2.1E-04	1.3E-03	7.0E-03	1.2E-04
4.7E-03	2.5E-02	4.2E-04	2.7E-03	1.4E-02	2.4E-04
7.1E-03	3.8E-02	6.3E-04	4.0E-03	2.1E-02	3.6E-04
6.8E-05	3.6E-04	6.1E-06	3.8E-05	2.0E-04	3.4E-06
1.4E-04	7.2E-04	1.2E-05	7.6E-05	4.0E-04	6.8E-06
2.7E-04	1.4E-03	2.4E-05	1.5E-04	8.1E-04	1.4E-05
4.1E-04	2.2E-03	3.6E-05	2.3E-04	1.2E-03	2.0E-05
8.1E-04	4.3E-03	7.3E-05	4.6E-04	2.4E-03	4.1E-05
1.6E-03	8.6E-03	1.5E-04	9.1E-04	4.8E-03	8.2E-05
3.5E-03	1.9E-02	3.2E-04	2.0E-03	1.0E-02	1.8E-04
7.0E-03	3.7E-02	6.3E-04	4.0E-03	2.1E-02	3.5E-04
1.1E-02	5.6E-02	9.5E-04	5.9E-03	3.1E-02	5.3E-04

Hazard Index RfD = 0.000045		Thyroid	Hazard Index RfD = 0.00018		Liver
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
0.07	0.35	0.01	0.02	0.09	0.00
0.13	0.70	0.01	0.03	0.18	0.00
0.27	1.41	0.02	0.07	0.35	0.01
0.40	2.11	0.04	0.10	0.53	0.01
0.80	4.22	0.07	0.20	1.06	0.02
1.59	8.45	0.14	0.40	2.11	0.04
3.45	18.30	0.31	0.86	4.58	0.08
6.91	36.61	0.62	1.73	9.15	0.15
10.36	54.91	0.93	2.59	13.73	0.23
0.13	0.70	0.01	0.03	0.18	0.00
0.27	1.41	0.02	0.07	0.35	0.01
0.53	2.82	0.05	0.13	0.70	0.01
0.80	4.22	0.07	0.20	1.06	0.02
1.59	8.45	0.14	0.40	2.11	0.04
3.19	16.90	0.29	0.80	4.22	0.07
6.91	36.61	0.62	1.73	9.15	0.15
13.82	73.21	1.24	3.45	18.30	0.31
20.73	109.82	1.86	5.18	27.46	0.46
0.20	1.05	0.02	0.05	0.26	0.00
0.40	2.10	0.04	0.10	0.52	0.01
0.79	4.20	0.07	0.20	1.05	0.02
1.19	6.30	0.11	0.30	1.57	0.03
2.38	12.60	0.21	0.59	3.15	0.05
4.76	25.20	0.43	1.19	6.30	0.11
10.30	54.59	0.92	2.58	13.65	0.23
20.61	109.18	1.85	5.15	27.29	0.46
30.91	163.77	2.77	7.73	40.94	0.69

Hazard Index RfD = 0.000009 Mean Conc.	Max. Conc.	Offspring Min. Conc.
0.33	1.76	0.03
0.66	3.52	0.06
1.33	7.04	0.12
1.99	10.56	0.18
3.99	21.12	0.36
7.97	42.24	0.71
17.27	91.52	1.55
34.54	183.03	3.09
51.82	274.55	4.64
0.66	3.52	0.06
1.33	7.04	0.12
2.66	14.08	0.24
3.99	21.12	0.36
7.97	42.24	0.71
15.94	84.48	1.43
34.54	183.03	3.09
69.09	366.07	6.19
103.63	549.10	9.28
0.99	5.25	0.09
1.98	10.50	0.18
3.96	21.00	0.35
5.94	31.49	0.53
11.89	62.99	1.06
23.78	125.98	2.13
51.51	272.95	4.61
103.03	545.89	9.23
154.54	818.84	13.84

Whitefish (All Sizes)

Tissue Concentration (85 + 86)	ppm
Mean	0.913
Max.	1.6
Min.	0.52
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean Conc.	Max. Conc.	Min. Conc.
0.114	1	0.000312	0.000004	0.000007	0.000002
0.114	2	0.000625	0.000008	0.000014	0.000005
0.114	4	0.001249	0.000016	0.000029	0.000009
0.114	6	0.001874	0.000024	0.000043	0.000014
0.114	12	0.003748	0.000049	0.000086	0.000028
0.114	24	0.007496	0.000098	0.000171	0.000056
0.114	52	0.016241	0.000212	0.000371	0.000121
0.114	104	0.032482	0.000424	0.000742	0.000241
0.114	156	0.048723	0.000635	0.001114	0.000362
0.228	1	0.000625	0.000008	0.000014	0.000005
0.228	2	0.001249	0.000016	0.000029	0.000009
0.228	4	0.002499	0.000033	0.000057	0.000019
0.228	6	0.003748	0.000049	0.000086	0.000028
0.228	12	0.007496	0.000098	0.000171	0.000056
0.228	24	0.014992	0.000196	0.000343	0.000111
0.228	52	0.032482	0.000424	0.000742	0.000241
0.228	104	0.064964	0.000847	0.001485	0.000483
0.228	156	0.097447	0.001271	0.002227	0.000724
0.34	1	0.000932	0.000012	0.000021	0.000007
0.34	2	0.001863	0.000024	0.000043	0.000014
0.34	4	0.003726	0.000049	0.000085	0.000028
0.34	6	0.005589	0.000073	0.000128	0.000042
0.34	12	0.011178	0.000146	0.000255	0.000083
0.34	24	0.022356	0.000292	0.000511	0.000166
0.34	52	0.048438	0.000632	0.001107	0.000360
0.34	104	0.096877	0.001264	0.002214	0.000720
0.34	156	0.145315	0.001895	0.003321	0.001079

Whitefish (All Sizes)

Upper Limit (95%) on Risk q* =7.6 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =4.27 Mg/Kg/Day-1		
Mean Conc.	Max. Conc.	Min. Conc.	Mean Conc.	Max. Conc.	Min. Conc.
3.1E-05	5.4E-05	1.8E-05	1.7E-05	3.0E-05	9.9E-06
6.2E-05	1.1E-04	3.5E-05	3.5E-05	6.1E-05	2.0E-05
1.2E-04	2.2E-04	7.1E-05	7.0E-05	1.2E-04	4.0E-05
1.9E-04	3.3E-04	1.1E-04	1.0E-04	1.8E-04	5.9E-05
3.7E-04	6.5E-04	2.1E-04	2.1E-04	3.7E-04	1.2E-04
7.4E-04	1.3E-03	4.2E-04	4.2E-04	7.3E-04	2.4E-04
1.6E-03	2.8E-03	9.2E-04	9.0E-04	1.6E-03	5.2E-04
3.2E-03	5.6E-03	1.8E-03	1.8E-03	3.2E-03	1.0E-03
4.8E-03	8.5E-03	2.8E-03	2.7E-03	4.8E-03	1.5E-03
6.2E-05	1.1E-04	3.5E-05	3.5E-05	6.1E-05	2.0E-05
1.2E-04	2.2E-04	7.1E-05	7.0E-05	1.2E-04	4.0E-05
2.5E-04	4.3E-04	1.4E-04	1.4E-04	2.4E-04	7.9E-05
3.7E-04	6.5E-04	2.1E-04	2.1E-04	3.7E-04	1.2E-04
7.4E-04	1.3E-03	4.2E-04	4.2E-04	7.3E-04	2.4E-04
1.5E-03	2.6E-03	8.5E-04	8.3E-04	1.5E-03	4.8E-04
3.2E-03	5.6E-03	1.8E-03	1.8E-03	3.2E-03	1.0E-03
6.4E-03	1.1E-02	3.7E-03	3.6E-03	6.3E-03	2.1E-03
9.7E-03	1.7E-02	5.5E-03	5.4E-03	9.5E-03	3.1E-03
9.2E-05	1.6E-04	5.3E-05	5.2E-05	9.1E-05	3.0E-05
1.8E-04	3.2E-04	1.1E-04	1.0E-04	1.8E-04	5.9E-05
3.7E-04	6.5E-04	2.1E-04	2.1E-04	3.6E-04	1.2E-04
5.5E-04	9.7E-04	3.2E-04	3.1E-04	5.5E-04	1.8E-04
1.1E-03	1.9E-03	6.3E-04	6.2E-04	1.1E-03	3.5E-04
2.2E-03	3.9E-03	1.3E-03	1.2E-03	2.2E-03	7.1E-04
4.8E-03	8.4E-03	2.7E-03	2.7E-03	4.7E-03	1.5E-03
9.6E-03	1.7E-02	5.5E-03	5.4E-03	9.5E-03	3.1E-03
1.4E-02	2.5E-02	8.2E-03	8.1E-03	1.4E-02	4.6E-03

Whitefish (All Sizes)

Hazard Index RfD = 0.000045 Mean Conc.	Max. Conc.	Thyroid Min. Conc.	Hazard Index RfD = 0.00018 Mean Conc.	Max. Conc.	Liver Min. Conc.
0.1	0.2	0.1	0.0	0.0	0.0
0.2	0.3	0.1	0.0	0.1	0.0
0.4	0.6	0.2	0.1	0.2	0.1
0.5	1.0	0.3	0.1	0.2	0.1
1.1	1.9	0.6	0.3	0.5	0.2
2.2	3.8	1.2	0.5	1.0	0.3
4.7	8.2	2.7	1.2	2.1	0.7
9.4	16.5	5.4	2.4	4.1	1.3
14.1	24.7	8.0	3.5	6.2	2.0
0.2	0.3	0.1	0.0	0.1	0.0
0.4	0.6	0.2	0.1	0.2	0.1
0.7	1.3	0.4	0.2	0.3	0.1
1.1	1.9	0.6	0.3	0.5	0.2
2.2	3.8	1.2	0.5	1.0	0.3
4.3	7.6	2.5	1.1	1.9	0.6
9.4	16.5	5.4	2.4	4.1	1.3
18.8	33.0	10.7	4.7	8.2	2.7
28.2	49.5	16.1	7.1	12.4	4.0
0.3	0.5	0.2	0.1	0.1	0.0
0.5	0.9	0.3	0.1	0.2	0.1
1.1	1.9	0.6	0.3	0.5	0.2
1.6	2.8	0.9	0.4	0.7	0.2
3.2	5.7	1.8	0.8	1.4	0.5
6.5	11.4	3.7	1.6	2.8	0.9
14.0	24.6	8.0	3.5	6.2	2.0
28.1	49.2	16.0	7.0	12.3	4.0
42.1	73.8	24.0	10.5	18.5	6.0

Whitefish (All Sizes)

Mean Conc.	Hazard Index RfD = 0.000009 Max. Conc.	Offspring Min. Conc.
0.5	0.8	0.3
0.9	1.6	0.5
1.8	3.2	1.0
2.7	4.8	1.5
5.4	9.5	3.1
10.9	19.0	6.2
23.5	41.2	13.4
47.1	82.5	26.8
70.6	123.7	40.2
0.9	1.6	0.5
1.8	3.2	1.0
3.6	6.3	2.1
5.4	9.5	3.1
10.9	19.0	6.2
21.7	38.1	12.4
47.1	82.5	26.8
94.1	165.0	53.6
141.2	247.5	80.4
1.3	2.4	0.8
2.7	4.7	1.5
5.4	9.5	3.1
8.1	14.2	4.6
16.2	28.4	9.2
32.4	56.8	18.5
70.2	123.0	40.0
140.4	246.0	80.0
210.6	369.1	119.9