The Effects of Toxaphene, Chlordane and 2,3,4,7,8-Pentachlorodibenzofuran on Lake Trout and White Sucker in an Ecosytem Experiment and the Distribution and Effects of 2,3,4,7,8-Pentachlorodibenzofuran on White Suckers and Broodstock Rainbow Trout in Laboratory Experiments

By

Peter David Delorme

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

Department of Zoology
University of Manitoba
Winnipeg, Manitoba

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Abstract

Samples of lake trout (*Salvelinus namaycush*) and white suckers (*Catastomus commersoni*) populations from a small lake (30 ha. surface area) located in the Experimental Lakes Area (ELA) in northwestern Ontario were treated with a single intraperitoneal injection of one of three toxicants, toxaphene (7 µg/g or 3.5 µg/g), chlordane (7 µg/g) or 2,3,4,7,8-pentachlorodibenzofuran (P_5CDF, 1 ng/g). Fish were tagged with individually coded tags, weighed, measured, treated, and released back into the lake. Fish were subsequently recaptured during spring (white sucker) and fall (lake trout) in 1989, 1990, 1991 1992, and, 1993. Meristic data were taken on recaptured fish and a subsample were sacrificed for residue and biochemical analyses. In 1990 and 1991 fish were spawned to assess reproductive success and survival of offspring.

Analysis of recapture data for white suckers showed decreased survival of toxaphene- and chlordane-treated suckers compared with controls, resulting in decreased abundance of treated groups. Survival of lake trout was lower than controls for toxaphene-, chlordane- and P_5CDF-treated fish, also resulting in decreased abundance. Clear decreases in growth were only evident in P_5CDF-treated white suckers. No differences were found in fertilization rate, total survival or incidence of deformities in eggs and fry from female lake trout treated with toxaphene or chlordane in 1990 and 1991. No differences were found in eggs from P_5CDF-treated fish in 1990, but there were suspected spawning failures in 1991 and 1992. Fertilization rates and survival to swim-up of white sucker eggs and fry from females in all treatment groups were decreased relative to controls in both 1990 and 1991. Residue analyses of parents and eggs for both suckers and lake trout showed maternal transfer of contaminant from female

to eggs; levels in eggs were much lower than in treated females but were still significantly higher than in controls. Residue analyses in tissues from adults showed that depuration of these contaminants was significantly slower than previously reported for both suckers and lake trout. Estimated half-lives for toxaphene were 314 and 793 days in lake trout and white sucker respectively, 553 and 569 days for chlordane and 3527 and 1469 days for P_5CDF. Biochemical analyses of P_5CDF-treated fish showed sustained induction of liver mixed function oxidase (MFO) enzyme activity (4 years) in both suckers and trout, and decreases in levels of retinoid stores in hepatic tissues.

In the laboratory, white suckers were injected with P_5CDF to examine the time course of its distribution among tissues and its effect on MFO enzymes. Samples were taken at weeks 1, 3, 7 and 12 after treatment and concentrations of P_5CDF were determined in muscle, liver, gonad, kidney, spleen, intestines and red blood cells. Visceral organs (spleen, intestine, kidney, liver and gonads) had similar concentrations in week 1, approximately 3 ng/g, while those in blood and muscle were lower. All tissues except muscle and gonad showed decreasing concentrations over time. Muscle and intestine accounted for 60 to 80% of total P_5CDF in each fish at the end of the experiment. Tissue distributions appear to be similar to those found in feeding studies.

Ethoxyresorufin-O-dethylase (EROD) activity was significantly induced at weeks 3, 7 and 12. Induction increased over time, with the greatest induction found 12 weeks after injection. EROD activity was correlated with liver concentrations of P_5CDF, but this relationship changed from week to week.

In a parallel laboratory experiment, broodstock rainbow trout (*Oncorhynchus mykiss*) were treated with P_5CDF and allowed to undergo gametogenesis. Despite large increases in MFO activity and decreases in retinoids, few effects were found on the reproductive competence of the adults. Fertilization success was slightly decreased in crosses from treated females and total survival was decreased in crosses with two treated parents.

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Special thanks to Dr. Scott Brown who has given unselfishly his time and expertise. It was at his urging that the rainbow trout laboratory study was conducted and that measurement of retinoids were done. Scott has spent many hours as a scientific foil throughout the course of my studies, often forcing me to refine my arguments and ideas.

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Preface

The research done for this thesis is an effort to take experimental toxicology beyond its traditional place in the laboratory to the field. The field experiment involved exposure of subsets of populations of either lake trout or white suckers to one of three contaminants; toxaphene, chlordane or 2,3,4,7,8-pentachlordibenzofuran (P_5CDF). Exposed and control groups were then examined over 5 years to obtain data on the effects of these treatments. Three disciplines within environmental toxicology were studied: ecology (growth, survival, reproduction), biochemistry (hepatic MFO activity, bone hydroxyproline levels, hepatic retinoids), and environmental chemistry (residue levels, depuration rate). In addition two laboratory experiments were conducted to help better understand certain aspects which could not be studied in the field.

For the major experiment, the field study, the thesis is divided into chapters roughly along the lines of the components of ecology, biochemistry and environmental chemistry Each chapter contains introduction, methodology, results and discussion. A general introduction is included at the beginning of the thesis to familiarize the reader with the background information relevant to the thesis as a whole. Following this, the first two chapters present the ecological results, survival and growth (Chapter 1) and reproduction (Chapter 2). Chapter 3 presents effects of P_5CDF on MFO activity and retinoid levels and chapter 4 presents the effects of toxaphene on bone biochemistry. Chapter 5 presents the environmental chemistry of the three toxicants used. The two laboratory studies are presented in Chapters 6 and 7. Chapter 8 summarizes the results and attempts an assessment of potential impacts on populations.

The discussions for each of Chapters 1 - 5 include references to the data presented in the other chapters. This was done in order to integrate and link all the results collected from the different field components.

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General Introduction

Toxicology is defined as the study of poisons and their effects. This encompasses many disciplines within the biological and chemical sciences since effects may occur at all levels of biological organization, from molecular through intervening levels to community and ecosystem ecology. Figure 1 illustrates the levels of biological organization, starting at the most fundamental, molecular biology. As one moves through successive levels of organization, each new level encompasses those below hence the level of complexity and the number of factors affecting results increases. In general, effects cascade through the various levels of organization. As described by Adams et al. (1990), effects of stress by contaminants are usually expressed first at the molecular/biochemical level, where pollutants affect the normal functioning of chemical processes in the body. These effects can be detected as biochemical changes (e.g. enzyme activities, changes in cell membranes, genetic material or in the availability of essential biomolecules). Changes at these sub-cellular levels can induce structural and/or functional responses at the next higher level of biological organization, and these can impair more complex processes such as hormonal regulation, metabolism, electrolyte balance and proper functioning of the immune system. These effects, in turn, may eventually alter the organism's ability to grow, survive and/or reproduce. Ultimately, irreversible and detrimental effects may be observed at any level including population, community or ecosystem.

The severity and time course of response by an organism will depend on the extent of exposure to toxicants (amount and duration of dose), the toxicity of the chemical and the susceptibility of the organism. The time it takes for a response to be manifested is also dependent on the level of organization being studied. Biochemical responses may be manifested in seconds to days, whereas effects on individuals may take

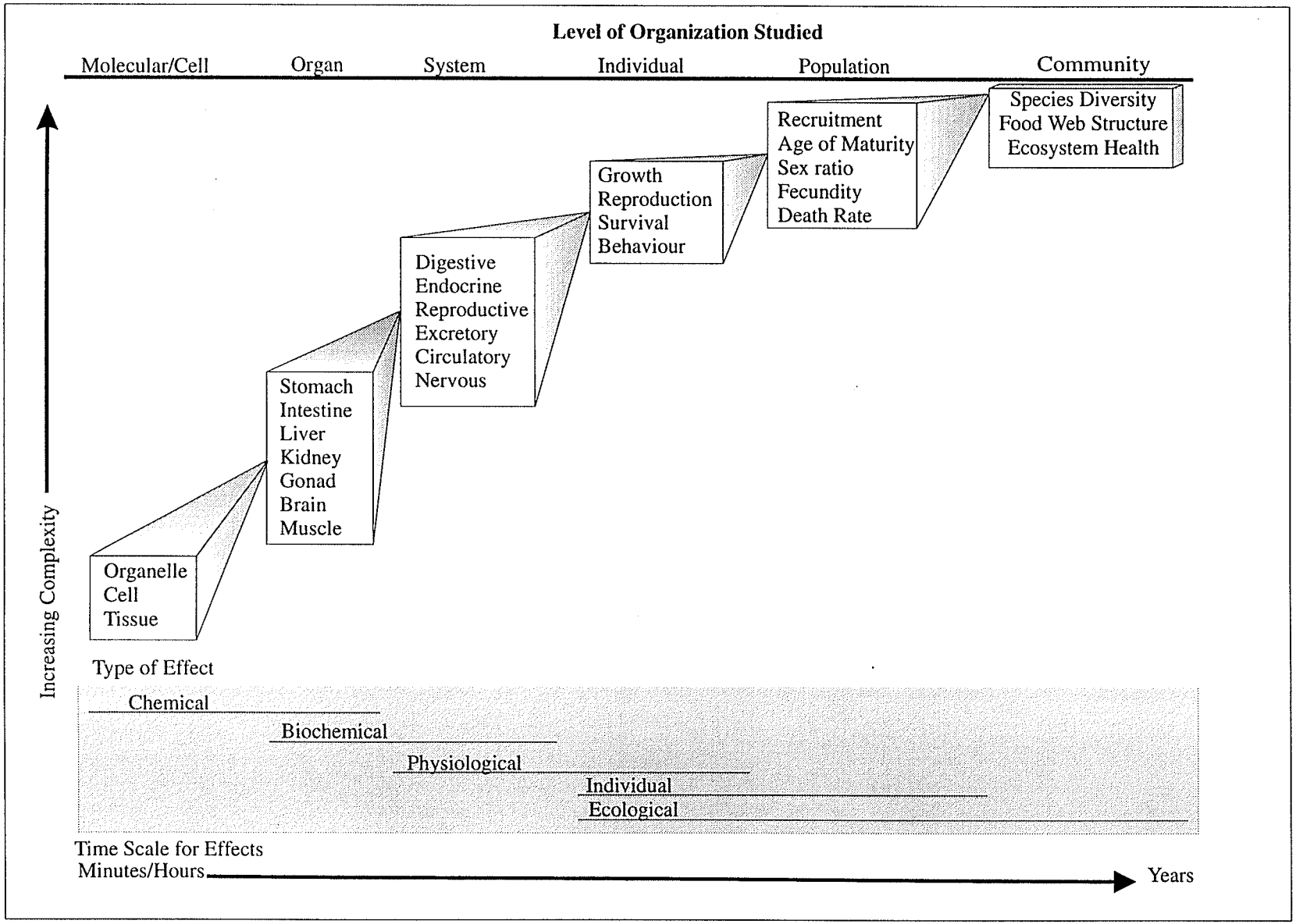

Figure 1. Levels of biological organization studied and affected by contaminants.

days to months, and perturbations in populations or communities may take years (Peakall 1992). Unfortunately, by the time effects are manifested at the higher levels of organization it is often difficult to ascertain the cause of the perturbation and it is usually too late for remediation of the problem (Mayer et al. 1992). It is also difficult to link responses at lower levels of biological organization to effects at higher levels of organization because of the complexity and number of factors which must be considered.

The traditional approach to studying the toxicity of chemicals has been to expose groups of organisms to a range of doses and report the responses. These studies, conducted in laboratories, typically look at responses within a particular level of biological organization, e.g. effects on hormones, effects on histology, effects on growth, etc., the most popular being mortality. Because of the limitations imposed by undertaking experiments with large animals in a laboratory, responses are limited to the level of the whole organism and below. The question of effects at higher levels of biological organization, (i.e. population, community and ecosystem) cannot be explored except for cases with very small organisms in simulated ecosystems (micro- and mesocosms). Laboratory research often identifies biological measures (biomarkers, bioindicators) which can be utilized to monitor exposure to and effects caused by contaminants in natural populations. Biomarkers can be defined as measurements of body fluids, cells or tissues that indicate in biochemical or cellular terms the presence of contaminants or the magnitude of an organism's response to contaminants (McCarthy and Shugart 1990).

The key requirement is to link biochemical and molecular biomarkers, which respond relatively quickly to contaminant exposure, to effects at higher levels of biological organization, which show effects over a longer time scale. A very limited amount of experimental toxicology has been done in "Real World" field settings, although a vast amount of monitoring has been done, both for contaminant

concentrations, and in recent times, for various biomarkers indicative of specific groups of contaminants.

Contamination of aquatic ecosystems with persistent chemicals has emerged as a global issue in the past 30 years. Levels of contamination have often exceeded toxic thresholds for aquatic life, leading to perturbations of ecosystems. Even in cases where acutely toxic levels have not been reached, the effects of continuous exposure to sublethal levels of environmental contaminants may be just as detrimental to long-term ecosystem stability as effects from short term high concentrations. However, in such cases the link between cause and effect is often very difficult to establish, given the complexity of most ecosystems and the number of factors, both natural and anthropogenic, which can cause ecological changes.

Many chlorinated pesticides and other chlorinated aromatic hydrocarbons that have been banned or are severely restricted in use still persist in the environment at sublethal levels which may be harmful to ecosystem health. Two such examples are toxaphene and chlordane. Toxaphene was withdrawn from the Canadian market in 1983. Chlordane has been restricted in use since 1985 and has been withdrawn from markets in the U.S. Despite decreased use, these compounds, and many other organochlorines, are still major contaminants in aquatic ecosystems in North America. Toxaphene is a major contaminant in the Great Lakes (Rice and Evans 1984) and in the Arctic (Muir et al. 1990a, Bidleman et al. 1993, Stern et al. 1992), and chlordane was determined to be widespread in recent (1976-1984) monitoring of pesticides in aquatic systems (Schmitt et al. 1985,1990), including the Great Lakes (Gooch et al. 1990) and in the Arctic (Norstrom et al. 1988).

Another group of contaminants are those which include (sometimes unknown) by-products of chemical manufacture (Rappe and Buser 1989), and compounds formed by chemical transformations during processes such as low temperature combustion (Olie et al. 1977). Polychlorinated-dibenzofurans (PCDF's) fall into this category and are

recognized as trace contaminants in the Great Lakes and other areas (Baumann and Whittle 1988, Hallet and Brooksbank 1986, Gardner and White 1990).

The chemical nature of these substances is such that they persist, they bioconcentrate in organisms and they biomagnify in aquatic food chains. All three of the contaminants used in this study have high octanol:water partition coefficients (K_{ow}); that is, they tend to partition from water to fatty material. Hence they become more concentrated in animals than in the surrounding water. They are also biomagnified; that is, organisms higher in the food chain have higher levels of contamination.

The effects of high body burdens of these chemicals on long-term growth, survival and reproduction of fish are unclear. Long term experiments (i.e. > 1 year) are lacking. As well, many of the toxicological methods used presently rely on introduction of the contaminant through exposure in the water column or contained in food. As previously noted these types of experiments are done in the laboratory, are short term and tend to use levels of toxicants which are higher than those found in ecosystems.

Current State of Knowledge

Toxaphene

Toxaphene is an insecticide that was heavily used in cotton-producing areas of the United States following the ban on the use of DDT in the early 1970's (Eisler and Jacknow 1985). It was also used as a piscicide in Canada and parts of the U.S. in the middle to late 1950's to control rough (undesirable) fish in lakes. This use was abandoned when it was found to be too persistent for successful restocking of lakes with desirable species (Stringer and McMynn 1958, 1960, Prevost 1960, Fukano and Hooper 1958, Terriere et al 1966).

Toxaphene is a mixture of more than 650 closely related compounds (Saleh 1983) of chlorinated camphene (Figure 2a). Because of this mixed composition it is difficult to identify and quantify residues in environmental samples. Identification of components is made more difficult by the interference of other xenobiotic compounds (such as PCBs) during analysis (Sullivan and Armstrong 1985). Toxaphene's water solubility is in the range of 0.5 - 3.0 mg/L (Paris et al. 1977, Brooks 1974), and its octanol:water partition coefficient has been reported to be relatively high, in the range of $10^{3.5}$ to $10^{6.4}$, therefore, toxaphene would be expected to bioconcentrate in organisms (Paris et al. 1977). The average elemental composition of toxaphene is $C_{10}H_{10}Cl_8$; however, individual compounds may contain from 6 to 10 chlorine atoms on the parent hydrocarbon structure (Sullivan and Armstrong 1985). As with other complex synthetic organic compounds like PCBs, dioxins and furans, the toxicity of toxaphene is related to the structures of the various components (Chandurkar et al. 1979).

Although never heavily used in Canada, toxaphene has become a major contaminant in the Great Lakes (Rice and Evans 1984) and in the Arctic (Lockhart et al. 1987, Muir et al. 1988,1990b). For example, lake trout from Lake Michigan have shown levels up to 10 mg/kg wet weight (Sullivan and Armstrong 1985), and open water values up to 0.7 ng/L have been measured. Burbot from the Mackenzie River drainage have up to 5 mg/kg in their livers (Lockhart et al. 1987). The primary source of toxaphene to aquatic systems in areas where it was not used is through aerial deposition (Rice et al. 1986, Bidleman and Olney 1975, Swackhammer and Hites 1988, Rappaport and Eisenreich 1986)

Given that toxaphene was the most heavily used insecticide in the U.S. and many parts of the world, relatively little information on effects in aquatic ecosystems is available when compared with contaminants such as DDT and PCBs. The best synopsis of information to date is contained in the review by Saleh (1991). Toxaphene is acutely

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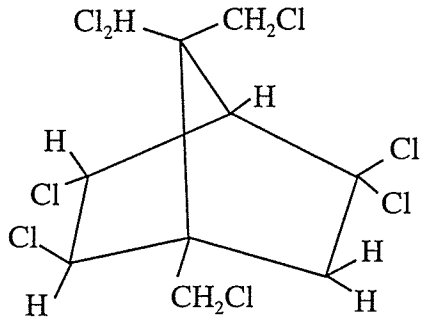

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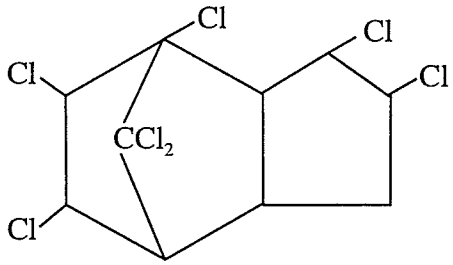

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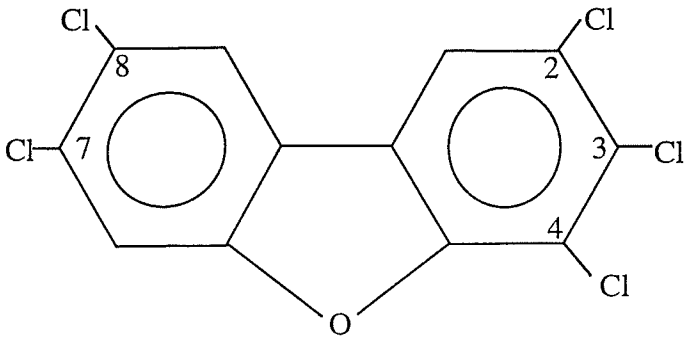


Figure 2. General structures of the three contaminants used in the study a) toxaphene, b) chlordane and c) 2,3,4,7,8-pentachlorodibenzofuran.

toxic to fish with a lethal concentration for freshwater fish in the range of 5 - 100 µg/L (Saleh 1991). It is also highly bioaccumulated and biomagnified in aquatic ecosystems. Effects in freshwater fish, documented in laboratory exposures, include behavioral disruptions in goldfish (Pollock and Kilgore 1978), reduced growth in fathead minnow, brook trout and channel catfish (Mayer et al. 1975, 1977), increased incidence of backbone abnormalities and mortality in developing fathead minnow and channel catfish (Mayer et al. 1977), and decreased viability of ova in brook trout (Mayer et al. 1977).

Chlordane

Chlordane (Figure 2b) is also an organochlorine insecticide which contains a mixture of compounds consisting primarily of cis- and trans-chlordane, trans-nonachlor and heptachlor. Originally used as a soil insecticide, again as a DDT replacement, it is now used primarily to control termites and similar pests (NRCC 1974). In Canada, application has been restricted since 1985 to the control of subterranean insects, and its use has been banned altogether in the United Stated. Despite limited use, levels in the aquatic environment appear to be increasing. It was identified in 38% of fish sampled in watersheds contributing to the Great Lakes (Veith et al. 1984) and all burbot (*Lota lota*) sampled from western and northern Canada (Lockhart et al. 1987). The range was from less than 1 to 2570 ng/g wet weight in the Great Lakes study and from 6 to 241 ng/g in livers of northern burbot. Chlordane was also identified as a major contaminant in aquatic ecosystems by the U.S. Fish and Wildlife Service during monitoring programs carried out across the United States (Schmitt et al. 1985,1990). Few if any data exist on chronic effects of chlordane on fish or on fish reproduction. Acute toxicity (96-h LC50) has been reported for several species of salmonids: coho salmon (14 µg/L), rainbow trout (42 µg/L), brown trout (11.1 µg/L) as well as for fathead minnows (115 µg/L) and

channel catfish (6.7 µg/L) (Johnson and Finley 1980). These tests were all done on juvenile fish between 0.6 g and 1.9 g wet weight.

Dibenzofurans

Polychlorinated-dibenzofurans (PCDFs) (Figure 2c) are produced through the combustion of municipal wastes, leaded automobile fuels and by low temperature combustion of PCBs (Buser et al. 1978, Clement et al. 1985, Marklund et al. 1987). They are dispersed globally and deposited through aerial deposition. In Canada, the major source appears to be as trace contaminants in chlorophenols, which are used as wood preservatives by the forest industry (NRCC 1984). PCDFs are also found in effluents from bleached kraft mills (Rappe et al. 1987, Servos et al. 1994). Levels of PCDFs in Great Lakes fish are reported to range from < 15 pg/g to > 300 pg/g wet weight (Baumann and Whittle 1988).

It is believed that the accumulation of PCDFs in lake trout and other predacious freshwater fish is entirely from food chain accumulation (Muir et al. 1992). It appears that 2,3,7,8 tetra- to hexachlorodibenzofurans are the only chlorinated dibenzofurans that biomagnify (increase in concentration up the food chain). PCDFs have been shown to be bioaccumulated in carp (Kuehl et al. 1987) but the effects of this bioaccumulation on reproduction have not been studied. 2,3,7,8-tetrachlorodibenzofuran (T_4CDF), was shown by Mehrle et al. (1988) to cause decreases in growth of rainbow trout fry exposed to water concentrations between 0.41 ng/L and 8.78 ng/L. Decreased growth was the most sensitive response to this PCDF congener because it occurred at the lowest level of exposure (0.41 ng/L). Behavioural changes were also noted in fish exposed to T_4CDF, including diminished feeding activity. Mehrle et al. (1988) concluded that 2,3,7,8-T_4CDF is about 1000 times more toxic than toxaphene. Muir et al. (1990c) showed sustained MFO induction in rainbow trout fed 2,3,4,7,8-P_5CDF, but no growth effects after 21 days.

Young chinook salmon taken downstream from pulp mills in the Fraser River showed very high concentrations of PCDDs and PCDFs and striking elevations in liver MFO enzymes (Rogers et al. 1989). Similarly, mature mountain whitefish from the Wapiti River also showed striking induction of MFO enzymes (Kloepper-Sams and Benton 1994).

Thesis Objectives

The research for this thesis represents a new approach to experimental toxicology. The major part of this thesis focuses on an experiment using naturally occuring fish populations in a lake to conduct a long term study following a single intraperitoneal injection of one of three organochlorine contaminants. This approach is unique in two ways; firstly thesub-populations of fish being studied are part of naturally occurring populations, subject to natural environmental fluctuations, and secondly the study extended over 5 years.

The data from the field study can be divided into three components, ecological effects (growth, survival, reproduction), biochemical effects (MFO activity, hydroxyproline levels, retinoid levels), and environmental chemistry (depuration rate, half-life). Each of these components can stand as a separate study, but in this case the results within each component can be integrated to provide a larger picture of the total response.

The objectives of the field study were:

1. To determine the effects of environmentally meaningful body burdens of toxaphene, chlordane and P_5CDF on the survival and/or growth of adult lake trout and white suckers in a natural environment.

2. To determine the effects of environmentally meaningful parental body burdens of toxaphene, chlordane and P_5CDF on hatching success and post-hatching survival of lake trout and white sucker eggs and fry.

3. To determine if there are changes in hepatic mixed function oxygenase activity levels, retinoids and tocopherol levels in adults exposed to P_5CDF.

4. To determine if there are changes in bone collagen, hydroxyproline and calcium levels in adults exposed to toxaphene.

5. To measure and describe the depuration rates of the three toxicants in adult lake trout and white suckers under natural conditions.

In addition to the field experiment, two studies were done in the laboratory to assist in the understanding of distribution and effects of toxicants on a shorter time scale (i.e.< 1 year) which could not be accommodated in the field study. The laboratory portion of the study has focused exclusively on 2,3,4,7,8-pentachlorodibenzofuran. The objectives of the first experiment were to:

1. Examine the time course of distribution of P_5CDF following intraperitoneal injection.

2. Examine the time course of induction of mixed function oxidase enzymes following intraperitoneal injection of P_5CDF.

The second experiment examined the effects of injection of brood stock rainbow trout with P_5CDF. This experiment was a parallel experiment to the field study, with the fish being maintained through a reproductive cycle. The objectives of this experiment were to:

1. Examine the effect of P_5CDF on hepatic MFO, retinoids and toxcopherol in parents.

2. Examine the effect of treatment with P_5CDF on reproductive processes in the parents.

3. Assess the effect of maternal transfer of toxicant to developing ova and the effects on the survival of offspring.

Chapter 1. Effects of a Single Intraperitoneal Injection of Toxaphene, Chlordane or 2,3,4,7,8-pentachlorodibenzofuran on Individual Growth and Survival of Natural Populations of Lake Trout (*Salvelinus namaycush*) and White Sucker (*Catostomus commersoni*).

Introduction

Communities within an ecosystem are continually changing. These changes may have their root causes in many different processes, for example, succession, competition, disturbance by weather, etc. Determining the agents causing ecological change is at best difficult and often not possible. The effect of anthropogenic influences on ecosystems further complicates the interpretation of community changes (Evans 1988).

The difficulties determining the agents of change are particularly clear in the case of fish communities within the Laurentian Great Lakes ecosystem. Fish communities in Lakes Erie and Ontario, have changed dramatically over the time that records have been kept (Smith 1968). Some of these community changes can be directly traced to commercial fishing pressures (Smith 1968); some can be attributed to the introduction of exotic species such as sea lamprey, alewife (*Alosa pseudoharengus*), zebra mussels (*Dreissena polymorpha*) and quaga mussels (*Dreissena sp.*), and others have been blamed on the presence of high concentrations of organochlorine contaminants within the ecosystem (Hartman 1988).

Toxic organic contaminants are suspected as a cause of recruitment failures of feral fish populations in various regions of the Laurentian Great Lakes (Mac and Gilbertson 1990). Despite considerable research on organic contaminants and reproduction in Great Lakes fishes (Berlin et al. 1981, Hilton et al. 1983, Morrison et al. 1985a,b,c, Mac and Edsall 1991, Van Der Kraak et al. 1992), it has not been possible to demonstrate convincingly, quantifiable cause and effect relationships between organic contaminants and reproductive effects. Moreover, chemical residue data, biochemical or

pathological impacts (biomarkers) have not yet been linked to changes in populations by concrete experimental evidence (Munkittrick 1993).

One problem in making the link between contaminants and changes in populations is the lack of long term (i.e. > 1 year) experiments which deal with more than a single phase of the life cycle of fish. While laboratory studies typically focus on one phase of the life cycle, egg, embryo, juvenile or adult, very few, if any, are true life cycle experiments (i.e. gametogenesis to death), especially with long-lived species of high economic value. Some of the obvious difficulties in undertaking such experiments are the length of time required, the physical setup to hold fish for this period of time and the problems associated with the treatment with contaminants over such a time period.

One approach to the problem of integrating population, community and ecosystem effects has been used at the Experimental Lakes Area (ELA), where over the past 25 years addition of chemicals to pristine whole lake systems, notably nutrients and acid, have been used to study their impacts (Schindler 1988). This design, while probably the best experimental approach, would seldom if ever be permitted with persistent organochlorines for ethical and regulatory reasons. One alternative to the whole lake addition approach is to treat specific components (i.e. fish, benthos) of the ecosystem or trophic structure and monitor effects of this treatment on organisms in their natural environment. In this way the contaminant is more contained, less contaminant is used and it is less likely that other organisms and substrates of the surrounding ecosystem will become contaminated. In addition, it is possible to remediate the lake by removing the treated fish or other treated components. This latter approach of exposing one component of an ecosystem is the experimental approach taken in this study. Two fish species within a small lake ecosystem in the ELA were treated by injecting known amounts of one of three toxicants into sub-groups of the white sucker and lake trout populations. These fish were then monitored over a five year period for growth, survival, three

biochemical parameters and contaminant burdens. As well, reproductive success was evaluated in two of the five years.

The field study reported in this work is unique in its use of natural populations to assess toxicological effects on survival and growth over a five year period. As previously stated very few if any toxicological studies on fish are done under natural conditions, other than monitoring of organisms for contamination and in some cases attempting to correlate "effects" with body concentrations of stable contaminant burden.

Materials and Methods

Study Site

The study was conducted at the ELA, a research site located in northwestern Ontario (49° 41´ 39" N, 93° 46´ 05" W) operated by the Department of Fisheries and Oceans, approximately 300 km east of Winnipeg (Figure 3). The ELA consists of 40 lakes and their associated watersheds which have been set aside for experimental and research purposes. This study was conducted in lake 260 (henceforth designated as L260), a small oligotrophic lake with a surface area of 32.4 ha. and a maximum depth of 14.6 m (Cleugh and Hauser 1971) (Figure 4).

Fish fauna in the lake consists of a single top predator, lake trout (*Salvelinus namaycush*), an omnivorous bottom feeder, white sucker (*Catastomus commersoni*) and 5 species of cyprinids, pearl dace (*Semotilus margarita*), fathead minnow (*Pimephales promelas*), fine scale dace (*Chrosomus neagrus*), northern redbelly dace (*Chrosomus eos*) and lake chub (*Couesius plumbeus*). L260 is a second order lake with a diffuse inflow (on the east side) and a diffuse outflow (along the north side) which do not allow for immigration or emigration of white suckers or lake trout to or from the lake. The adult lake trout population in the lake has been estimated to be from 325 to 375

Figure 3. Location of the Experimental Lakes Area (ELA)

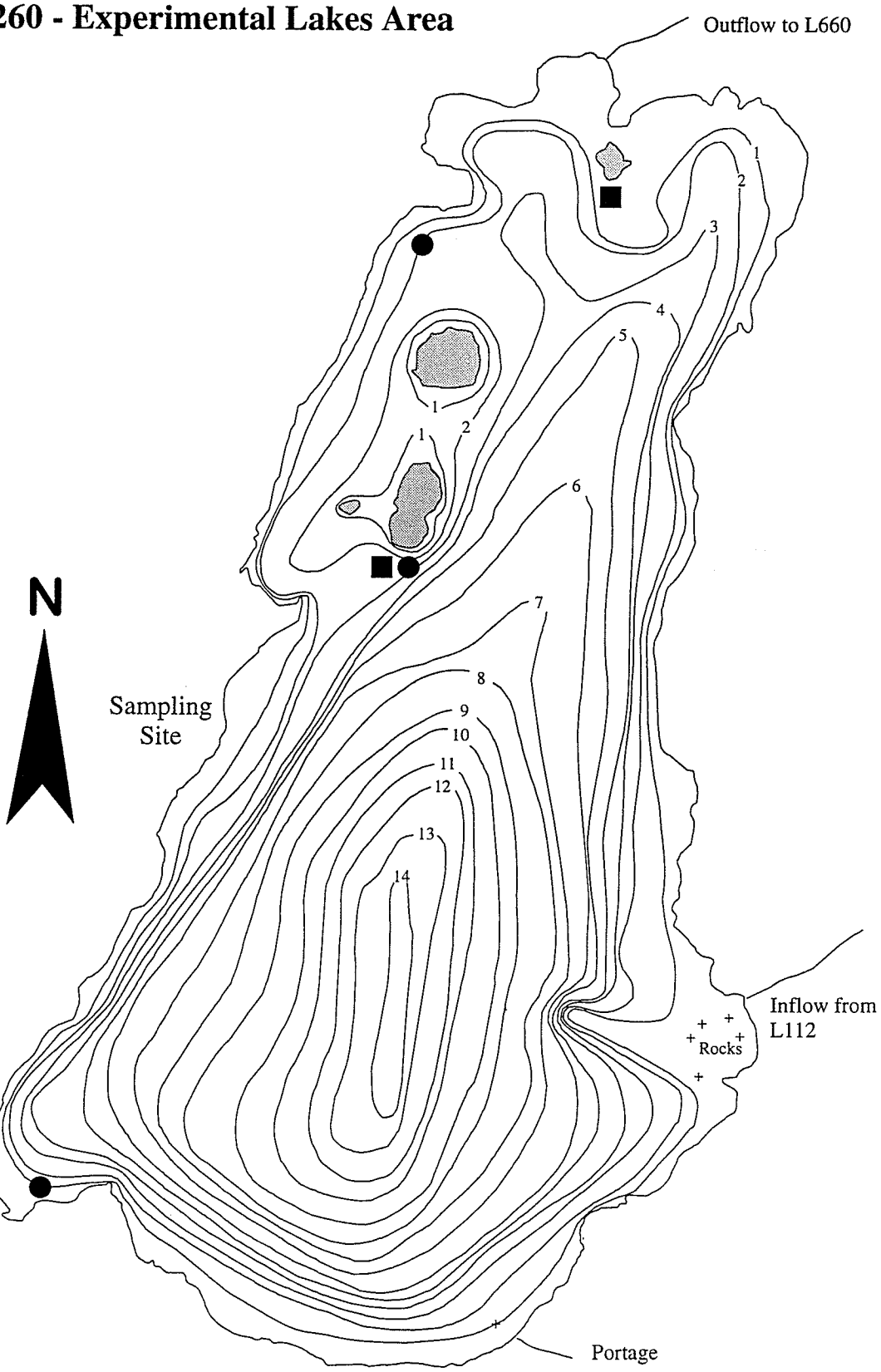


Figure 4. Bathymetry of L260 in the Experimental Lakes Area (ELA). Sampling locations for white suckers (●) and lake trout (■) are indicated.

individuals and the white sucker population is estimated to be from 2200 to 2500 (K.H. Mills, pers. com.). Each of the species studied has a single spawning site within the lake.

Capture and Sampling of Fish

Lake trout and white suckers were captured using several methods including modified trap nets, short (20 minute) sets of small mesh (3.8 cm stretched mesh) gill nets and angling. Trap nets were set in two areas for each species (see Figure 4) and were emptied every second day. Gill netting took place in the early evening as long as the water temperatures remained below 10°C. Fish captured by angling were caught using barbless lead-head jigs baited with minnows. Fish caught by angling or gill netting were held in floating holding pens (1.96 x 1.22 x 1.22 m), until the next day when they were sampled. The maximum density in any pen was 30 fish.

When sampling lake trout or white suckers, fish were removed from pens and/or trap nets and were transported to the sampling site where they were held in tubs until sampled. Fish were anesthetized with tricaine methanosulfonate (also known as MS-222, approximately 0.20 mmol/L) and weighed to the nearest gram using a portable electronic balance (AND, Model 12KD). Total length (TL) and fork length (FL) were measured and recorded for each fish. All lake trout and white suckers were batch marked by scarring dorsal fin rays (Welch and Mills 1981), with the fin ray scarred being incremented by 1 for each capture period. In addition to scarring dorsal fins, each fish was individually tagged with a modified spaghetti tag (White and Beamish 1972) with a unique numeric code. Anal fins were also batch marked to identify the treatment group of each fish in case of tag loss. In years when no injections were done, fish > 400 mm FL were given this type of tag. From the fall of 1988 through to the present (1994) all lake trout were also tagged using a visual implant (VItag®, Northwest Marine Technology, Shaw Island,

WA) which had a unique alpha numeric code. This tag is a 1 x 2 mm piece of film which is injected into the clear adipose tissue posterior to the eye.

Chemicals

Toxaphene and chlordane were obtained from Chem Service (Westchester, Pa.). Each was mixed with corn oil (Sigma Chemical, St. Louis, Mo.) on a weight:volume basis and mixed on a wrist action shaker for 1h. Samples of this preparation were analyzed by the method described in Delorme et al. (1993) to confirm final concentrations of 7 mg/mL corn oil for both toxaphene and chlordane. The doses of toxaphene and chlordane were chosen to be representative of concentrations in fish from moderately to highly contaminated areas such as the Laurentian Great Lakes (Schmitt et al. 1985, 1990, Devault et al. 1988, Swackhammer and Hites 1988)

Uniformly ring-labeled ^{14}C-2,3,4,7,8-pentachlorodibenzofuran (P_5CDF, specific activity = 2.07 X 10^{12} Bq/mol) was obtained from Chemsyn Science Laboratories Ltd. (Lexana, Kansas). The stock solution was purified to >99% by reverse phase thin-layer chromatography (Whatman RP18 plates and a solvent system of acetone:water, 80:20). A corn oil solution was prepared by evaporating an acetone solution of P_5CDF to near dryness under N_2 and then mixing with corn oil. Aliquots of the corn oil solution were assayed by liquid scintillation counting (LSC) to verify a concentration of 1.0 µg/mL.

Injection of Fish

Adult lake trout and white suckers chosen at random were given intraperitoneal injections of corn oil containing either toxaphene, chlordane, or P_5CDF. Injections were done on the ventral surface of the fish approximately one third of the distance from the pectoral to the pelvic fins at a rate of 1 ml/kg wet weight, for nominal doses of 7 µg/g wet weight for toxaphene or chlordane and 1 ng/g of P_5CDF. A sub-group of each species were also injected as controls at the same rate (1 ml/kg) with corn oil. Four months after

the beginning of the study it appeared as if major mortality had occurred in the toxaphene injected lake trout. To maintain an experiment with toxaphene an additional number of lake trout were injected with 3.5 μg/g toxaphene at the next sampling time.

Survival and Abundance

Estimates of survival and abundance of injected fishes were made using a Jolly-Seber death-only model (Jolly 1965). Overall effects of treatment on survival were tested for by using the program RELEASE (Burnham et al. 1987) which uses maximum likelihood estimates of parameters in the models. For the purposes of these analyses each group of injected fish for each species was treated as a separate population. The assumptions of the death-only model (individuals randomly selected, equal probability of capture or recapture, no tag loss, no mortality from tagging procedure, no immigration or emigration) are implicit in the experimental design because no immigration into the populations is possible except through the injection of more individuals and emigration of adults from the lake is not possible.

Calculation of Growth

Growth rates were calculated as instantaneous rates of increase (G) (Ricker 1975). Growth rates were calculated for each recapture of an individual fish. The calculation was as follows:

$$G = \frac{\ln(w_{rc}) - \ln(w_{ic})}{\Delta t}$$

where w_{ic} is the weight at time of initial capture and injection, w_{rc} is the weight at subsequent recapture time(s) and Δt is the number of days between date of recapture and the date of injection. Individual values were averaged to calculate mean instantaneous growth rates for each treatment group (e.g. toxaphene, chlordane, etc.) for each recapture

interval after injection. Only data from those recaptures which were confirmed by tag numbers were used; data from recaptures with tag losses were excluded from these analyses.

Aging

Fish were aged by the method described in Chalanchuk (1984) from pectoral and pelvic fins rays taken when fish were initially tagged. In brief, fins were air dried and then subsequently embedded in epoxy, sectioned using a Buehler ISOMET Low Speed saw and mounted on glass slides with Accu Mount 280 (Baxter Scientific). Ages were validated by removing additional rays when fish were recaptured at least one year after marking, using the method of Mills and Beamish (1980).

Statistical Analyses

Comparison of initial weight and age distributions was done using a Kolmogorov-Smirnov two sample test (Daniels 1978). ANOVA was used in the analysis of initial age and weight. Equality of variance was assessed prior to ANOVA using Bartlett's test (Zar 1984) and, where appropriate, transformations were done based on Taylor's power law (Elliot 1977).

Estimates of survival probability for each recapture period derived from Jolly-Seber mark-recapture modeling were tested for differences between treated and controls for a given recapture period using a Z-test for the normal deviate (i.e. overlap of confidence intervals) (Mills 1981). Overall differences in survival between treated and control groups were tested for by using the program RELEASE (Burnham et al. 1987). This model is based on Jolly-Seber capture-recapture models which deal with multiple recaptures of marked animals. The program was originally designed for the analyses of release-recapture data pertaining to fisheries issues associated with hydroelectric

development along the Columbia River. The authors state that the general theory employed in their model development can be applied to any experiment involving treatment and control groups of marked animals, including the assessment of chronic effects of contaminants on survival (Burnham et al. 1987).

Mean instantaneous growth rates were compared between each treated and control group for each recapture period using ANOVA. Prior to testing for differences in growth rates, the mean weights of treated and control fish calculated for each recapture period were tested for equality (ANOVA), to ensure that calculated growth rates were not biased by one group being significantly larger or smaller than the other. No comparisons were made in cases where 2 or fewer individuals were recaptured in a treatment group in a given recapture interval.

Results

Initial Capture and Treatment

Initial injections of toxaphene and chlordane were done in May of 1988. Injections with P_5CDF were in May, 1989. Because of low recapture rates of toxaphene-injected lake trout in the fall of 1988, it was decided to inject more fish but with half (3.5 mg/kg) the original dose. These injections were done in the fall of 1989. The numbers of fish treated and the dose of each contaminant are presented in Tables 1 and 2. There were no significant differences in the initial mean length, mean weight or mean age of the fish among any of the treated or control groups for either lake trout (Table 1) or white suckers at the beginning of the study (Table 2) (ANOVA, $p \leq 0.05$). Mean age and weight of lake trout and white suckers which were injected in 1989 were compared with controls which had been recaptured in 1989. There was also no difference in age or weight between treated and control fish (ANOVA, $p \leq 0.05$).

Distribution of weights and ages were not different at the time of injection for the different sub-groups of lake trout (Figure 5 & 6) or white suckers (Figure 7 & 8)(Kolmogorov-Smirnov Two Sample test, $p \leq 0.05$).

Lake Trout Survival and Abundance

Toxaphene

Survival of lake trout treated with 7.0 mg/kg was significantly reduced overall ($p=0.0449$) when compared with control fish. Comparison of survival among recapture periods showed significantly decreased survival only in the first 4 months post-treatment (Figure 9). Of the 30 lake trout initially treated only 1 individual was recaptured in the first recapture period after treatment (see Appendix 1 for Jolly-Seber formatted data). Of the 30 treated individuals a total of 14 were recaptured over the 5 recapture periods, whereas 24 of the 30 lake trout injected with corn oil were subsequently recaptured. It appears that survival was not affected after the first year following treatment. Survival was reflected in the estimated population numbers (Figure 10), where a large initial decrease is followed by a gradual decline, similar to that seen in control fish.

Survival of lake trout treated at the lower dose of 3.5 mg/kg toxaphene was not decreased by the treatment with toxaphene (Figure 11). All fish treated with this dose level were seen at least once post-treatment. Estimated population size appeared to be stable throughout the study (Figure 12). The apparent better survival of this treated group of fish compared with controls may have been the result of better handling procedures which were simply the result of increased experience in tagging and injecting fish or it may have been the lower dose.

Table 1. Contaminant dose, numbers injected, mean weight, length and age of lake trout in each group.

Treatment	Dose (µg/g)	Number Injected	Weight (g) ±SE	Length (mm) ±SE	Age (years) ±SE
Corn Oil (Controls)	-	32	638 ± 37	392 ± 10	10.1 ± 1
Toxaphene	7.0	34	639 ± 33	392 ± 9	11.2 ± 1
Chlordane	7.0	32	670 ± 41	399 ± 10	11.0 ± 1
P_5CDF	0.001	25	620 ± 28	385 ± 6	10.1 ± 1
Toxaphene	3.5	20	693 ± 28	404 ± 6	10.8 ± 1

Table 2. Contaminant dose, numbers injected, mean weight, length and age of white sucker in each group.

Treatment	Dose (µg/g)	Number Injected	Weight (g) ±SE	Length (mm) ±SE	Age (years) ±SE
Corn Oil (Controls)	-	69	593 ± 43	343 ± 7	9 ± 0.2
Toxaphene	7.0	74	578 ± 39	339 ± 8	9 ± 0.2
Chlordane	7.0	68	597 ± 40	340 ± 9	9 ± 0.2
P_5CDF	0.001	35	783 ± 48	384 ± 8	10 ± 0.5

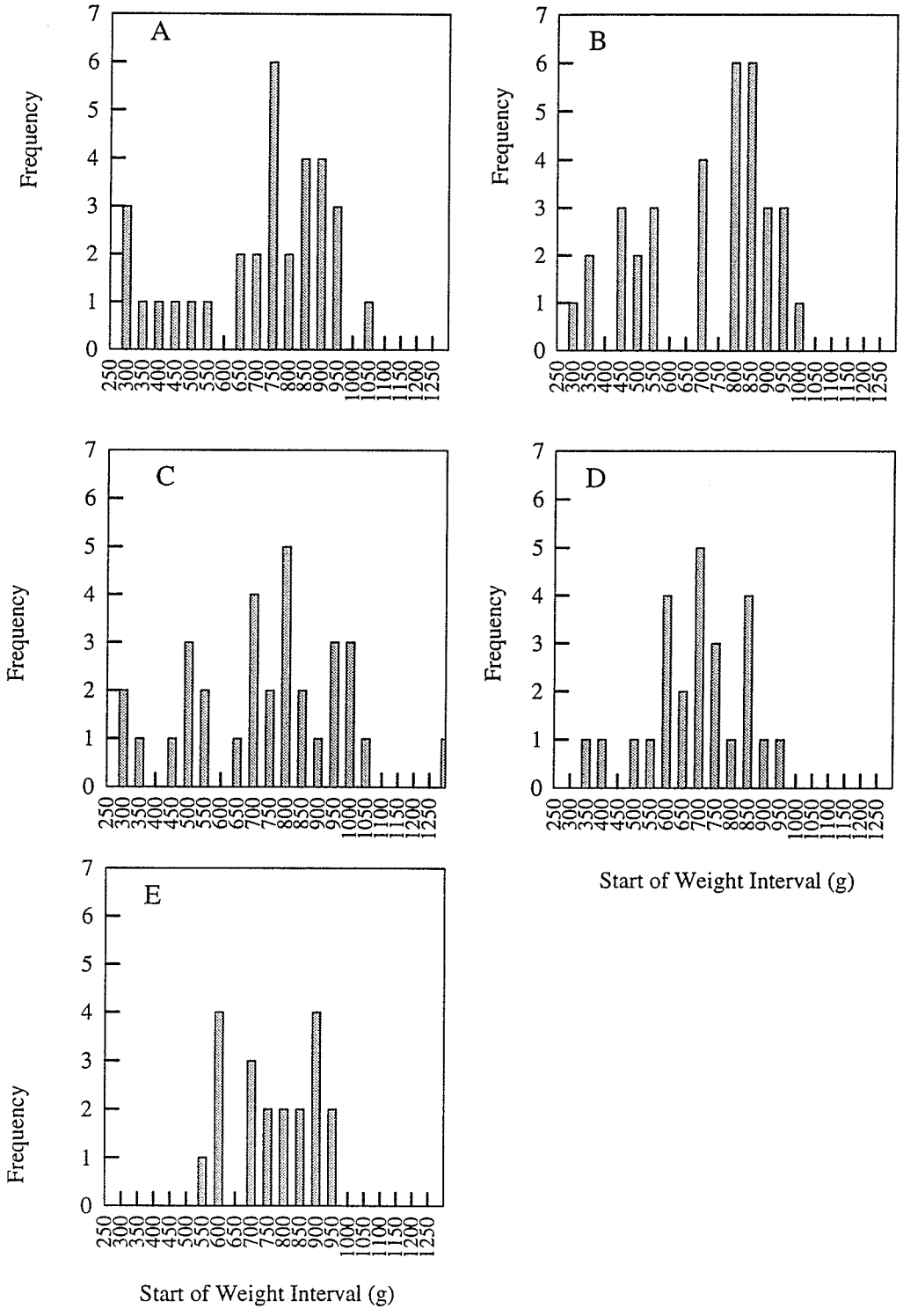


Figure 5. Frequency distribution of lake trout weights at the time of treatment for each treated group: (a) controls, (b) toxaphene 7.0 µg/g, (c) chlordane, (d) P_5CDF, (e) toxaphene 3.5 µg/g.

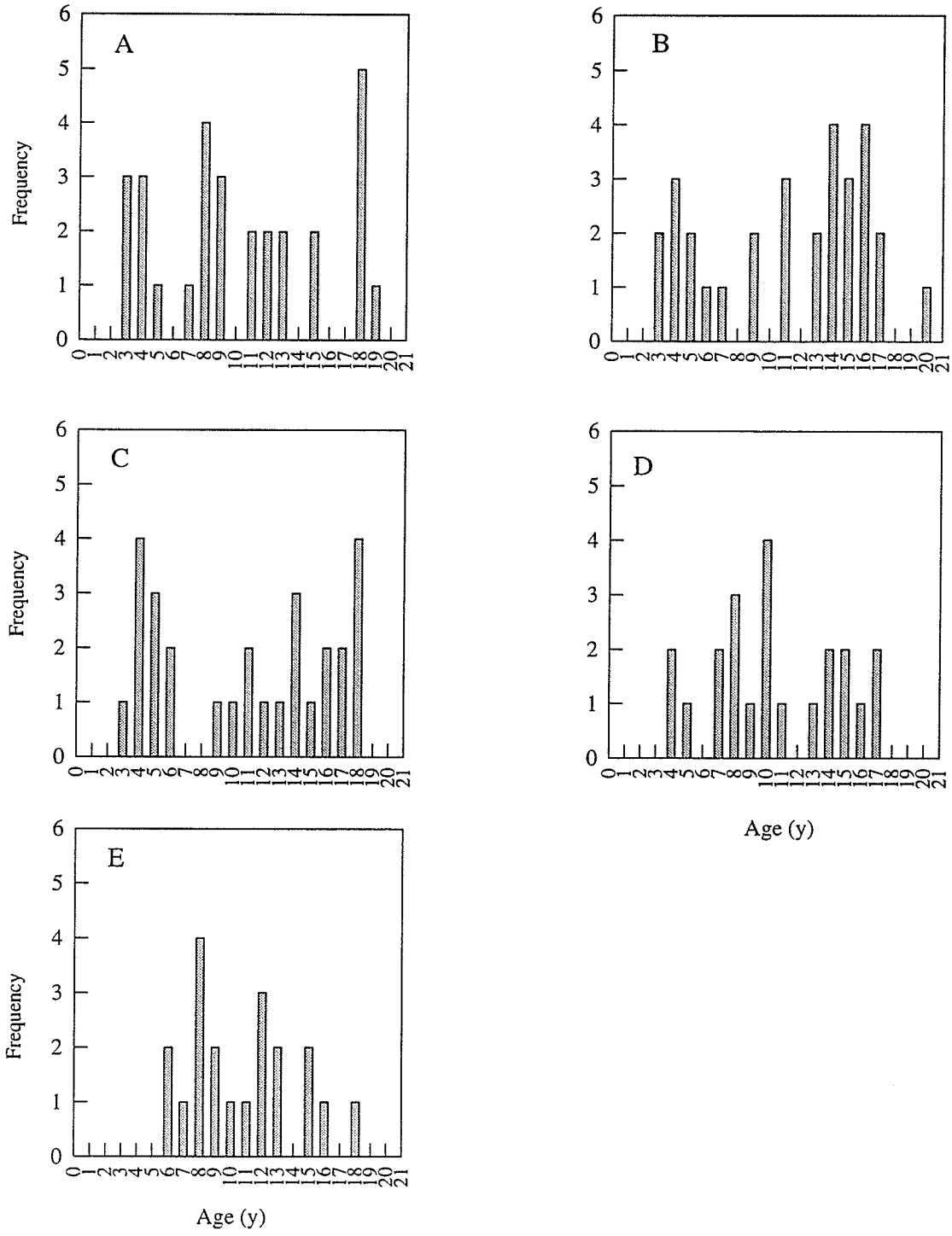


Figure 6. Frequency distribution of lake trout ages at the time of treatment for each treated group: (a) controls, (b) toxaphene 7.0 µg/g, (c) chlordane, (d) P_5CDF, (e) toxaphene 3.5 µg/g.

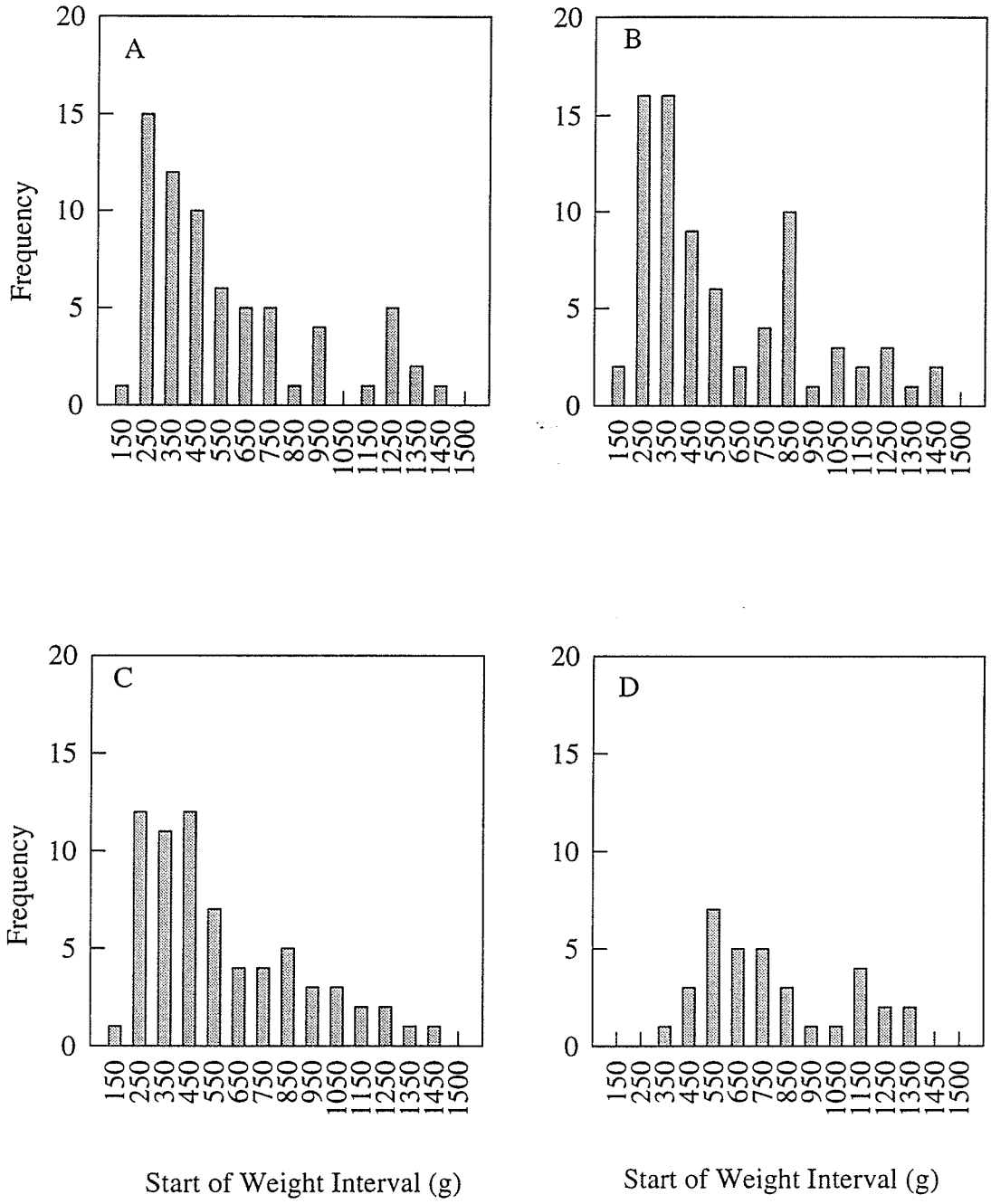


Figure 7. Frequency distribution of white sucker weights at the time of treatment for each treated group: (a) controls, (b) toxaphene, (c) chlordane, (d) P_5CDF.

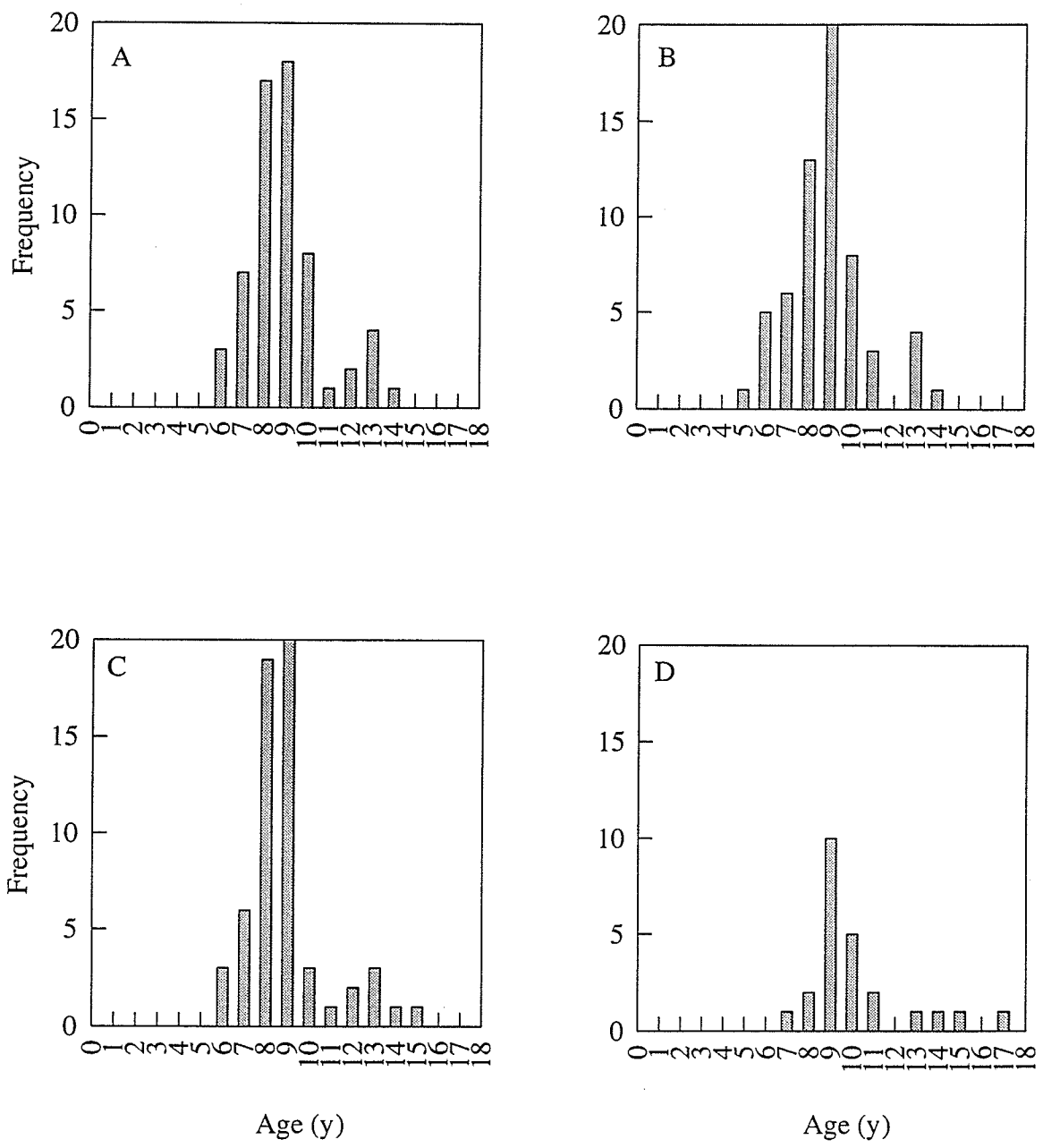


Figure 8. Frequency distribution of white sucker ages at the time of treatment for each treated group: (a) controls, (b) toxaphene, (c) chlordane, (d) P_5CDF.

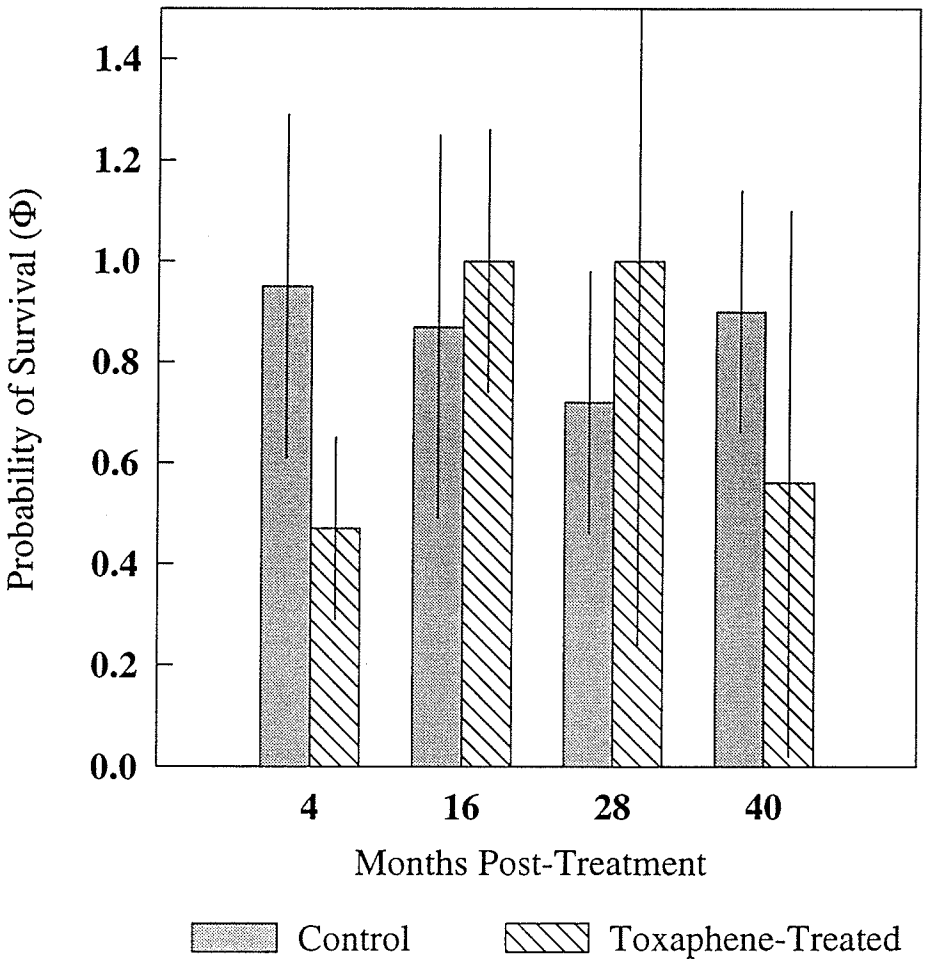


Figure 9. Jolly-Seber estimates of survival probability for control and 7.0 µg/g toxaphene-treated lake trout.

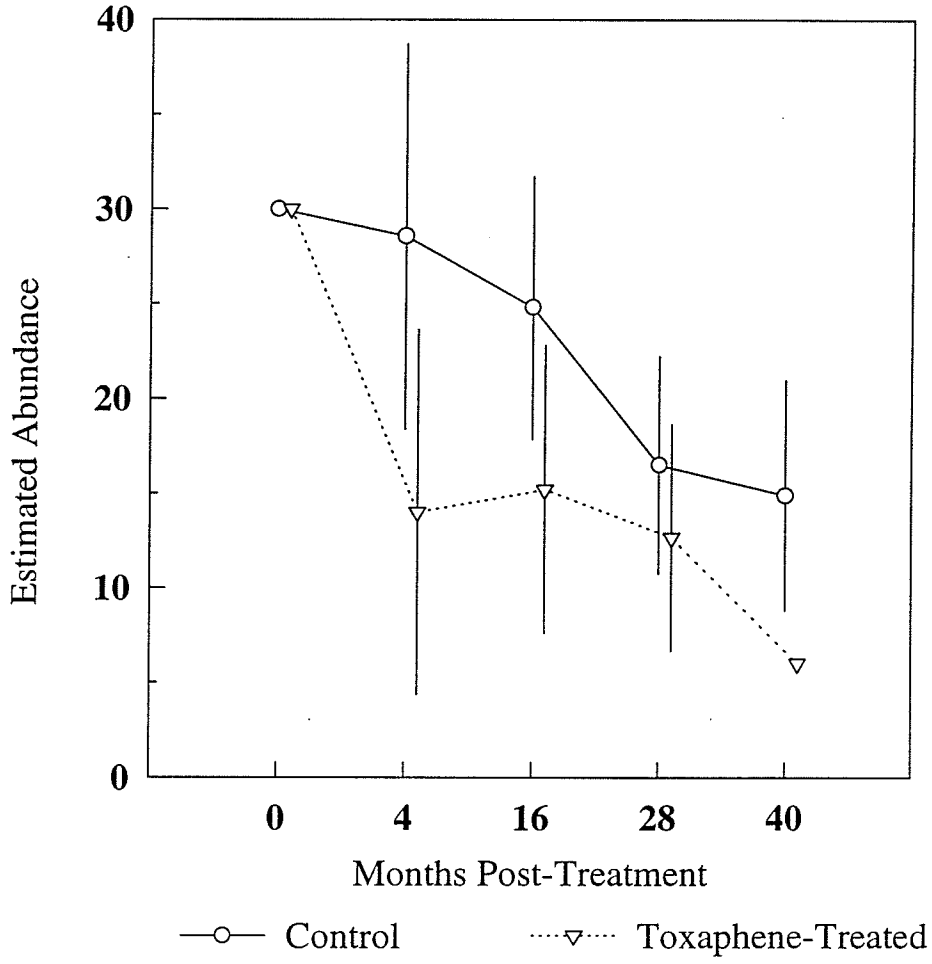


Figure 10. Jolly-Seber estimates of abundance for control and 7.0 µg/g toxaphene-treated lake trout.

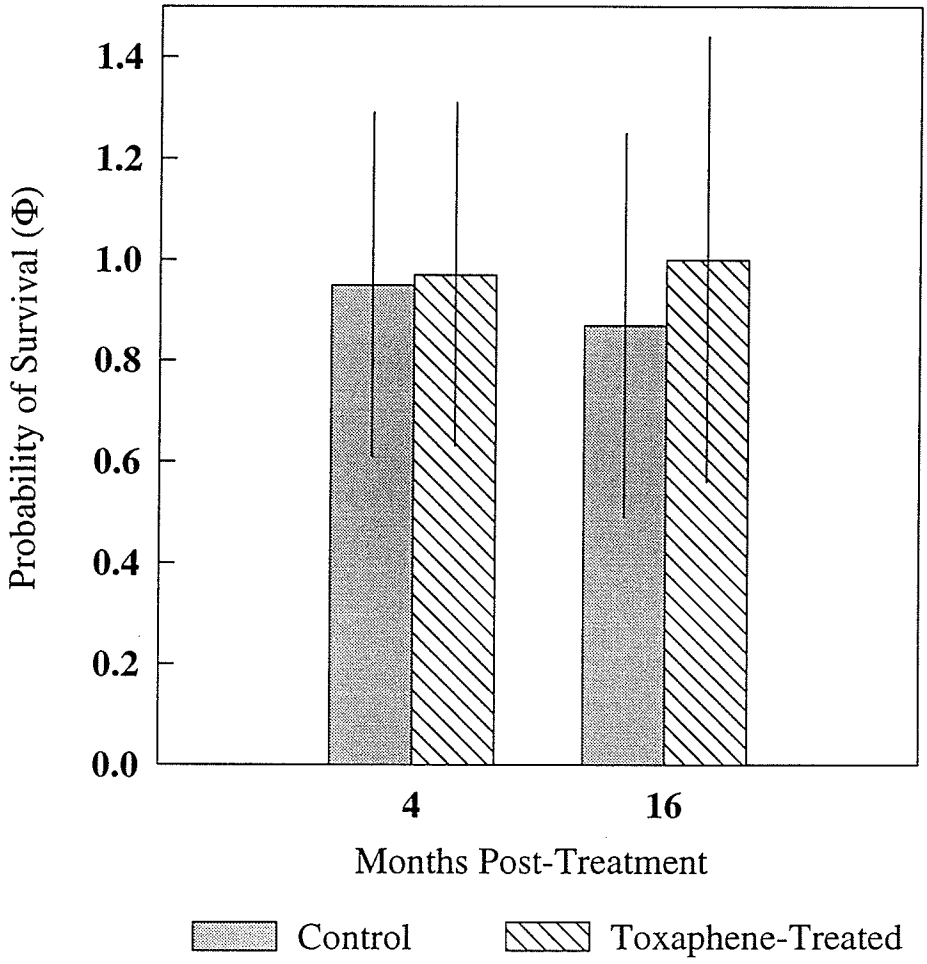


Figure 11. Jolly-Seber estimates of survival probability for control and 3.5 µg/g toxaphene-treated lake trout.

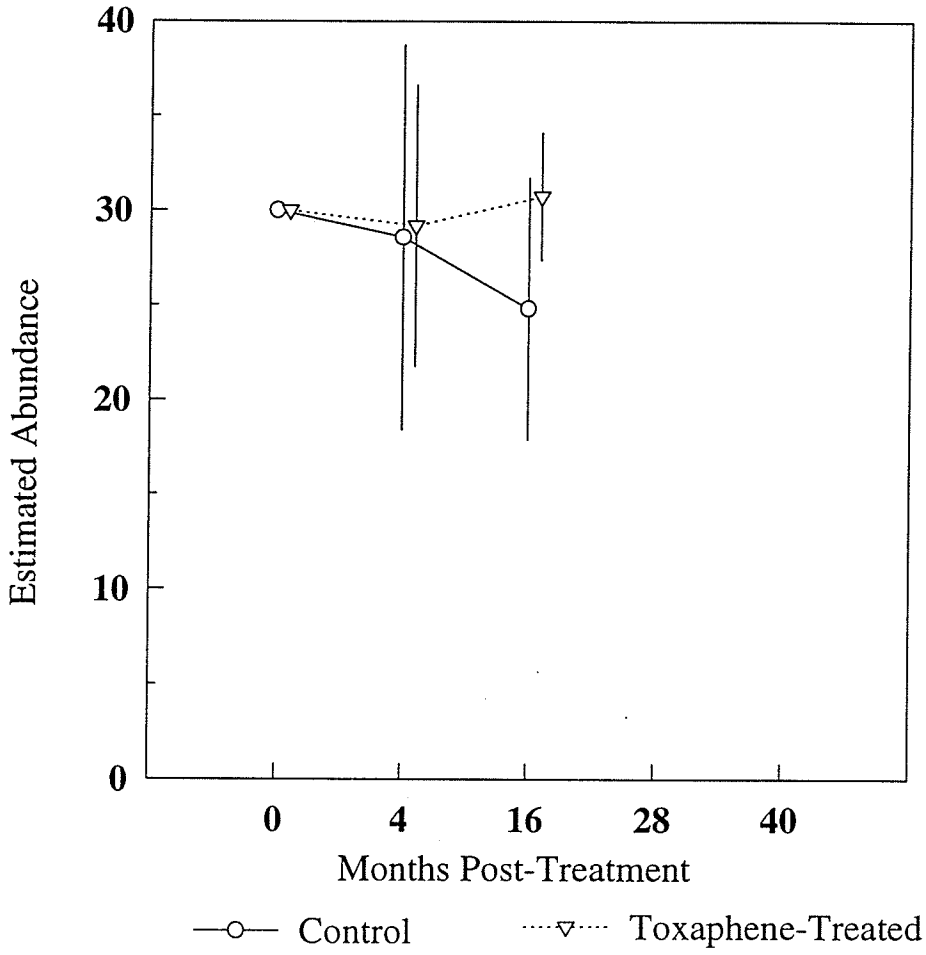


Figure 12. Jolly-Seber estimates of abundance for control and 3.5 µg/g toxaphene-treated lake trout.

Chlordane

Lake trout treated with chlordane had decreased survival in the first four months after treatment. However this decrease was not statistically significant (Figure 13). Overall survival was not significantly different between chlordane-treated and control lake trout. However, the lower survival in the first year resulted in a smaller population throughout the balance of the experiment (Figure 14).

Furan

P_5CDF-treated lake trout had marginally decreased overall survival ($p=0.0674$). Although survival was reduced in both recapture periods after treatment, it was not statistically different from controls (Figure 15), but it resulted in overall lower abundance (Figure 16).

White Sucker Survival and Abundance

Toxaphene

Survival of toxaphene-treated white suckers was similar to controls in the first year post-treatment, but significantly lower in the second year (Figure 17). Survival was lower in the third and fourth years but the difference was not significant. Overall survival was not different between toxaphene-treated and control suckers. Estimated population sizes for control and toxaphene-treated fish (Figure 18) show an initial decrease in numbers of both control and toxaphene-treated suckers in the first year post-treatment. The toxaphene-treated fish continued on this downward trend whereas the control fish numbers stabilized, with the exception of the estimate for the last year. The initial decrease in control-treated sucker's survival and estimated population size suggests a handling effect although one could not be detected statistically (Arnason and Mills 1987).

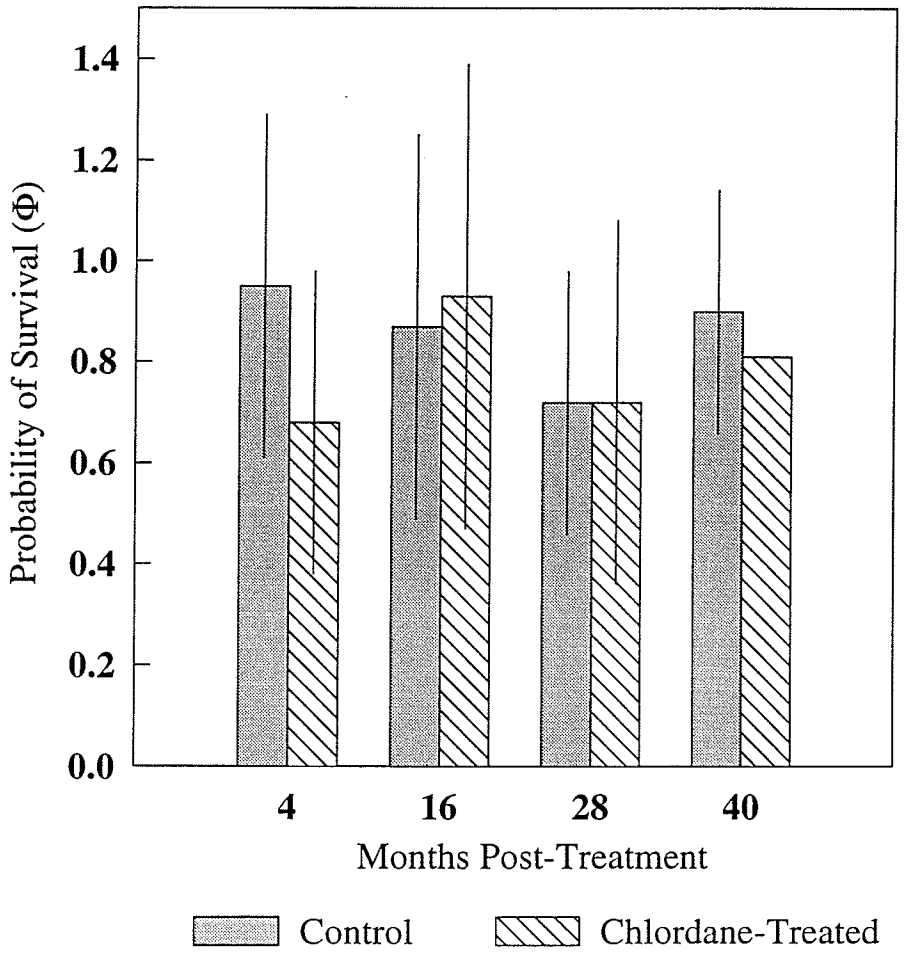


Figure 13. Jolly-Seber estimates of survival probability for control and chlordane-treated lake trout.

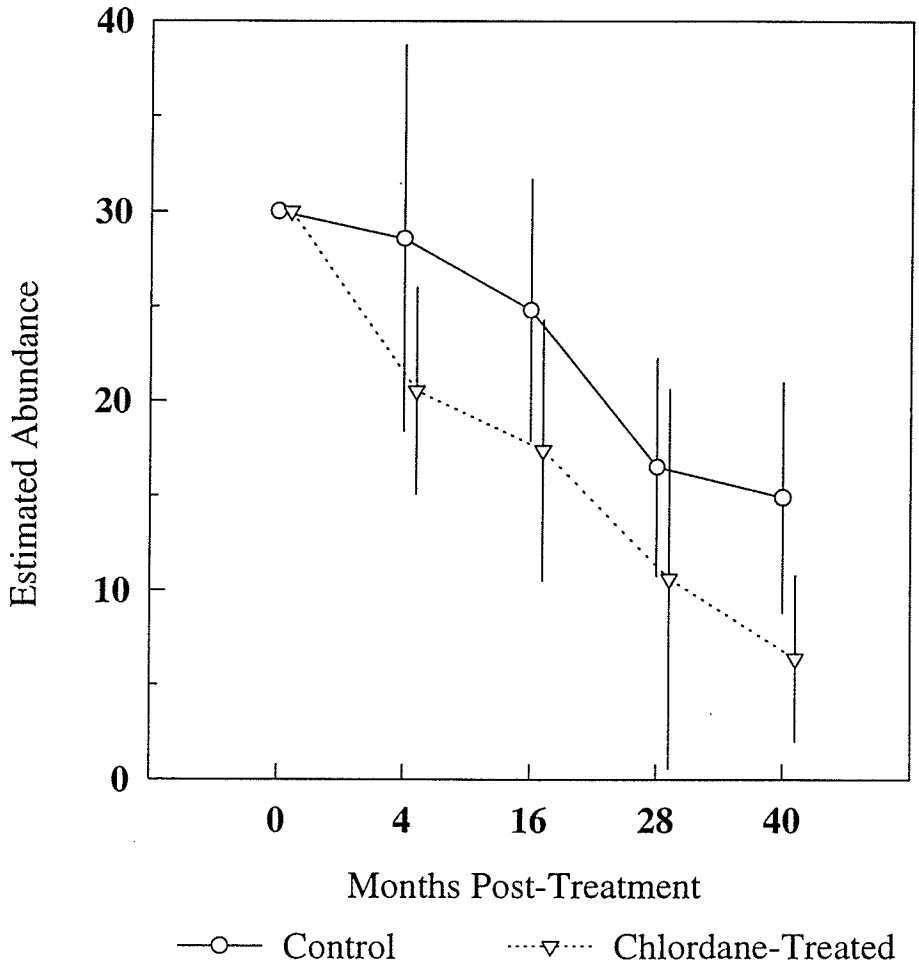


Figure 14. Jolly-Seber estimates of abundance for control and chlordane-treated lake trout.

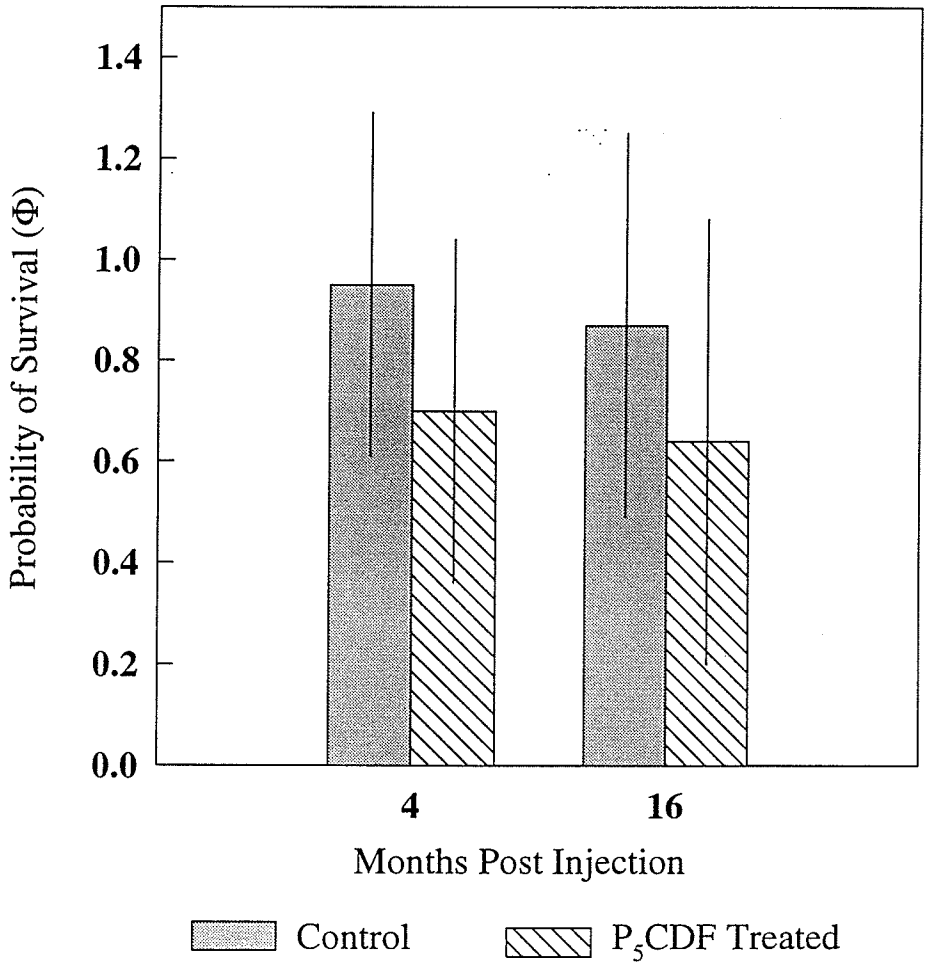


Figure 15. Jolly-Seber estimates of survival probability for control and P_5CDF-treated lake trout.

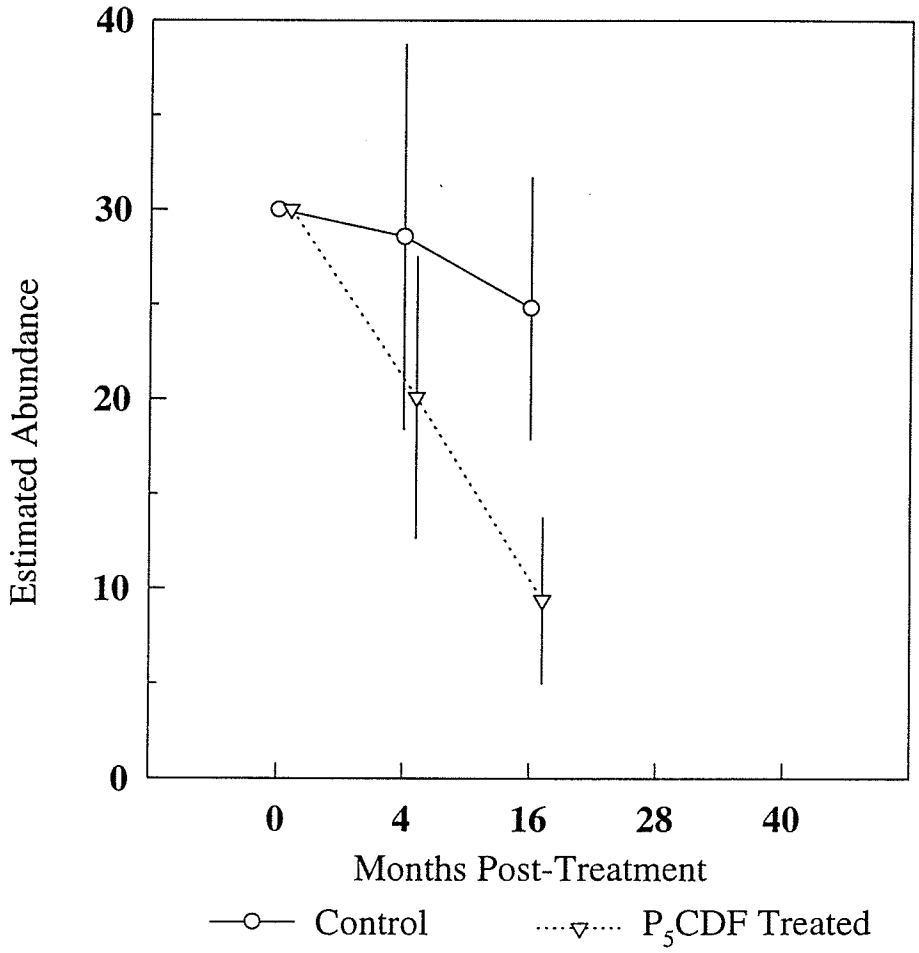


Figure 16. Jolly-Seber estimates of abundance for control and P_5CDF-treated lake trout.

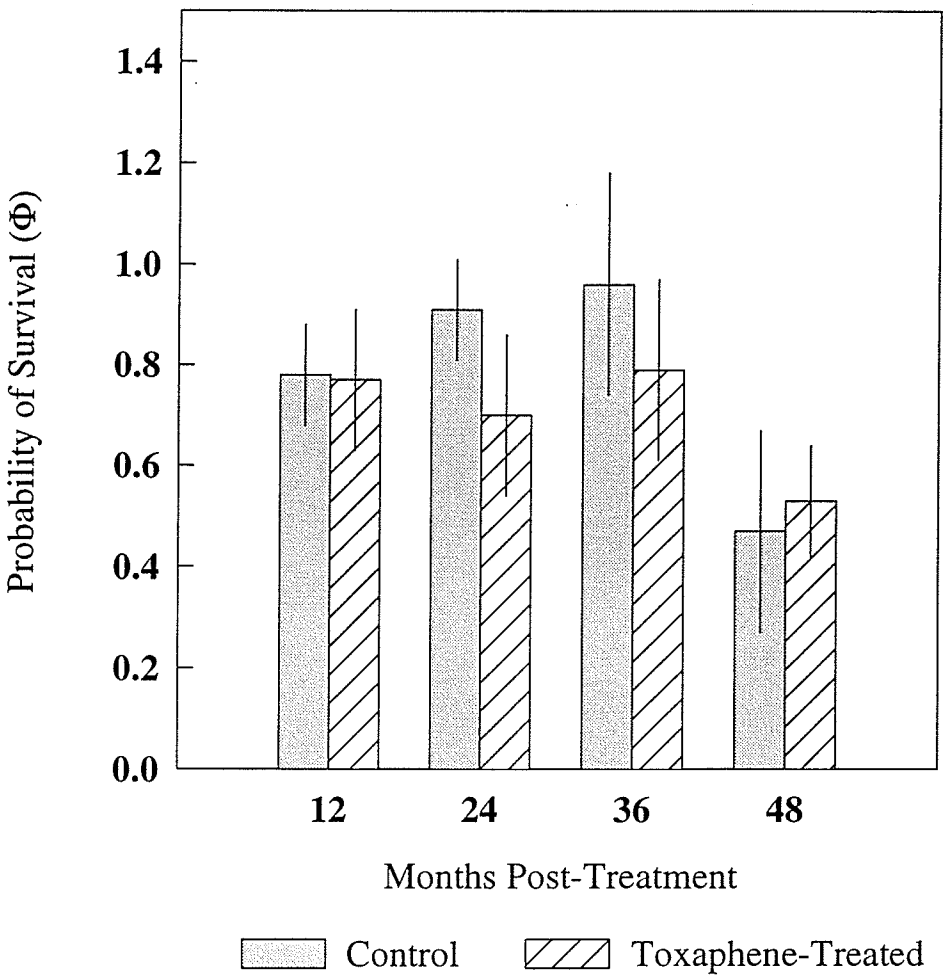


Figure 17. Jolly-Seber estimates of survival probability for control and toxaphene-treated white suckers.

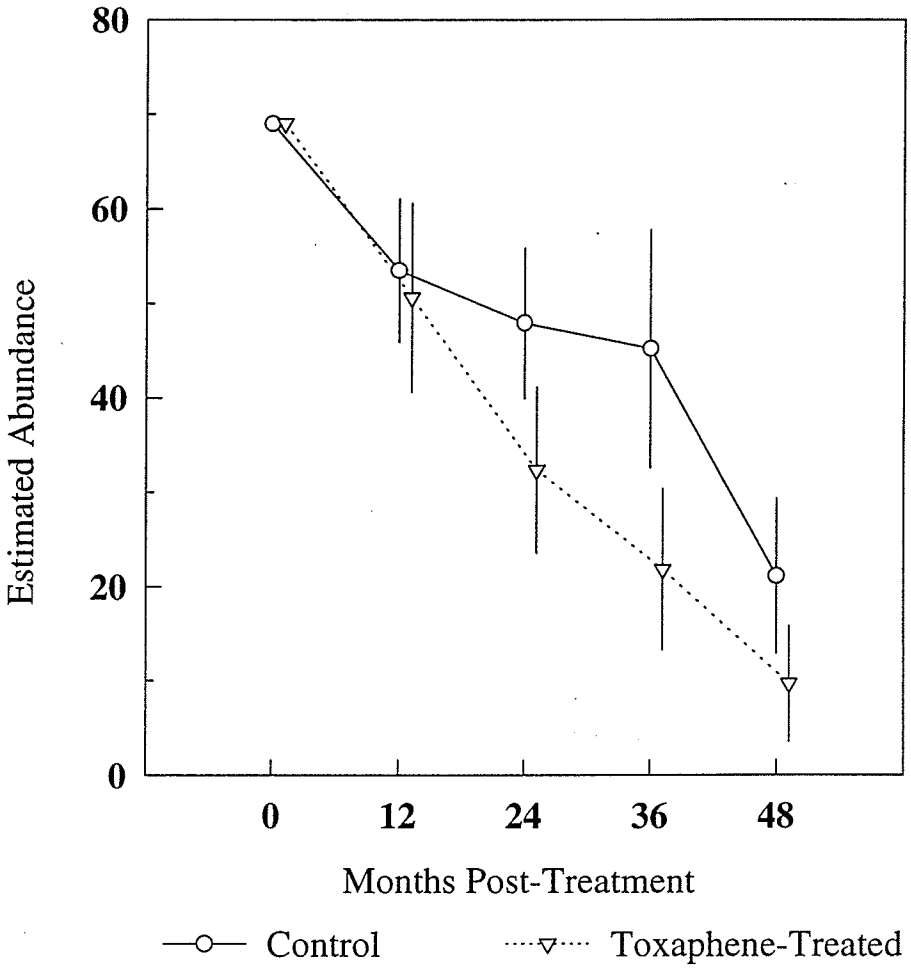


Figure 18. Jolly-Seber estimates of abundance for control and toxaphene-treated white suckers.

Chlordane

Initial survival of chlordane-treated white suckers was significantly lower than control fish (Figure 19). Survival in years 2 and 3 post-treatment was similar to controls. The estimated population of chlordane-treated suckers (Figure 20) followed a pattern similar to control fish, with the exception that estimated numbers were lower after the first 12 months.

Furan

Estimated survival probabilities of P_5CDF-treated white suckers were compared with survival probabilities of control fish estimated for the same recapture interval (i.e. 12, 24 and 36 months post-treatment) to account for the treatment of this group in 1989 instead of 1988. Survival probabilities were significantly higher for P_5CDF-treated fish in the first interval and similar for all other intervals (Figure 21). Overall survival was significantly different at the 90% level (p=0.0647), the result of greater survival of treated fish over the first year of treatment. The population estimates for P_5CDF-treated and control-treated white suckers are similar (Figure 22). As with the lake trout injected with the lower dose of toxaphene, this group showed better survival than controls in the first year post-treatment, and may be a reflection of more experience in the handling the fish.

The estimate of probability of survival of control suckers was lower in the first year post-treatment than at other times during the study. This lower survival may have been caused by the increased handling necessary to tag and inject the fish. In subsequent recapture periods, survival was greater, with the exception of the fourth recapture interval between 36 and 48 months post-treatment. For all groups of white suckers the numbers released (s_i) to recaptured (R_i) ratios (s_i/R_i) were lower in the recapture period at 48 months (1991) than they were in all other recapture periods during the experiment. This resulted in a increased estimate of abundance (N_i) for 1991,

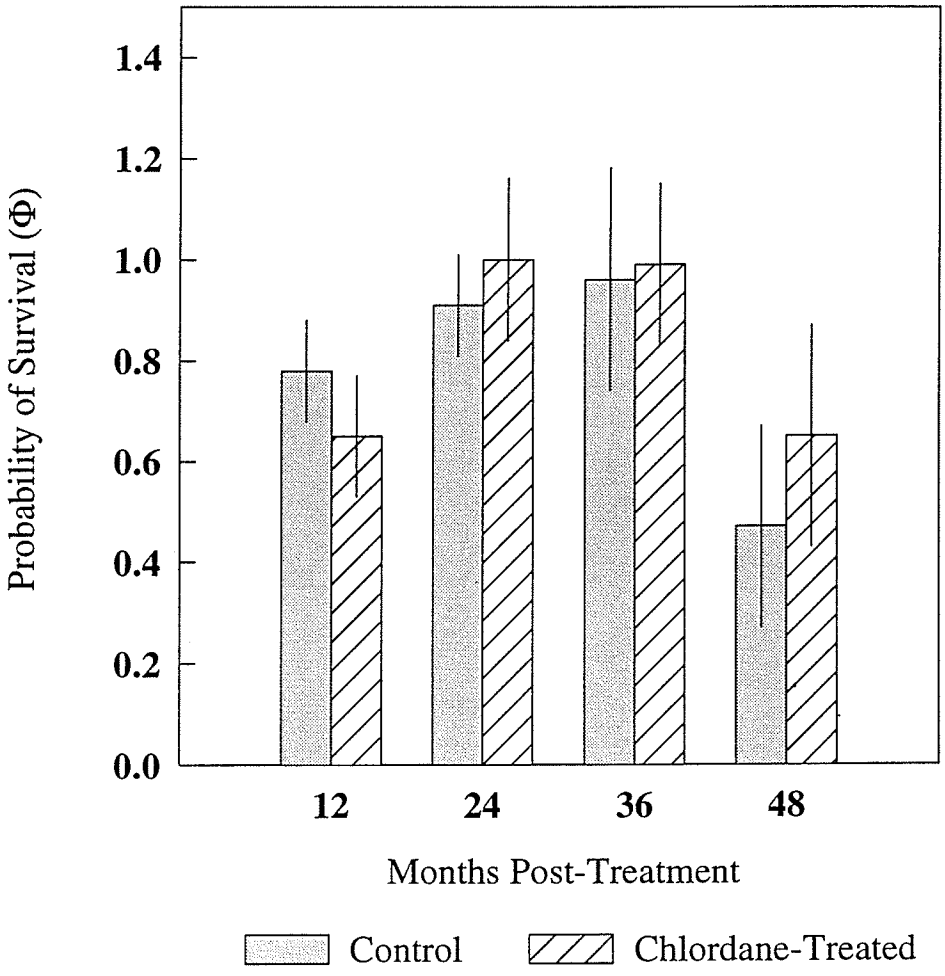


Figure 19. Jolly-Seber estimates of survival probability for control and chlordane-treated white suckers.

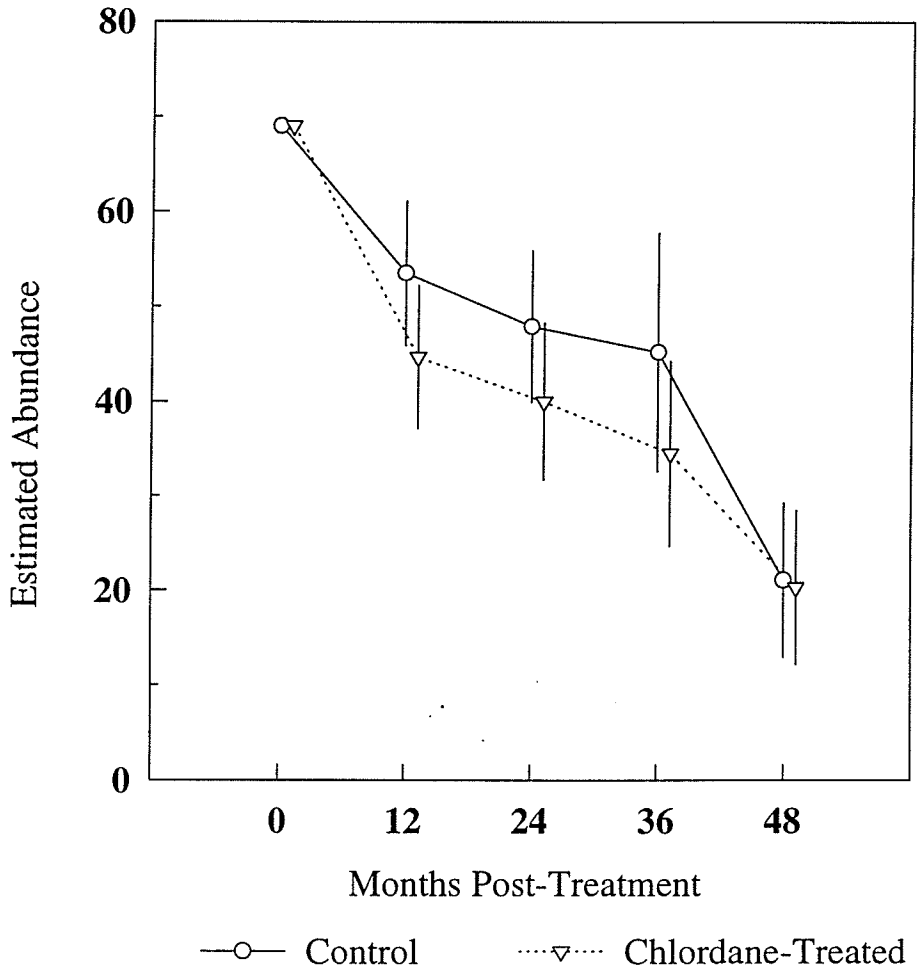


Figure 20. Jolly-Seber estimates of abundance for control and chlordane-treated white suckers.

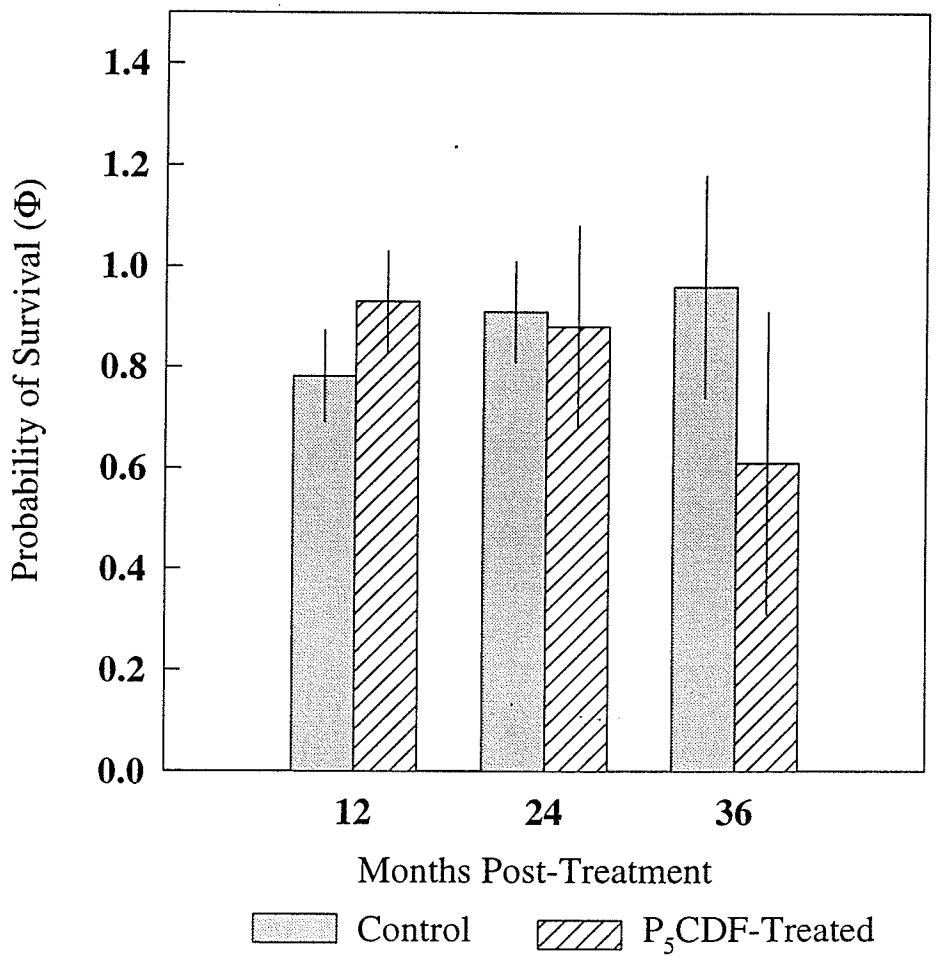


Figure 21. Jolly-Seber estimates of survival probability for control and P_5CDF-treated white suckers.

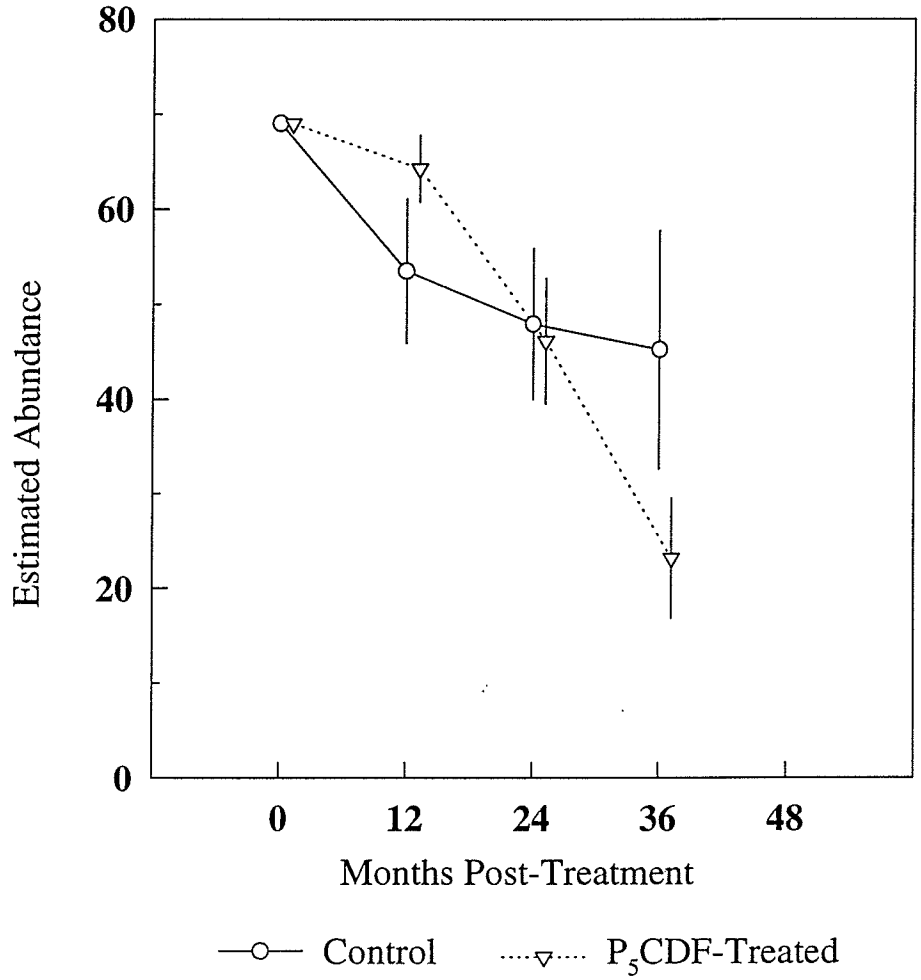


Figure 22. Jolly-Seber estimates of abundance for control and P_5CDF-treated white suckers.

which results in a decrease in the estimated survival probability (Φ_1) for the same period and an increased estimate of Φ_1 for the preceding recapture period. Since this bias appears to be consistent through all groups, the most probable explanation of decreased recaptures is that a handling mortality occurred in those fish captured in 1991, resulting in lowered recaptures and increased estimate of N_i for 1991 (Arnason and Mills 1987). Since this bias is consistent through all groups, it only affects the absolute estimate of survival. The relative differences between treated and control groups should remain unaffected.

Lake Trout Growth

Growth of lake trout treated with corn oil was significantly lower in the first interval between the time of treatment and the first recapture (Table 3). Mean growth rates in subsequent years were not significantly different from each other. The lower growth in the first recapture interval indicates that initial handling affected growth of the lake trout.

There were no differences in mean growth rate of lake trout treated with 7.0 µg/g toxaphene-treated and control fish in the second, third and fourth recapture intervals. Differences in growth were not tested for in the first recapture interval because only a single fish was recaptured in this treated group (Table 3). Lake trout treated with 3.5 mg/kg toxaphene had no differences in growth in any of the sampling intervals (Table 3).

Growth of chlordane-treated lake trout was not significantly different from controls over the first two sampling intervals, but it was significantly lower over the third sampling time (Table 3).

Table 3. Mean daily instantaneous growth in weight and weights (g) of treated and control lake trout in each treatment group for each recapture interval. Values are means ± Std. Err.

Treatment		Months Post Treatment 4	16	28	40	52
Controls	Growth	-0.0000478 ± 0.0001199[a]	0.0001636 ± 0.0001208[a]	0.0002916 ± 0.0001116[a]	0.0001335 ± 0.0000235[a]	0.0002512 ± 0.0000580[a]
	Weight	739 ± 43	774 ± 30	794 ± 33	867 ± 40	970 ± 33
	N	8	15	11	9	11
Toxaphene 7.0 ug/g	Growth	-0.0002986 ± [nt]	0.0003272 ± 0.0001636[a]	0.0002977 ± 0.0001400[a]	0.0003442 ± 0.0001295[a]	0.0005455 ± 0.0002512[nt]
	Weight	386 ±	630 ± 46	754 ± 11	872 ± 47	959 ± 76
	N	1	8	6	5	2
Chlordane	Growth	-0.0000754 ± 0.0002530[a]	0.0001660 ± 0.0001151[a]	0.0000495 ± 0.0000241[b]	0.0003359 ± 0.0002241[a]	0.0001973 ± 0.0000346[a]
	Weight	662 ± 61	803 ± 30	820 ± 37	1023 ± 35	861 ± 59
	N	7	8	10	3	3

		Months Post Treatment 4	12	24	36
Toxaphene 3.5 ug/g	Growth		-0.0000052 ± 0.0000487[a]	0.0002661 ± 0.0000621[a]	0.0002217 ± 0.0000407
	Weight		700 ± 36	824 ± 32	870 ± 29
	N		13	11	11
P_5CDF	Growth	-0.0001023 ± 0.0005089[nt]	0.0000560 ± 0.0000926[a]	0.0003382 ± 0.0000853[a]	0.0003418 ± 0.0000711[a]
	Weight	424 ± 124	724 ± 21	817 ± 22	873 ± 55
	N	2	9	6	4

[a,b] Values with different letters have significantly different growth from control from T_0 to time of recapture, ANOVA, $p<0.05$

[nt] Not tested for differences due to small sample size

There were no differences in mean growth rate of lake trout treated with P_5CDF and control treated fish in the second and third recapture intervals. Differences in growth were not tested for in the first recapture interval because only two treated fish were recaptured (Table 3).

White Sucker Growth

Growth of white suckers treated with corn oil did not differ among any of the recapture intervals (Table 4), indicating that the initial treatment did not alter growth of suckers. Mean growth rate of white suckers treated with injections of toxaphene was lower at the second recapture time (Table 4). Growth of white suckers treated with chlordane did not differ from control treated fish in any of the recapture intervals (Table 4). White suckers treated with P_5CDF had significantly decreased growth from controls in all recapture intervals (Table 4).

Discussion

Evidence of the contamination of remote ecosystems with chlorinated organics is relatively recent. The aerial transport and deposition of contaminants has resulted in the continuous low level contamination of food webs. Toxicity testing done for many pesticides has centered on short term tests of mortality which are suitable to predict effects from over spray, runoff or large spills into aquatic systems but which have limited relevance to chronic low-level exposure. The long term persistence, accumulation and transport of chlorinated organics such as PCBs, DDT, toxaphene and chlordane to remote areas was not foreseen until the late 1960s. As well, the recent discovery of previously unknown trace contaminant by-products such as dioxins and furans associated

Table 4. Instantaneous growth rate (/day) and weights (g) of treated and control white suckers in each treatment group for each recapture interval. Values are means ± Std. Err.

Treatment		Months Post-treatment			
		12	24	36	48
Controls	Growth	0.0004924 ± 0. 0000770[a]	0.0005461 ± 0.000067[a]	0.0005055 ± 0.000061[a]	0.0004495 ± 0.000067[a]
	Weight	816 ± 48	953 ± 38	1033 ± 35	1198 ± 18
	N	35	32	25	13
Toxaphene	Growth	0.0004135 ± 0.0000858[a]	0.0003696 ± 0.0000528[b]	0.0003394 ± 0.0000783[a]	0.0004168 ± 0.0000807[a]
	Weight	738 ± 49	868 ± 52	994 ± 54	1147 ± 72
	N	33	27	13	9
Chlordane	Growth	0.0004994 ± 0.0000800[a]	0.0005544 ± 0.0000725[a]	0.0004745 ± 0.0000808[a]	0.0005375 ± 0.0000450[nt]
	Weight	812 ± 48	884 ± 47	1001 ± 37	1045 ± 61[*]
	N	33	26	16	12
P_5CDF	Growth	0.0000976 ± 0.0000968[b]	0.0002325 ± 0.0000519[b]	0.0002527 ± 0.0000788[b]	0.0001275 ± 0.0000877[b]
	Weight	870 ± 45	948 ± 56	1005 ± 78	1024 ± 187
	N	28	20	10	3

[a,b] Values with different letters have significantly different growth than controls for the same recapture period, ANOVA, $p<0.05$
[nt] Not tested for differences due to small sample size
[*] Weight significantly different from controls, ANOVA, $p<0.05$

with the synthesis of various chemicals or with other industrial processes was unexpected. This has resulted in few data on the long term effects of high body burdens accumulated through food chain biomagnification on basic ecological parameters such as growth and survival for many contaminants.

Survival

Overall survival of lake trout was lower in two of four treated groups, the group treated at 7.0 mg/kg of toxaphene and in the group treated with P_5CDF. Although overall survival was not significantly lower in the chlordane-treated group, lower initial survival rates contributed to a lower estimated population size after four years. There were no differences in overall survival in any of the treated groups of white suckers. However, there were intervals where survival of treated groups was lower than controls. This contributed to the general trend that treated groups had smaller estimated population sizes at the end of sampling than did controls.

The toxicity of toxaphene in water was known to fisheries managers, who in the middle to late 1950's and early 60s used toxaphene as a piscicide to control undesirable species of fish. This use was abandoned when toxaphene was found to be too persistent for quick restocking of lakes (Stringer and McMynn 1958, 1960, Prevost 1960, Fukano and Hooper 1958). Previous experiments to determine the effect of toxaphene on survival of fish have mainly investigated exposures in water, because this was reasoned to be the major route of exposure following the application on agricultural crops. Maximal acceptable concentrations of toxaphene in water are reported as 25 to 54 ng/L for fathead minnows (*Pimephales promelas*), 49 to 72 ng/L for channel catfish (*Ictalurus punctatus*), and <39 ng/L for brook trout (*Salvelinus fontinalis*) (Mayer et al. 1975 1977). Effects of high body burdens on growth and survival over a long period of time have not previously been investigated.

Survival of lake trout treated with 7.0 µg/g toxaphene was decreased in the first interval between treatment and spawning. Although it is not known exactly when the mortality took place, it is possible that it occurred at spawning. Mayer et al. (1975) reported increased mortality in brook trout during spawning for fish which had been continuously exposed to toxaphene in the water. Concentrations of toxaphene measured in fillets of these brook trout after 161 days of exposure were 0.87 µg/g and 4.0 µg/g in fish exposed to 270 and 500 ng/L exposures respectively and were 3.0 and 11 µg/g in the internal organs and 2.4 and 8.0 µg/g in whole body residues. In these exposure groups increased mortality at spawning time (50% and 100% respectively) was reported. The concentrations reported in whole body are similar to the 7.0 µg/g and 3.5 µg/g used in this experiment. Based on the depuration curves from this experiment (see Chapter 5), lake trout treated with 7.0 µg/g would have had 4.8 µg/g at first spawning after treatment. This type of mortality may be species-specific because no increased mortality was seen in channel catfish and fathead minnows during spawning under similar exposure conditions (Mayer et al. 1977).

The concentration of toxaphene used in this study is similar to levels reported in lake trout from the Great Lakes. Concentrations in lake trout from Lake Michigan ranged from 5 to 10 µg/g, and from 5to7 µg/g in Lake Superior (Schmitt et al. 1985, 1990). High concentrations have also been reported in burbot liver (2.8 µg/g) and lake trout muscle (0.7 µg/g) in Lake Laberge in the Yukon Territory (Kidd et al. 1993).

Despite the widespread occurrence of chlordane in aquatic ecosystems including the Great Lakes, (Gooch et al. 1990, Schmitt et al. 1990, Borgman and Whittle 1991, Miller et al. 1992), the Arctic (Muir et al. 1988) the Antarctic (Kawano et al. 1986) and the Baltic (Jansson and Wideqvist 1983), few, if any, data exist on long term effects in fishes. In fact, few data other that LD50s exist on effects in fish. Johnson and Finley (1980) report and LD50 of 0.042 mg/L. Chlordane is also reported to be toxic to rainbow

trout and bluegills at 5 - 200 ug/L (NRCC 1974). In the present experiment, chlordane-treated lake trout did not have significant differences in survival, although survival was decreased in the first recapture interval compared with controls. White suckers had decreased survival only in the first recapture interval after treatment. The concentration of chlordane used in this study and found in the fish after the first interval is similar to what occurs in the environment today. Total concentrations of five of the major chlordane components in Lake Michigan lake trout ranged from 1.20 - 2.00 μg/g in lake trout belly flaps and 0.26 - 0.60 μg/g in fillets (Gooch et al. 1990). Lake trout from Sisikiwit Lake on Isle Royale in Lake Superior had 17.4 ng/g in fillets and 0.12 μg/g in belly flap samples.

Much of the research relevant to the effects of P_5CDF has been done with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (T_4CDD) or 2,3,7,8-tetrachlorodibenzofuran (T_4CDF). Decreases in survival following exposure via various routes (feeding, I.P. injection, waterborne) to T_4CDD or T_4CDF have been reported for several different species of fish including rainbow trout (Giesy et al. 1993), guppies (Norris and Miller 1974), juvenile rainbow trout (Mehrle et al. 1988, Spitsbergen et al. 1988) and juvenile coho salmon (Miller et al. 1979). Typically, mortality following exposure to TCDD or like compounds (furans, non-ortho substituted PCBs) is delayed (Cook et al. 1991, 1993). Estimated survival probability of P_5CDF-treated lake trout was 74% of controls in both recapture intervals. Mortality appears to have occurred at a relatively constant rate throughout the study.

The injected dose of 1 ng/g (1000 pg/g) P_5CDF was about 16 times higher than congener specific levels of P_5CDF recently reported for salmonids from Lake Ontario (Whittle et al. 1992) and the Baltic (Wiberg et al. 1992). If 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-TEQs) (Safe 1990) are considered, the initial dose represents about 500 pg TCDD-TEQ/g. Recent reports show that 200 to 1800 pg TCDD-TEQ/g

have been detected in fishes from Lake Ontario (Niimi and Oliver 1989, Huestis et al. 1993) and Lake Michigan (Smith et al. 1990). Depuration of P_5CDF was slow in both lake trout and white suckers, with mean whole body concentrations (growth-corrected) of 688 pg/g (344 pg TCDD-TEQ/g) and 291 pg/g (145 pg TCDD-TEQ/g) in trout and suckers respectively 3 years after treatment (see Chapter 5). These values are in the range of TCDD toxic equivalent concentrations currently found in Great Lakes fish.

Growth

Growth of lake trout was not affected to any great degree by any of the contaminants. Decreased growth was only found in chlordane treated fish during one recapture interval. This is not surprising given that growth in adult fish is generally considered to be one of the less sensitive indicators of exposure (Woltering 1984). Growth of control fish was more variable in lake trout than in white suckers. This increased variability as well as the smaller numbers of treated and recaptured fish greatly reduces the ability to detect differences. In general growth of lake trout appeared to be more affected by the initial handling than growth of suckers.

Growth of suckers was affected by treatment with toxaphene, but not consistently in every recapture interval. Mayer et al. (1975) and Mehrle and Mayer (1975) reported decreased growth in yearling brook trout (*Salvelinus fontinalis*) exposed continuously to 502 and 288 ng/L for 6 months. Transient decreases in growth have been reported in adult fathead minnows exposed to more than 97 ng/L toxaphene. Growth was decreased 35 and 98 days after starting exposure but not at 295 days. The effect of toxaphene on growth is also dependent on the life stage exposed. Growth is consistently decreased in fry or juvenile fish. Mayer et al. (1975, 1977) reported decreased growth in brook trout, fathead and catfish fry, but not in adult catfish. As previously noted the concentrations of

toxaphene in the treated lake trout and white suckers are similar to levels recently reported in Great Lakes fish and in Arctic fish.

Growth of both species was initially unaffected by treatment with chlordane in this study. However, lake trout showed significantly reduced growth in the third recapture interval. The effect on growth may have been caused by decreased feeding. Little et al. (1990) reported that juvenile rainbow trout consumed less food when exposed to 0.0002 - 0.02 mg/L chlordane for 96h.

Although no effects on growth were initially seen, it may be that the type of exposure affected the results. Gooch et al. (1990) reported that two stable metabolites of chlordane, heptachlor epoxide and oxychlordane, were three to five times more toxic than technical chlordane to lake trout. Because the chlordane was administered as an intraperitoneal injection, it would not have immediately been subjected to normal metabolic pathways in the liver as it would have been if it had been given in food (see Chapter 6). Eventually the contaminant would be subject to metabolism, but not until much later.

Decreased growth was initially reported as one of the more sensitive responses of fish to exposure to TCDD or TCDD analogues such as P_5CDF (Mehrle et al. 1988). Although it is not clear what causes the decreased growth, feeding inhibition has been reported in several different species fed or exposed to TCDD, such as rainbow trout (Giesy et al. 1993, Mehrle et al. 1988), coho salmon, (Miller et al. 1979), and lake trout (Walker et al. 1994). Not all studies report decreased growth. Muir et al. (1990c) found no growth effects in juvenile rainbow trout fed 0.82 - 9.00 ng/g of P_5CDF. In the current study, clear effects on growth were seen in white suckers, but not in lake trout. Because of the interval between sampling (1 year) it is difficult to assess if there were transient undetected growth effects in lake trout. This may be an instance where effects seen in

laboratory experiments are not manifested in a field situation. Instantaneous growth rates in P_5CDF suckers were 50% or less of those for control fish (Table 4).

General

The effects of reduced growth and decreased survival of adults on populations could be quite substantial. Decreases in overall population size (abundance) would translate into fewer individuals to contribute to subsequent generations. It also represents a decrease in the genetic diversity of the stock because fecundity is related to size in fish (Bagenal 1978). Decreases in the size of individuals may also result in decreases in overall population size as slower growth in fish will result in a decrease in egg production over the reproductive years of the fish (Shuter 1990, Weatherly and Gill 1987). Little is known about the effects of such contaminants on the age of first reproduction, but white suckers exposed to effluent from pulp and paper mills show a delayed maturity (Munkittrick et al. 1992). This type of effect could also contribute to decreases in abundance.

One must also consider what effect changes in population will have on the dynamics of contaminant distribution within a food web. It has been shown that lake trout populations which are exploited tend to have larger fish with a higher condition factor (Day 1983). This may also result in higher contaminant levels, especially in top predators. For example, Kidd et al. (1993) reported that lake trout from Lake Laberge, which have been exploited for several decades, had higher levels of toxaphene than did fish from nearby unexploited lakes. The results of the current study indicate that high body burdens can result in subtle population level effects. It could be hypothesized that exploited populations may have an increased risk of failure caused by the double-ended pressure of exploitation of adults and the resulting increases in contaminant burden,

which may lead to decreases in growth, survival and reproductive success in the remaining individuals making up the population.

All three of the contaminants used in this study altered growth or survival to some extent. Although the experiment was designed to be as realistic as possible, each contaminant was tested separately. One question which remains to be addressed concerns the effects of having more than one contaminant present at the same time. It is not known if effects from a single contaminant are additive, synergistic or antagonistic to those of other contaminants which may be present.

Chapter 2. Reproductive Success of Natural Populations of Lake Trout and White Suckers treated with toxaphene, chlordane or [^{14}C]-2,3,4,7,8 - pentachloro-dibenzofuran.

Introduction

There has been concern in the past 20 years that persistent organochlorine contaminants in freshwater and marine environments affect the ability of fish to maintain viable populations. Populations already subject to over harvesting and changes to habitat, face an additional stress from persistent chemcial contaminants. The problem is to determine what effects, if any, these persistent compounds have on their reproductive success.

The basis of maintaining a viable population of any organism lies in its ability to reproduce successfully. If the net reproductive rate (R_O) of a population should fall below one, (i.e. a population just replacing itself), then the population would no longer be replacing itself, and would be in danger of becoming extinct.

Rosenthal and Alderdice (1976) probably best summarize the problem from an ecological perspective when they state that environmental changes fall into two basic zones, those which can be tolerated indefinitely and those which ultimately result in death. Between these zones of tolerance and resistance lies a boundary region of sublethal response, where the survival potential of the organism is reduced, indirectly at a later stage. Sublethal effects are of concern because of their subtle nature. They may often occur but go unrecognized at the individual level and proceed until their significance is interpreted at the population level, after the damage is done. This may very well be the case with reproduction in many of the salmonids in the Great Lakes. They further state that several stages of fish are more susceptible to environmental and pollution stress. These are germ cells, the highly plastic early embryo and the stage of larval transition between reliance on endogenous and exogenous sources of food.

A variety of stress-stressor systems may trigger a limited number of responses in the organism. These often appear to involve the same physiological and biochemical mechanisms. Most sublethal effects are based at the biochemical level and are caused by physical and chemical changes; they may be expressed as histological, morphological, physiological or behavioral responses (Rosenthal and Alderdice 1976). Responses of these sorts either in parents or in developing embryos and fry can result in decreased reproductive success.

Organochlorine contaminants have been implicated in reproductive failure of fish stocks from the early 1960's (Burdick et al. 1964, Hopkins et al. 1969). Mortalities in hatching lake trout (*Salvelinus namaycush*) in a New York state fish hatchery led to the conclusion that DDT was the likely cause of the mortality (Burdick et al. 1964). Since then, many other organochlorine contaminants, such as PCB's (polychlorinated biphenyls), DDT (Wilford et al. 1981), toxaphene (Mayer et al. 1975,1977), chlordane, mirex (Giesy et al. 1986) and more recently dioxins and furans (Helder 1980, 1981, Walker et al. 1992,1994) have been implicated in reproductive problems with fish. These compounds all persist in the environment, both terrestrial and aquatic; most are classes which consist of many different stereo isomers and a number of different congeners (i.e. they vary in the number of chlorine atoms). Certain of these isomers and/or congeners are more persistent than others. Persistence is related to a combination of the 3-dimensional stereochemical configuration and the degree of chlorine substitution. Certain configurations are more hydrophobic (for example chlorinated aromatics, chlorinated aliphatics) than others and thus have a greater tendency to partition into the lipids in the environment. This is the fraction which has the potential to most affect organisms and their reproduction, and this is especially true in fish, most of which rely on lipids as a major constituent of eggs. It is known that toxic substances become concentrated in lipids of fish eggs and can become more concentrated in the remaining

yolk as the fry absorbs the yolk, so that the fry receives a large dose of contaminant when the last of the yolk is absorbed (Atchison 1976, Guiney et al. 1980)

It has been established in laboratory studies (Bengtsson 1980, Burdick et al. 1964, 1972, Freeman and Idler 1975, Guiney et al. 1979) and in field studies (Ankley et al. 1989, Cross and Hose 1988, Eisenberg and Topping 1985, Lockhart et al 1977, Mac et al. 1985, Niimi 1983, Noguchi and Hesselberg 1991, Spies and Rice 1988 and von Westernhagen et al. 1981) that maternal transfer of contaminants occurs in fish. The amount transferred will depend on several factors including the nature of the toxicant and the species of fish. Niimi (1983) found that the amount of PCB transferred to eggs depended on the level of lipid transferred to eggs and that this in turn was species dependent. Indeed, spawning has been used by several authors as a mechanism to explain decreases in overall body burdens of contaminants (Guiney et al. 1979, Vodicnik and Peterson 1985). However, Niimi (1983) stated that whole body burdens may be increased or decreased by spawning depending on the proportion of total body weight made up by eggs and the amount transferred. He determined that total PCB body burdens in rainbow trout and white suckers increased whereas body burdens in small mouth bass and yellow perch decreased. It should be pointed out that using changes in overall body burdens is somewhat misleading as concentrations on a tissue basis would likely remain the same but the entire body burden would increase or decrease depending on the lipid content, relative concentration and proportion of body weight represented by eggs.

Giesy et al. (1986) tried to correlate chlorinated hydrocarbon concentrations in eggs with rearing mortality of chinook salmon from Lake Michigan. They found that toxaphene and PCB concentrations were negatively correlated with survival of fry to the swim up stage. Concentrations were approximately 3.5 µg/g eggs for toxaphene and 9.1 µg/g total PCBs.

Studies are usually conducted under laboratory conditions, giving the investigator more control over the dosage of contaminant, duration of exposure, stage exposed etc.

(Giesy et al. 1986). Some laboratory studies with eggs and/or fry have been conducted at unrealistically high concentrations. Direct exposure of eggs or fry by adding toxicants to the water may be inappropriate because the absorption and disposition of the toxicant within the animal may not be similar to natural transfer from the female. Safe et al. (1982) have argued that although these types of study can provide insight into the mechanisms of toxic action and relative toxicities, they are not useful for determining effects of mixtures of residues deposited in eggs due to long term exposure of adults to (low) concentrations in both water and food.

Reproductive success in fish is inherently variable, hence changes from the norm are often difficult to detect in laboratory studies and they are even more difficult to establish in field situations. Often many other environmental variables can influence the success or failure of reproduction, including but not limited to handling, water chemistry, water temperature, etc (Lam 1983).

There are several times during the life cycle of fish where toxicants can affect reproductive effort. The premature death of adults will obviously limit the contribution of an individual to the next generation. Interference of contaminants with reproductive success, through effects on endocrine systems, for example, can also alter reproductive success of adults. Direct transfer of contaminants from parents to offspring, which subsequently potentially affects the development and survival of the young, also affects reproductive success. Finally, the direct effects of non-parentally transferred (environmental) contaminants on developing eggs and fry can influence egg, embryo and fry survival.

This chapter addresses the question of potential effects caused by parental transfer of contaminants from adults to eggs, and indirectly deals with premature death of adults and interference with reproductive effort.

Materials and Methods

Capture and Treatment of Fish

The study site, capture and treatment methods are described in Chapter 1.

Fertilization of Eggs

The same method was used to fertilize white sucker eggs and lake trout eggs. After transport to the sampling site, fish were sorted by sex and treatment group and ripe fish were set aside. It was preferable to spawn fish which had not been anaesthetized in order to avoid the possibility of getting MS 222 in with the spawn. Female fish were removed from the water, and excess water was removed with a towel to avoid getting slime in with the eggs. The fish was held in a semi-upright position with a second person holding the tail while eggs were expressed into a clean stainless steel bowl by applying gentle pressure while stroking towards the vent. With the eggs in the bowl, a male fish from the appropriate treatment group was picked and the procedure repeated. The mixture of eggs and milt was gently stirred together using fingers (making sure they were rinsed if they had been in water with MS-222). As a precaution, if possible, an extra male was kept ready in case the first one chosen was not sufficiently ripe. Eggs and milt were allowed to sit for 2 - 3 minutes for fertilization to take place and then clean water was added. Water was changed approximately every 10 - 15 minutes, more frequently if water temperatures were warm. The eggs were allowed to water-harden for a minimum of 45 minutes, usually an hour, before being moved, . Expressing eggs, fertilization and water-hardening took place out of direct sunlight

Transport of Lake Trout Eggs

Freshly fertilized and water hardened eggs were transported to animal care facilities in the Department of Zoology, University of Manitoba, Winnipeg, within 12h of

fertilization. Eggs were transported in modified 500 mL Rubbermaid® sandwich containers. Containers were modified by cutting out the raised, square portion of the lid. Eggs were carefully added to the container, a piece of cheese cloth was placed over the container and the modified top snapped in place. The containers were then placed into a cooler and the cooler was filled with lake water. In order to prevent excessive movement of containers within the cooler, it was necessary to place a piece of wood at the top of the cooler. The cooler was then carried from the lake in a back pack. Water in the cooler was renewed at least once prior to departing for Winnipeg, but care was taken to ensure that temperature changes of more than 1 °C were avoided. A small amount of ice was added to the water in the cooler to stabilize the temperature for the 4h trip to Winnipeg. Once in Winnipeg the water temperatures were allowed to equilibrate with those in the incubation trays. This was done by floating the containers with eggs in the incubation trays. Once the temperature difference was less than 1 °C eggs were carefully transferred to incubation trays.

Incubation and Care of Lake Trout Eggs

Eggs were incubated in standard Heath trays with each incubation unit composed of 8 flow through trays stacked vertically. Water supply was dechlorinated City of Winnipeg water, with a flow rate of 7 - 10 L/min. Eggs were initially incubated as close to lake water temperature as possible, usually 10 °C ± 1 °C. After all crosses had been made and all eggs were in Winnipeg, (about 5 to 7 days after onset of spawning), the water temperature was adjusted to 4 °C ± 1 °C over a period of 7 to 10 days. Eggs were maintained at this temperature for the remainder of the incubation period.

Incubation trays were covered with dark plastic. Dead eggs were removed at least once every 10 days, usually once a week. Dead eggs were preserved in Davidsons

fluid for later determination of fertility. Deformed individuals were also picked when they were observed and preserved in Davidson's fluid. Fry were killed when an estimated 90% of the yolk sac had been resorbed. All viable fry remaining at this point were euthanized in MS 222 and then preserved in Davidson's fluid.

Incubation and Care of White Sucker Eggs

White sucker eggs were incubated in an in situ hatchery designed in the course of this experiment. In brief, 1000 - 1500 sucker eggs, measured volumetrically (Bagenal 1978), were placed in modified 500 mL Rubbermaid® sandwich containers. Containers were modified by the addition of a glass tube in one side which ran the length of container with the end bent at approximately a 15° degree angle towards the bottom of the container (Figure 23). The glass tube was friction fitted into the disconnect side of a Nalgene® quick-disconnect hose fitting, which had been placed through a hole drilled through the side of the container. The fitting was held in place by a 1 cm piece of rubber tubing which was stretched over the fitting in the inside of the container. The fitting was connected to a water line to allow water circulation with in the container (Figure 23). The lid of the container was modified by removing a 8 x 8cm piece from the centre of the lid. A piece of 500 µm Nitex® mesh was placed between the container and the lid. The entire assembly was then turned on edge with the glass tubing pointing towards the bottom, the water supply entering from the top and secured in a rack.

This rack was suspended 45 - 60 cm underwater off the end of a dock. Water flow of 600 - 800 $mL \cdot min^{-1}$ of lake water per incubation container was supplied by two submersible pumps (March Mfg Ltd.) feeding a thirty-six port manifold. Lake water was

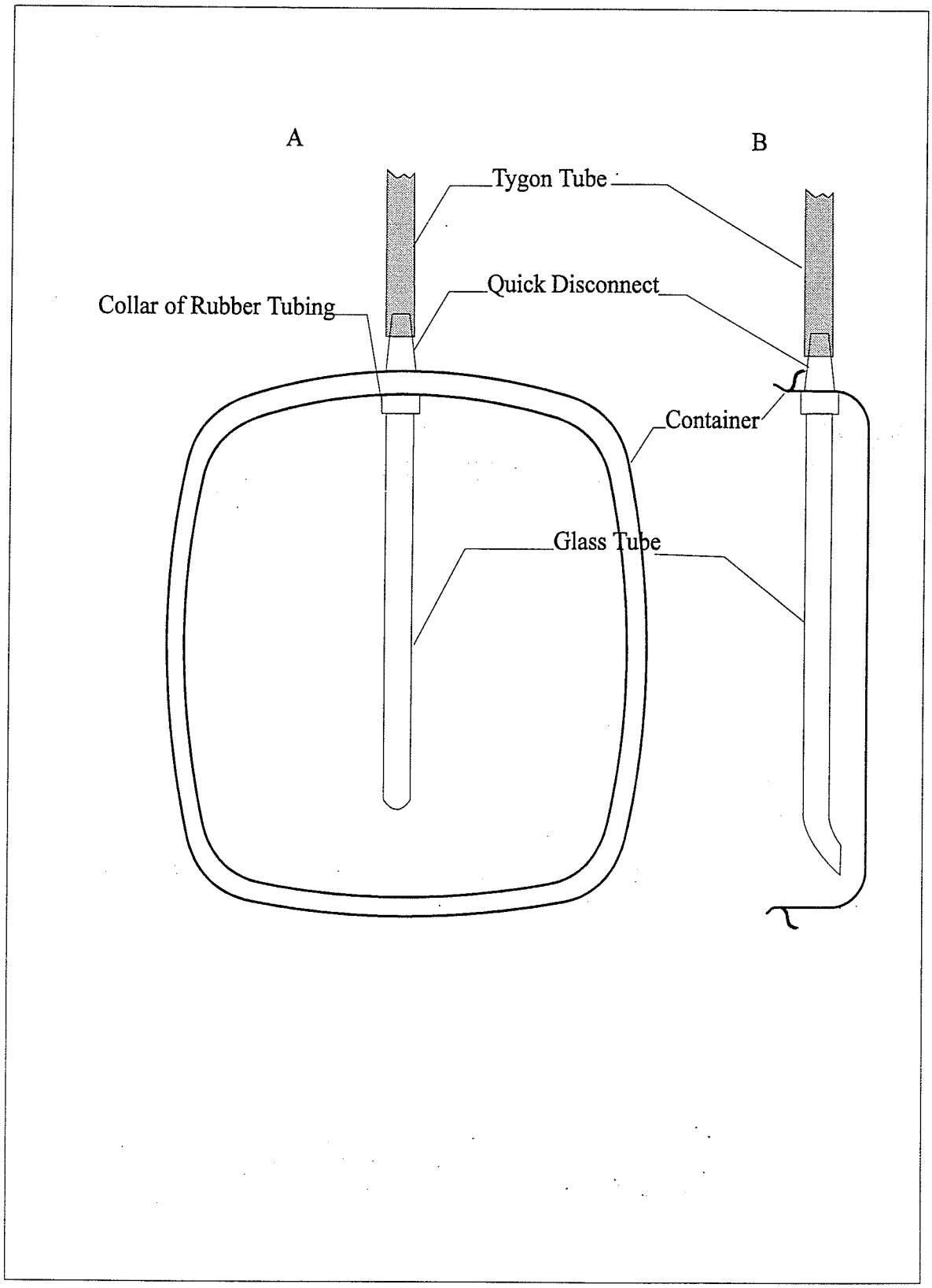


Figure 23. Schematic diagram of setup of sucker hatching containers

partially filtered by attaching plastic containers with 1 mm Nitex® mesh to the intakes of each pump.

Each incubation container was examined daily and dead eggs were removed. Individuals with obvious deformities and dead individuals were removed as soon as they appeared and were preserved in Davidsons fluid for enumeration and examination. Fry were terminated by euthanizing them in MS-222 when the gas bladder was apparent.

Data Analysis

The various parameters calculated from egg/embryo mortality and survival numbers from each cross were calculated as follows:

$$\%\text{Fertilization} = \frac{\#\text{Fertilized Eggs}}{\#\text{Eggs Spawned}} x100$$

$$\%\text{Non - Viable} = \frac{\#\text{ dead embryos } + \#\text{ dead sac - fry}}{\#\text{Fertilized Eggs}} x100$$

$$\%\text{Survival of Fertilized} = \frac{\#\text{viable sac - fry at termination}}{\#\text{Fertilized Eggs}} x100$$

$$\%\text{Total Survival} = \frac{\#\text{viable sac - fry at termination}}{\#\text{Spawned Eggs}} x100$$

All proportions were transformed using the arcsine of the square root of the proportion (Zar 1984) and differences between control crosses and each treated cross were analyzed by Student's t-test using SYSTAT (Wilkinson et al. 1992).

Residue Determination in Eggs

Sub-samples of eggs were taken following water-hardening of the eggs for measurement of contaminant levels. Samples were placed in labelled Whirlpac® bags and

frozen as soon as possible, usually within 4h. Analysis for toxaphene, chlordane and P_5CDF were done using the methods described in Chapter 5.

Results

General

Attempts to spawn both lake trout and white suckers in 1989 were unsuccessful. For white suckers, the first field hatchery design, simple suspension of cages in the water column, did not allow enough water circulation to prevent fungal growth on the eggs. With the lake trout, water temperatures could not be kept low enough and the fish developed a generalized edema similar to blue-sac in all crosses (Symula 1990). Improved technical set-ups in subsequent years allowed the successful rearing of both white sucker and lake trout eggs and fry.

Reproductive Assessments for Lake Trout

Controls

Fertilization success, proportion of non-viable embyos/sac-fry, survival of fertilized eggs and survival of spawned eggs from crosses using eggs and milt from untreated or corn oil-treated lake trout were not significantly different between the two years when reproduction was assessed (Table 5).

Toxaphene

The proportion of lake trout eggs successfully fertilized from toxaphene-treated female lake trout was not different in either of the years in which reproduction was assessed (Table 5). There were also no differences in the proportion of non-viable embyros or sac-fry or in the total survival of larvae from fertilization through to swim-up.

Table 5. Means of reproductive measures (± S.E.) for crosses of treated and control lake trout done in 1990 and 1991. Results of t-test comparisons are reported as p-values.

Female	Male	Year	Number of Crosses	% Fertilization	p[c]	% Non-viable Embryo/Sac-Fry	p	% Survival of Fertilized Eggs	p	% Total Survival of Spawned Eggs	p
Control[a]	Control	1990	5	94.96 ± 1.83		8.72 ± 3.02		90.18 ± 4.01		86.57 ± 2.61	
		1991	7	94.57 ± 0.92	0.633[b]	6.01 ± 3.02	0.360[b]	93.99 ± 3.02	0.390[b]	88.89 ± 3.02	0.519[b]
Chlordane	Control	1990	3	89.31 ± 5.55	0.404	14.05 ± 2.31	0.246	85.95 ± 2.31	0.246	76.44 ± 3.64	0.093
		1991	1	77.27		11.42		88.58		68.45	
Control	Chlordane	1990	3	95.00 ± 1.44	0.908	15.09 ± 3.14	0.217	84.91 ± 3.14	0.217	80.53 ± 1.97	0.189
		1991	3	94.84 ± 0.61	0.544	1.85 ± 0.37	0.372	98.15 ± 0.37	0.389	93.09 ± 0.91	0.639
P_4CDF	Control	1990	3	91.19 ± 1.63	0.242	6.44 ± 1.85	0.649	93.56 ± 1.85	0.649	85.41 ± 3.17	0.829
		1991	0								
Control	P_4CDF	1990	3	92.86 ± 1.63	0.435	2.95 ± 1.09	0.153	97.05 ± 1.09	0.153	90.17 ± 2.44	0.431
		1991	2	95.47 ± 3.57	0.544	14.20 ± 12.24	0.389	85.80 ± 12.25	0.389	82.35 ± 14.76	0.639
Toxaphene	Control	1990	3	83.92 ± 6.90	0.198	14.70 ± 5.98	0.470	85.30 ± 5.98	0.470	71.81 ± 8.88	0.229
		1991	3	96.66 ± 0.26	0.153	11.78 ± 2.13	0.168	88.22 ± 2.13	0.073	85.28 ± 2.09	0.329
Control	Toxaphene	1990	3	93.85 ± 1.20	0.574	3.41 ± 0.80	0.199	96.59 ± 0.80	0.347	90.63 ± 1.01	0.199
		1991	3	89.65 ± 1.87	0.035	6.90 ± 1.90	0.623	93.10 ± 1.90	0.623	83.51 ± 3.17	0.273

[a] Control fish were either corn oil injected or untreated fish.
[b] p value for controls refers to comparison of 1990 vs. 1991 control crosses.
[c] all p values for treated crosses are for comparison with control cross from the same year.

Fertilization success of eggs from untreated females when spawned with milt from toxaphene-treated male lake trout was not decreased in 1990 but was decreased in 1991 crosses. The magnitude of the change was relatively minor from 94.6% fertilization with control males to 89.7% fertilization with toxaphene-treated males (Table 1). There were no differences in the proportions of non-viable embryos/sac-fry, survival of fertilized eggs or in total survival of eggs spawned in crosses using toxaphene-treated males in either of the years.

Although no crosses were made 1988, one point to note was the absence of toxaphene-treated fish on the spawning grounds. As reported in chapter 1, only 1 lake trout treated with toxaphene was recaptured in the fall 1988 recapture period. Apparently few, if any, toxaphene-treated lake trout spawned in the spawning period immediately following treatment.

Chlordane

There were no significant differences in proportions of fertilization, non-viable eggs/sac-fry or in survival of fertilized eggs from crosses using chlordane-treated female lake trout in 1990 (Table 5). There was a marginal decrease in the total survival of eggs/embryo/sac-fry from chlordane-treated females from 94.0% to 89.3% ($p=0.093$) in the 1990 crosses (Table 5). Although no statistical testing was done on the 1991 data since only one cross was performed in that year, fertilization and total survival were decreased compared to control values (Table 5). For crosses done using chlordane-treated male lake trout, there were no differences in fertilization, survival of fertilized eggs, proportion of non-viable embryos and sac-fry, or in the survival of spawned eggs in either year (Table 5).

P_5CDF

Perhaps one of the most interesting results from the experiment was found in the reproduction of lake trout treated with P_5CDF. No differences were found in any of the reproductive measures made on eggs spawned from P_5CDF-treated female lake trout in 1990 (Table 1) or in crosses which were done using P_5CDF-treated male lake trout and untreated females (Table 1). However, in 1991 no ripe P_5CDF-treated females could be found to spawn despite the fact that four of the seven P_5CDF-treated fish caught were females. One female was sacrificed on the last day of sampling and she showed no evidence of having undergone oogenesis that year. In the following year, of three females recaptured and sacrificed, two had not undergone oogenesis and the third had undergone only partial formation of eggs. Records of fish recaptured from this lake indicate that females usually spawn every year. In fact, the female sacrificed in 1991 had been successfully spawned the previous year. This suggests that oogenesis was disrupted by the P_5CDF very early in the reproductive cycle. Evidently when oogenesis was initiated, it resulted in viable eggs, but for some reason it was not initiated in all P_5CDF-treated females..

Reproductive Assessments for White Sucker

Controls

The proportion of non-viable embyos/sac-fry, survival of fertilized eggs and survival of spawned eggs from crosses using eggs and milt from untreated or corn oil-treated white suckers were significantly different between the two years when reproduction was assessed (Table 6). Fertilization success was not different between the two years, however, it was lower and more variable in the first year (Table 6).

Table 6. Means of reproductive measures (± S.E.) for crosses of treated and control white sucker done in 1990 and 1991. Results of t-test comparisons are reported as p-values.

Female	Male	Year	Number of Crosses	% Fertilization	p[c]	% Non-viable Embryo/Sac-Fry	p	% Survival of Fertilized Eggs	p	% Total Survival of Spawned Eggs	p
Control[a]	Control	1990	5	40.68 ± 15.60		25.62 ± 6.04		73.42 ± 6.35		25.07 ± 11.10	
		1991	8	62.31 ± 12.00	0.257[b]	5.52 ± 1.69	0.006	94.48 ± 1.69	0.006	60.65 ± 12.09	0.045
Chlordane	Control	1990	7	23.43 ± 8.27	0.290	43.98 ± 14.17	0.991	55.98 ± 14.21	0.079	11.88 ± 4.91	0.259
		1991	5	31.39 ± 12.39	0.064	20.63 ± 7.99	0.071	79.37 ± 7.99	0.036	25.45 ± 9.52	0.028
Control	Chlordane	1990	2	18.38 ± 10.59	0.448	53.11 ± 36.36	0.339	46.89 ± 36.36	0.347	12.47 ± 11.65	0.429
		1991	2	14.64 ± 7.57	0.035	14.95 ± 4.26	0.042	85.05 ± 4.26	0.042	12.55 ± 6.19	0.036
P$_4$CDF	Control	1990	3	18.49 ± 6.32	0.353	26.16 ± 6.75	0.887	73.37 ± 6.51	0.914	14.32 ± 5.48	0.527
		1991	3	13.56 ± 6.83	0.012	2.63 ± 1.25	0.332	97.37 ± 1.25	0.332	19.82 ± 1.65	0.072
Control	P$_4$CDF	1990	5	40.91 ± 11.91	0.993	14.51 ± 7.26	0.286	79.87 ± 7.04	0.572	32.53 ± 10.53	0.607
		1991	2	17.64 ± 1.79	0.047	5.23 ± 2.50	0.983	94.77 ± 2.50	0.983	16.84 ± 1.36	0.055
Toxaphene	Control	1990	5	23.79 ± 7.51	0.352	26.52 ± 17.82	0.963	73.33 ± 17.79	0.944	21.02 ± 6.76	0.723
		1991	5	12.04 ± 7.36	0.002	47.10 ± 20.30	0.024	52.90 ± 20.30	0.024	12.43 ± 8.42	0.007
Control	Toxaphene	1990	4	26.87 ± 11.88	0.483	22.14 ± 10.11	0.659	77.59 ± 10.02	0.642	16.66 ± 6.37	0.575
		1991	3[d]	15.65 ± 18.69	0.017	9.33		90.67		43.93	

[a] Control fish were either corn oil injected or untreated fish.
[b] p value for controls refers to comparison of 1990 vs. 1991 control crosses.
[c] all p values for treated crosses are for comparison with control cross from the same year.
[d] n=3 only for fertilization data, all others n=1 as only 1 cross had sufficient fertilization for rearing.

Toxaphene

Toxaphene significantly affected the fertilization, viability of fertilized embryos and sac-fry and survival of eggs/sac-fry from toxaphene-treated female white suckers in crosses made in 1991, but not those done in 1990 (Table 6). In all measures, proportions were greater in crosses done in 1990 than in 1991.

No effects were found in crosses using toxaphene-treated males in 1990. Although the proportion of eggs fertilized was half of the control value, it was not significantly different (Table 6). In crosses done in the second year, only the proportion of eggs fertilized was tested for significance. Mean fertilization rate was significantly different and was approximately 25% of the control fertilization rate (Table 6). Of the three crosses made using toxaphene-treated male white suckers, only one had a sufficient number of eggs fertilized for further rearing. The other crosses had less than 1% fertilization success. The successful cross had a 43% survival rate of spawned eggs compared with a mean of 60% survival for control crosses (Table 6).

Chlordane

Although proportions of the reproductive measures were similar for crosses made with chlordane-treated white sucker females in both years, only those from the second year differed significantly from control crosses (Table 6). Fertilization was 50% of control values as was the overall survival of spawned eggs. The mean proportion of embryos and sac-fry which were classified as non-viable was four times the mean value for control crosses (Table 6).

Crosses made with chlordane-treated males showed significant effects only in the second year of reproductive assessment (Table 6). All proportions were significantly affected when chlordane-treated male white suckers were crossed with untreated females.

P_5CDF

Eggs from P_5CDF-treated female white suckers were significantly affected in 1991 but not in 1990. The proportion of fertilized eggs was decreased from control proportions in both years, however, only that from 1991 differed significantly from controls (Table 6). There were no increases in the percentages of non-viable embryos and sac-fry in either year. Results were similar for crosses made using P_5CDF-treated male white suckers spawned with untreated females. The proportion of fertilized eggs was significantly reduced in the 1991 samples, but was similar to control values in 1990 (Table 6).

Residues in Eggs

Residues were measured using water-hardened eggs a choice which results in lower concentration because of the increased weight of these eggs. Concentrations were converted to non-water-hardened values (i.e. ovarian egg concentrations) by multiplying by a factor representing the average gain in weight for water-hardened versus ovarian eggs. For lake trout it was determined that, on average, water-hardened eggs were 1.43 times heavier than freshly spawned eggs. For white suckers, water-hardened-eggs were 4.89 times the weight of freshly spawned eggs.

Mean concentrations of the three contaminants determined from sub-samples of the spawned eggs from both control and treated females of both species are presented for both water-hardened values in Table 7a and converted to non-water hardened values in Table 7b.

In general, concentrations were greater in lake trout eggs than white sucker eggs. With the exception of controls, concentrations decreased in eggs from 1990 to 1991. Residue determinations were not made in eggs sampled in 1991 from P_5CDF-treated white suckers.

Table 7a. Mean contaminant concentrations (± S.E.) in water hardened eggs from control and treated lake trout and control and treated white suckers.

		Eggs from Control Females			Eggs from Treated Females				
Species	Year	n	Chlordane (ng/g)	Toxaphene (ng/g)	n	Chlordane (ng/g)	n	Toxaphene (ng/g)	n	P_5CDF (pg/g)
Lake Trout	1990	2	8.24 ± 0.89	0.60 ± 0.06	2	477.00 ± 56.00	3	147.00 ± 32.00	2	38.9 ± 27.5
	1991	9	5.60 ± 0.60	3.36 ± 0.46	1	148.00	3	58.00 ± 12.00	0	
White Sucker	1990	3	3.79 ± 2.78	0.77 ± 0.14	3	20.95 ± 7.81	3	48.38 ± 40.00	2	2.8 ± 1.1
	1991	3	0.23 ± 0.05	n.d.	4	10.24 ± 3.71	3	5.86 ± 4.68	0	

n.d. - Not detected

Table 7b. Adjusted mean contaminant concentrations (± S.E.) in eggs from control and treated lake trout and control and treated white suckers. Values were adjusted to factor out increased weight of eggs from water hardening.

		Eggs from Control Females			Eggs from Treated Females				
Species	Year	n	Chlordane (ng/g)	Toxaphene (ng/g)	n	Chlordane (ng/g)	n	Toxaphene (ng/g)	n	P_5CDF (pg/g)
Lake Trout	1990	2	11.78 ± 1.27	0.86 ± 0.09	2	682.11 ± 80.08	3	210.21 ± 45.76	2	55.6 ± 39.3
	1991	9	8.01 ± 0.86	4.80 ± 0.66	1	211.64	3	82.94 ± 17.16	0	
White Sucker	1990	3	18.53 ± 13.59	3.77 ± 0.68	3	102.45 ± 38.19	3	236.58 ± 195.60	2	13.6 ± 5.5
	1991	3	1.12 ± 0.24	n.d.	4	50.05 ± 18.16	3	28.67 ± 22.88	0	

n.d. - Not detected

Discussion

Controls

A major difference between the two years was the weather. In 1990 the lake was extremely slow to warm up which resulted in white suckers spawning two weeks later than normal. It is possible that fish were not quite ready or alternatively that they had started to resorb eggs because of the delayed spawning. As well, when the water did warm, temperatures increased very quickly. At the beginning of incubation, water temperatures were 16°C and this reached 21°C by termination, with development taking 7 days, compared with a change in water temperatures from 12°C to 17°C over 11 days in 1991.

The differences between the white sucker control crosses in the two years when reproduction was assessed made interpretation of reproduction effects more complicated. In many cases similar proportions for a given measure were found in treated crosses for both years, but because of decreased fertilization and survival and increases in the numbers of non-viable embryos and sac-fry in the 1990 control data, values were not significantly different from controls.

Toxaphene

Toxaphene caused significant decreases in the fertilization of eggs from white suckers. The mean concentration of toxaphene in white sucker eggs associated with a mean decrease of 80% in fertility was 28.7 ng/g wet weight for non-water hardened eggs (Table 7). In addition, toxaphene decreased the efficiency of milt from treated male white suckers to fertilize eggs from untreated females. Mean toxaphene concentrations in male white suckers were 1552±679 and 852±800 ng/g, respectively, in the two years when reproductive effects were assessed. In addition to the effects on fertilization, treatment with toxaphene caused increases in the proportion of non-viable white sucker

embryos and sac-fry, with 47% of fertilized eggs being non-viable in the 1991 crosses. This decrease, coupled with the decrease in fertilization, resulted in a total survival of 12% of spawned eggs compared to a 60% survival rate for control crosses.

A decrease small decreased (5%) was also found in the ability of milt from toxaphene-treated male lake trout to fertilize untreated eggs. Mean concentrations of toxaphene in whole fish homogenates of treated male lake trout were 922 and 508 ng/g in the two years respectively.

Two laboratory studies have previously examined the effects of toxaphene on reproduction in fish. Mayer et al. (1977) found no effect on hatching success in fathead minnows (*Pimepheles promelas*) exposed to five different concentrations of toxaphene in the water (0 to 173 ng/L). Significant decreases were found in the success of channel catfish eggs from females exposed to 630 ng/L and this was accompanied by a decrease in the amount of proteinaceous matrix surrounding the eggs.

Brook trout (*Salvelinus fontinalis*) eggs exposed for 22 days in a flow-through system to a concentration range of 0 - 502 ng/L toxaphene had decreased viability at levels higher than 68 ng/L (Mayer et al. 1975, 1977). Toxaphene measured in eggs ranged from 400 ng/g to 5200 ng/g, with viability being reduced at levels greater than 900 ng/g. Lake trout eggs from toxaphene-treated females in the current study contained between 35 and 203 ng/g, values much less than those reported by Mayer et al. (1975) to cause decreased viability in brook trout eggs. Concentrations of toxaphene in white sucker eggs (corrected for water hardening) were similar to those found in lake trout. Effects were much more pronounced, indicating that white sucker eggs are more sensitive to toxaphene than salmonid eggs.

Mayer et al. (1975) and Mehrle et al. (1977) reported that adult brook trout exposed to high levels of toxaphene had a higher mortality rate just prior to spawning than unexposed fish. This offers a plausible explanation for the apparent increased mortality of toxaphene-treated lake trout in the first interval (4 months) after treatment

which resulted in a lack of spawning adults from the toxaphene-treated group in the first spawning period following treatment.

Chlordane

Despite the widespread use of chlordane in the years following the ban of DDT there are no previous reports in the literature on effects of chlordane on reproduction in fishes.

Changes in fertilization and survival of eggs and embryos from chlordane-treated white suckers were similar to effects seen in toxaphene-treated fish. Decreased fertilization of eggs occurred in crosses using both chlordane-treated females and males. Fertilization of eggs from treated females was reduced by roughly 50% relative to control crosses in each year corresponding to mean egg concentrations of 105 and 50 ng/g (non-water hardened) in 1990 and 1991 respectively.

Fertilization of eggs by chlordane-treated males was also reduced in both years, to 50 and 75% respectively in 1990 and 1991 relative to control crosses. Mean concentrations in treated male white suckers were relatively high at 5465 and 1471 ng/g in the two years respectively.

In addition to decreased fertilization, increases in the proportions of non-viable embryos and sac-fry were found with crosses which used either chlordane-treated female or male white sucker. The combined effect resulted in total survival of spawned egg of 12 to 25% compared with 60% in control crosses.

Effects of chlordane on lake trout reproduction were less pronounced than effects on white sucker. No effects were found in crosses between untreated females and chlordane-treated male lake trout. A marginal decrease (10%) was found in total survival of spawned eggs from 1990 crosses using chlordane-treated females. In this case there was not a large difference from a lack of fertilization or from increases in the numbers of

unviable embryos and sac-fry, but rather a cumulative effect of the two. Because of difficulty in recapturing chlordane-treated female lake trout in 1991, only one cross was done, making statistical comparison with controls impossible. However the survival of eggs from this cross was consistent with the decreased total survival noted in the previous year.

P_5CDF

A number of researchers have focused their attention on the effects of chlorinated dioxins and furans on reproduction and development in fish. Specifically they have concentrated on the planar configurations which are chlorinated in the 2,3,7,8 positions. The mode of toxicity and the effects are believed to be the same for dioxins and furans chlorinated at the 2,3,7,8 positions, differing only in the degree of potency of a given congener. The similarity of effects caused by dioxins, furans and non-ortho substituted PCBs has led to the development of toxic equivalent factors (TEFs), using embryo mortality as the endpoint. These factors allow the comparison of effects caused by different dioxin, furan and PCB congeners.

Effects of dioxins and furans include developmental and growth retardation in embryos and fry of rainbow trout and pike, increased incidence of skeletal malformations, (Helder 1980a, 1981), increased mortality rates at hatch due to half hatching in rainbow trout, lake trout and Japanese medaka (Helder 1980,1981, Spitsbergen et al. 1991, Walker et al. 1990, Wisk and Cooper 1990a) and a lethal generalized edema which has been termed blue-sac disease (Walker et al. 1990).

In this study no effects were seen in reproductive success of eggs from P_5CDF-treated lake trout in 1990. Mean concentration of P_5CDF in eggs was 38.9 pg/g. Use of a TEF for the 2,3,4,7,8 substituted furan congener of 0.700 (Walker and Peterson 1991) gives a equivalent TCDD concentration of 27.8 pg/g. Walker et al. (1994) report a no

observable adverse effects level (NOAEL) of 23 pg/g and a lowest observable effects level of 50 pg/g for maternally transferred TCDD in lake trout. On the basis of these values, no effects would be expected in the eggs from treated female lake trout spawned in 1990.

Perhaps the most surprising finding from this study was the lack of oogenesis in P_5CDF-treated female lake trout in 1991 and 1992. Although the sample may seem quite small, the four fish which are known to have been affected (1 in 1991, 3 in 1992), represent 25% of the female treated population. The cessation of oogenesis has previously been reported in zebrafish (*Brachydanio rerio*) exposed to TCDD (Wannemacher et al. 1992). In Wannemacher's study, fish treated with a single dose of ≥ 5 ng TCDD showed a rapid decrease in the number of eggs/spawning, and failed to produce eggs after 1 - 2 spawnings. Histological examination of ovaries from exposed fish revealed a significant dose-dependent increase in the number of immature previtellogenic oocytes and a decrease in mature vitellogenic follicles (Wannemacher et al. 1992). These effects are similar to those seen in P_5CDF-treated female lake trout in the current study; namely cessation of oogenesis (3 fish) or in the case of a single fish, only partial oogenesis, were not seen until after one or two reproductive cycles. Mean P_5CDF concentration in non-spawning female lake trout (whole fish homogenate) was 435 pg/g, with a range of 90 - 486 pg/g.

Treatment with P_5CDF also affected reproduction in white sucker. Crosses made with eggs from P_5CDF-treated female white sucker had a mean reduction of 50 - 80% in fertilization success. There was no increase in the mortality of fertilized eggs, but because of the low fertilization rate, survival of spawned eggs was reduced 45 - 60%. Mean egg concentrations in 1990 were 13.6 ng/g for non-water hardened eggs which is lower than concentrations found in lake trout eggs. This indicates that white sucker eggs may be even more sensitive to effects of P_5CDF on viability than lake trout eggs. Unfortunately, because the 1991 samples were lost, a year-to-year comparison cannot be

made. If we assume that the transfer of contaminant to eggs from body stores of P_5CDF is similar between years, then concentrations in the 1991 spawned eggs would be lower by approximately 25% based on a half life ($t_{0.5}$) of roughly 2 years for P_5CDF in white sucker (see Chapter 5 for $t_{0.5}$ estimation). Thus the viability of white sucker oocytes may be compromised at very low levels of contamination.

General

From an ecological viewpoint, the ultimate measure of reproductive success is taken to be the survival of an egg from spawning through to first feeding. The stages between spawning and first feeding are likely the most sensitive to insult from pollutants. The hydrophobic nature of the contaminants in this study was such that concentrations in water would not normally be of concern. Thus once fish begin feeding, natural mortality likely outweighs any potential mortality from contaminants. However, the possibility cannot be ruled out that exposure to the contaminants as a developing embryo will affect future reproductive potential. Effects on the F1 generation have been show in flag fish (*Jordanella floridae*) cultured from parents exposed to methoxychlor (Holdway and Dixon 1986), and effects have also been reported in male mice exposed *in utero* to TCDD (Mably et al. 1992).

The reproductive ecology of each of the species may influence the seriousness of the effects caused by the contaminants. Lake trout reproduce using the strategy of fewer larger eggs which develop over an extended period of time (5 months) whereas white suckers reproduce using a large number of small eggs which develop over a very short period of time (7 - 10 days). These differences in allocation of reproductive effort may account for some of the differences between species found in this study. Overall, reproductive success in white suckers appeared to be more sensitive than in lake trout. Effects on white suckers were much more pronounced and generally occurred at lower

concentrations of contaminants. The most important post-spawning factor in the success of sucker reproduction was fertilization, with viability of gametes being affected in both male and female white suckers. Because effects on reproduction were assessed independently for males and females, the possibility remains that these effects may be additive, so that in a situation where both males and females are contaminated, the result may be even lower overall reproductive success.

Chapter 3. Effect of an I.P. Injection of 2,3,4,7,8 - Pentachlorodibenzofuran on Mixed Function Oxygenase (MFO) Activity, Retinoids and Tocopherol Levels in Natural Populations of Lake Trout and White Suckers.

Introduction

Biomarkers can be defined as measurements of body fluids, cells or tissues that indicate in biochemical or cellular terms the presence of contaminants or the magnitude of an organisms response to contaminants (McCarthy and Shugart 1990). The use of biochemical indices (biomarkers) has become an important tool in the field of toxicology in the early detection of potential problems. Biomarkers may indicate exposure of organisms to various toxicants long before effects are manifested at higher levels of organization, such as population, community or ecosystem. As such, biochemical indicators are useful as an early warning of potential problems. However, in many cases they offer little in the way of information as to the duration of exposure or seriousness of the effect. The consequences of long term changes in various biochemical processes have not been well studied.

Mixed Function Oxygenase

Induction of mixed function oxygenase (MFO) enzymes, through the aryl-hydrocarbon receptor complex (Ah receptor), is generally recognized as a sensitive biochemical indicator of exposure of fish to planar halogenated aromatic hydrocarbons (PHAHs) such as non-ortho substituted PCBs, dioxins and furans, and polycyclic aromatic hydrocarbons (PAHs) (James and Bend 1980, Goksoyr and Forlin 1992). Induction of MFO enzymes in fish is well documented from both laboratory studies (Muir et al. 1990c, Parrot et al. 1995, Stegeman et al. 1981) and from field observations (Andersson et al. 1988, McMaster et al. 1991, Munkittrick et al. 1991, Spies et al. 1988, Rogers et al. 1989, Monosson and Stegeman 1991). The intensity of induction has been

shown to depend on both dose and structure (Melancon and Lech 1983, Safe 1990, Tillit et al. 1991). This relationship between structure and dose has been utilized in the development of toxic equivalence factors (TEFs) among compounds with similar pharmacologies (Ahlborg et al. 1994, Goldstein and Safe 1989, Safe 1990, 1991, 1992). These are used to calculate toxic equivalent concentrations (TECs) in environmental samples where mixtures of contaminants exist (e.g. Williams et al. 1992).

Although increases in MFO activity provide a sensitive indication of exposure of fish and other animals to PHAHs such as dioxins, furans and non-ortho substituted PCBs, the toxicological and ecological relevance of increased enzyme activity has not been established in fish. Furthermore, little is known about the kinetics of induction. It is assumed that, as concentrations decrease following uptake, the level of enzyme activity will also decrease. Although the relative potencies of some compounds have been well studied, the ability of these compounds to sustain induction and the effects associated with this induction have not been extensively studied.

Retinoids and Tocopherol

Both retinoids and tocopherol have recently received attention as indicators of exposure to different environmental contaminants in both fish (Palace and Brown 1994, Spear et al. 1992) and birds (Spear et al. 1990, 1989). Retinoids are required in animals for vision, cell development and cell growth and cell differentiation (Halver 1982, Moore 1957). Tocopherol is an important antioxidant involved in the protection of cellular and sub-cellular membranes (Burton and Traber 1990).

Interest in these essential nutrients stems from studies in mammals (Chen et al. 1992, Hakkanson et al. 1991, Spear et al. 1988, Thunberg et al. 1979), birds (Spear et al. 1990) and fish (Spear et al. 1992, Palace and Brown 1994). These studies indicate that chemicals which interact with the Ah-receptor may alter the metabolism of retinoids and tocopherol.

Biomarkers measured in the current experiment were examined to study the magnitude and duration of change in biochemical measures (hepatic MFO levels, and hepatic levels of retinoids and tocopherol) in naturally occurring lake trout and white sucker adults exposed to P_5CDF. A further goal was to determine if any link exists between responses seen at the biochemical level and those reported at the individual level (growth, reproduction) and the population level (abundance).

Materials and Methods

Treatment of Fish

The study site, capture and treatment methods are described in Chapter 1.

Sampling of Fish

Sub-samples of fish chosen at random for each species from each treatment and control group were sacrificed with an overdose of MS-222. Livers were removed immediately upon death and placed in labeled bags. Each sample was then frozen quickly between two blocks of dry ice. Samples were stored on dry ice until transport to Winnipeg, where they were stored at -80°C until analyzed.

Microsome Preparation for EROD Determination

All sample handling was done in a controlled environment room at 2 °C. Samples were transferred from the freezer to the CE room and any sub-sampling was done immediately on the frozen sample. If sub-sampling was required, any remaining sample was immediately returned to the freezer. The sample to be analysed (approx. 1 gram) was accurately weighed and allowed to semi-thaw at 2 °C. The sample was then cut into small pieces with scissors and transferred to a 15 ml conical glass tissue homogenizer. The tissue was homogenized in a HEPES-KCl buffer (0.02 M HEPES, 0.15 M KCl, pH 7.5)

at the rate of 4 ml of buffer per gram of tissue using 5-7 passes of a motor driven Teflon pestle. The resulting homogenate was centrifuged at 12,350 x g for 20 minutes at 2 °C (10,000 RPM in a Sorval SM-24 rotor in a Sorval RC-2B superspeed centrifuge). The resulting postmitochondrial supernatant was centrifuged at 100,000 x g for 75 minutes at 2°C to pellet the microsomes (T1270 rotor in Sorval RC-60 ultracentrifuge at 39,000 RPM). The microsomal pellet was rinsed three times with the resuspension buffer (0.1 M Tris, 1 mM dithiothreitol, 1 mM EDTA, 20 % (by vol) glycerol, pH 7.4) and then resuspended in sufficient buffer to make a protein concentration range of 5-10 mg/ml. The resuspended microsome samples were then frozen and stored in liquid nitrogen until analysis.

Ethoxyresorufin O-Deethylase (EROD) Determination

The determination of EROD was based on the assay described by Pohl and Fouts (1980). The reaction was carried out in disposable 16 x 100 mm culture tubes. The reaction mixture consisted of buffer (1100 µL Hepes buffer 0.1 M, pH 7.8), magnesium sulphate (10 µL, 0.154 g/ml), and NADPH generating system (10 µL, NADP, 98.3 mg/mL, 50 ul bovine albumin, 0.04 g/mL, 10 µL isocitric acid - trisodium salt, 193.58 mg/mL, and 10 µL (1 unit) isocitric dehydrogenase). These were mixed and allowed to stand a minimum of 10 minutes prior to the addition of 50 µL of resuspended microsomes. The samples were then incubated for a minimum of 5 minutes at 25°C prior to the addition of ethoxyresorufin (10 µL ethoxyresorufin in dimethyl sulphoxide, 1.33 mg/ml). After the ethoxyresorufin addition, the samples were incubated for 2 minutes (accurately timed) at 25 C and then the reaction was stopped with 2.5 ml of HPLC grade methanol. Blanks had methanol added prior to the addition of the ethoxyresorufin.

Samples analysed prior to September, 1991, were done in 16 x 100 mm Corex centrifuge tubes and centrifuged at 15,500 x g for 5 minutes to precipitate the protein.

Samples analysed after August 1991 were centrifuged at 3500 x g for 15 minutes to precipitate the protein. (Both procedures are effective in pelleting the precipitated protein and yield the same activity results.) The resorufin produced was then measured in the supernatant fluorometrically.

Samples analysed prior to April, 1992 were analysed using an Aminco-Bowman spectrofluorometer with excitation and emission wavelengths of 530 and 585 nm. Triplicate assays and triplicate blanks were run for each sample and the mean blank for that particular sample was used in the enzyme activity calculation. After April, 1992, fluorescence of the resorufin produced was measured in the supernatant using a Perkin-Elmer LS50 luminescence spectrofluorometer with an excitation wavelength of 530 nm with an emission wavelength of 585 nm and excitation and emission slit widths of 9 nm. One reagent control and triplicate assays were run for each sample. The values obtained for the reagent controls from all samples were averaged and this average value was used in the calculations of enzyme activity for all samples in the batch.

Activity was calculated using a response factor from standards containing known amounts of resorufin added to the reaction matrix and measured identically to the samples. The results are expressed in nanomoles of resorufin produced per milligram of protein in the reaction mixture per minute of incubation time (nmoles/mg/min).

Protein Analysis

The assay is based on the modification of the Lowry procedure (Lowry 1951) by Markwell et al. (1981) Microsome samples required dilution to 1/50 or 1/100 with distilled water prior to analysis. All samples were analysed in triplicate. Reaction reagent was prepared daily by mixing reagent A (2 % Na_2CO_3, 0.4 % NaOH, 0.16 % sodium tartrate, 1.0 % sodium dodecyl sulphate) and reagent B (4 % $CuSO_4.5H_2O$) in the proportions 100:1. Sixty (60) µL aliquots of sample were added to 1.0 ml of the reaction

reagent and allowed to stand for at least 10 minutes at room temperature. One hundred (100) µL of Folin-Ciocalteu reagent (BDH Chemicals, Toronto, diluted 1:2 with redistilled water) was added to each tube. The samples were allowed to stand for at least 30 minutes at room temperature and then the absorbance was read at 600 nm using a Beckman DU-7 spectrophotometer.

A standard curve was prepared using bovine serum albumin. Quality control was maintained by analysing aliquots of two bulk samples of microsomes and a lyophilized human quality control serum with each batch of samples along with the standards. (Prior to January, 1993 a single standard response factor had been determined for each batch of reagents for use in the calculation of protein concentration. After that date a full standard curve as well as the QC's were run with each batch of samples.) Appropriate reagent controls were run with each batch of samples.

Determination of Retinoids and Tocopherol

Determination of hepatic levels of retinol, dehydroretinol retinyl palmitate and tocopherol was done using the method described in Palace and Brown (1994). In brief, 100 mg of tissue were homogenized in 2.0 mL of distilled-deionized water. To 200 µL of the homogenate 200 µL of HPLC-grade ethanol were added to precipitate the protein. Samples were then extracted with 500 µL (3:2, v/v) ethylacetate:hexane. These were then dried and residues from the ethylacetate/hexane extracts were redissolved in mobile phase (acetonitrile: methanol:water 70:20:10 v/v/v) for HPLC analysis.

Samples (10 µL) were injected onto a 3 µm bead-size Adsorbosphere HS C_{18} column (4.6 mm i.d., 150 mm length) with an attached 10-mm Adsorbosphere guard column (Alltech Associates, Deerfield , IL). The column was kept at a constant 26°C and samples and standards were eluted isocratically with acetonitrile:methanol:water (70:20:10 v/v/v) with a flow rate of 1 mL/min. A model 116 dual channel UV

absorbance detector was used (Gilson Medical Electronics, Milwaukee, WI). The detector was set at 292 nm for tocopherol and tocopherol acetate detection and 325 nm for retinol and retinyl palmitate detection. Tocopherol acetate was used as an internal standard as a check for extraction efficiencies.

Determination of Hepatic P_5CDF

Determination of liver residues was done using the method described for determining muscle concentrations described in Chapter 5. Sub-samples (1-2 g) of liver were freeze dried and then homogenized in toluene using a Polytron Homogenizer (Kinematica). Samples were thoroughly mixed, allowed to sit 24h, then centrifuged to separate particulate matter from the toluene. A 1 mL aliquot of toluene was counted by LSC as described in Chapter 5.

Results

Mixed Function Oxygenase Enzyme Activity

Activities of MFO enzymes, as represented by ethoxyresorufin-O-deethylase (EROD), were significantly increased ($p<0.05$) in lake trout treated with P_5CDF (Figure 24). Increased enzyme activity was sustained throughout the experiment (roughly 4 years) at a level approximately 100 times greater than control levels.

EROD activity was also significantly ($p<0.05$) increased in white suckers treated with P_5CDF (Figure 25). As with the lake trout, the increased enzyme activity was sustained throughout the experiment, but, with the exception of the first year, the level of induction was only 10 to 15 times greater than control levels (Figure 25).

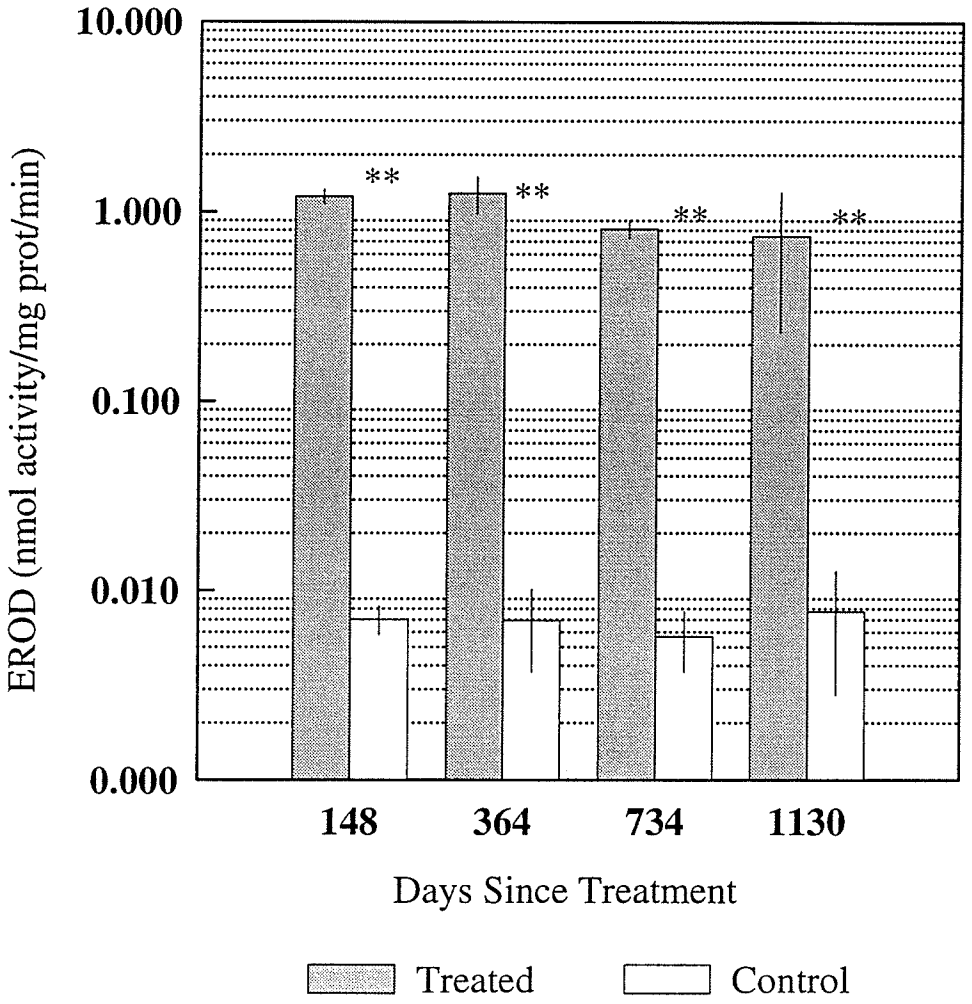


Figure 24. Mean (± S.E.)hepatic EROD activity in P_5CDF-treated and control lake trout sampled in each recapture period. Significant differences ($p < 0.05$) are indicated by **.

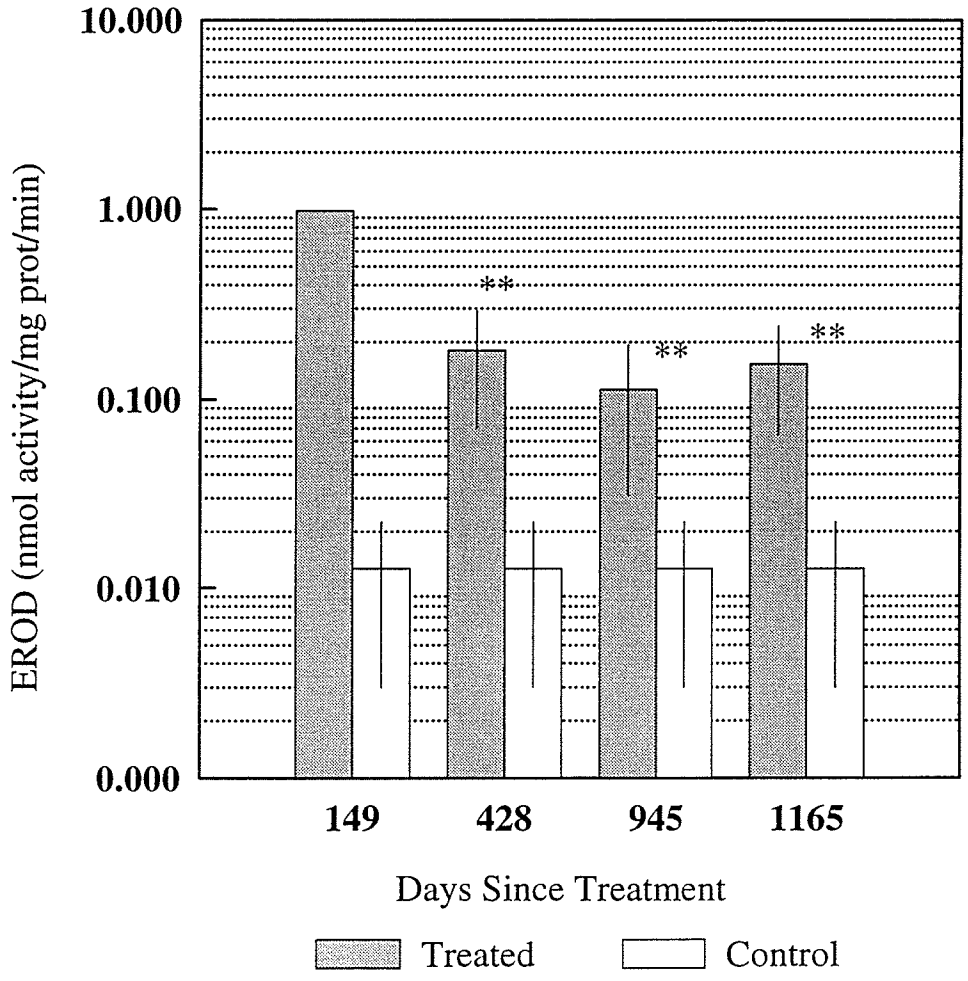


Figure 25. Mean (± S.E.)hepatic EROD activity in P_5CDF-treated and control white sucker sampled in each recapture period. Significant differences ($p < 0.05$) are indicated by **. N=1 for day 149 of P_5CDF-treated all other n≥3.

Retinoids and Tocopherol

Measurement of hepatic levels of dehydroretinol, retinol and retinyl palmitate in liver tissue from P_5CDF-treated and control lake trout showed reduced levels of dehydroretinol and retinol in males but not in females (Table 8). The storage form of vitamin A, retinyl palmitate, was significantly reduced in both sexes of lake trout (Table 8). Tocopherol (vitamin E) levels were not different between control and treated fish, but females had decreased levels when compared with males (Table 8).

No differences were found in the levels of any of the retinoids or tocopherol between P_5CDF-treated and control white suckers (Table 8).

Relationship Between EROD Activity, Retinoids and P_5CDF Concentrations

EROD activity in treated lake trout was significantly correlated (Pearson's Correlation Coefficient, $p<0.05$) with both whole body and liver concentrations of P_5CDF (Figure 26a and 26b). Liver concentrations were also correlated with whole body concentrations (Figure 26c). Mean yearly EROD activities were highly correlated with mean yearly whole body concentrations (Figure 26d)

EROD activity in treated white suckers was not correlated with mean muscle or liver concentrations of P_5CDF. Muscle or liver concentrations did not show any relationship with EROD activity when raw data were used (Figure 27a, b, c). However, there was a positive correlation between mean EROD activities and mean whole body concentrations using the three points available ($r = 0.995$, $p=0.071$) (Figure 27d).

Treated and control lake trout had retinol and retinyl palmitate negatively correlated ($r = -0.466$ and $r = -0.402$ respectively $p<0.05$) with EROD activity (Figure 28). Because no differences were found in white sucker retinoids or tocopherol, no attempt was made to test for relationships among the various measures.

Table 8. Mean (± S.E.) dehydroretinol, retinol, retinyl palmitate and tocopherol levels in P_5CDF-treated and control lake trout and white sucker.

	n	Dehydroretinol (µg/g)	Retinol (µg/g)	Retinyl Palmitate (µg/g)	Tocopherol (µg/g)
Lake Trout					
Females					
Control	6	99 ± 10	57 ± 7	3249 ± 875	981 ± 136
Treated	5	88 ± 16	57 ± 8	1004 ± 373*	786 ± 131
Males					
Control	8	123 ± 14	56 ± 7	3881 ± 834	1952 ± 235
Treated	7	6 ± 12*	34 ± 8*	1225 ± 270*	2426 ± 754
White Suckers					
Females					
Control	6	9 ± 2	23 ± 5	1867 ± 331	145 ± 34
Treated	6	8 ± 5	22 ± 13	1799 ± 451	176 ± 58
Males					
Control	5	10 ± 3	17 ± 5	1711 ± 233	358 ± 50
Treated	7	13 ± 4	27 ± 7	1434 ± 117	243 ± 37

* Significantly different from controls $p < 0.05$

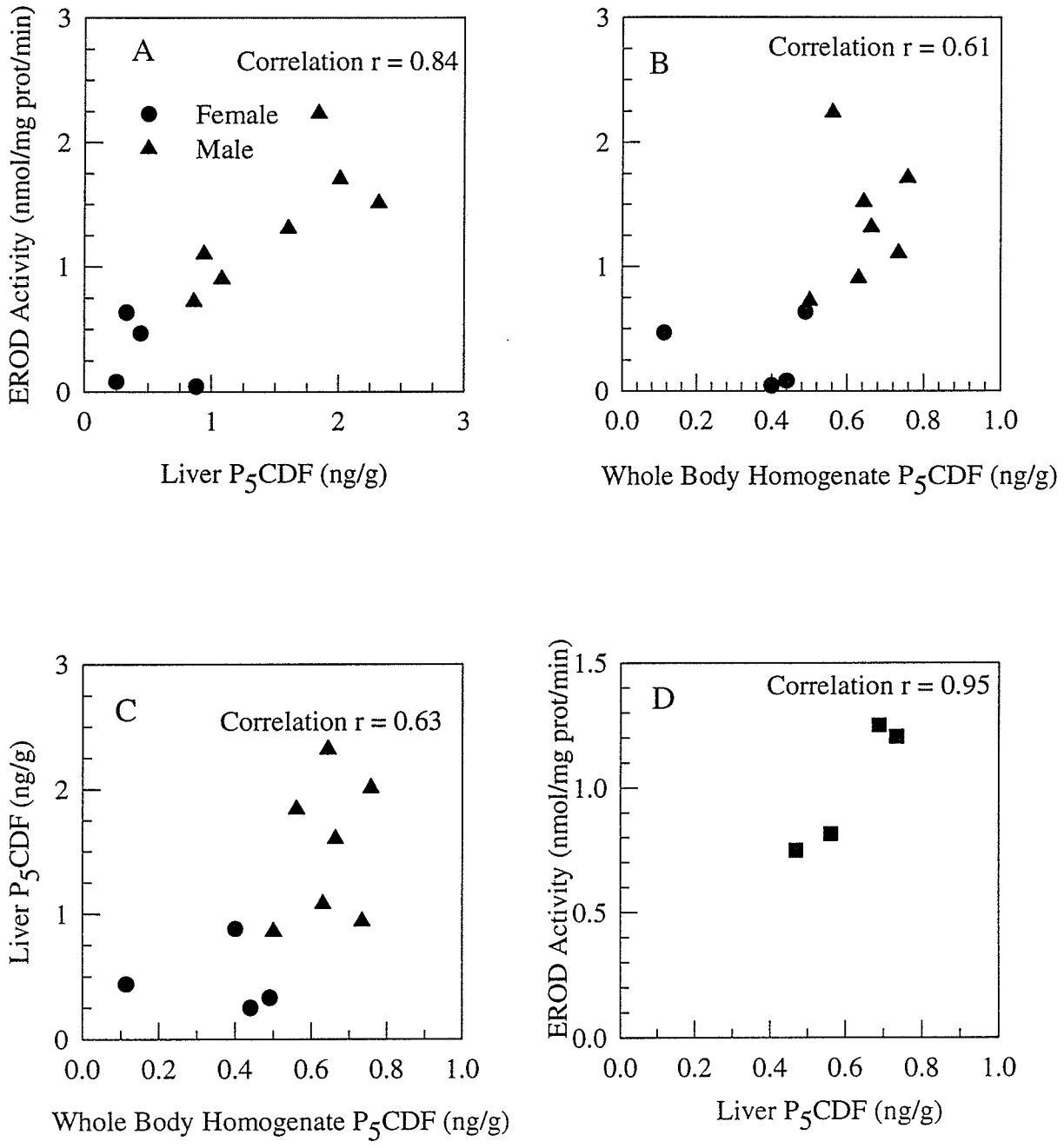


Figure 26. Relationships between (A) hepatic EROD activity and P_5CDF concentrations in liver tissue, and (B) whole body homogenates from P_5CDF-treated lake trout. Relationship between (C) P_5CDF concentrations in liver tissue and whole body homogenates and (D) relationship between mean yearly EROD activity (pooled male and female) and mean hepatic P_5CDF concentrations in P_5CDF-treated lake trout.

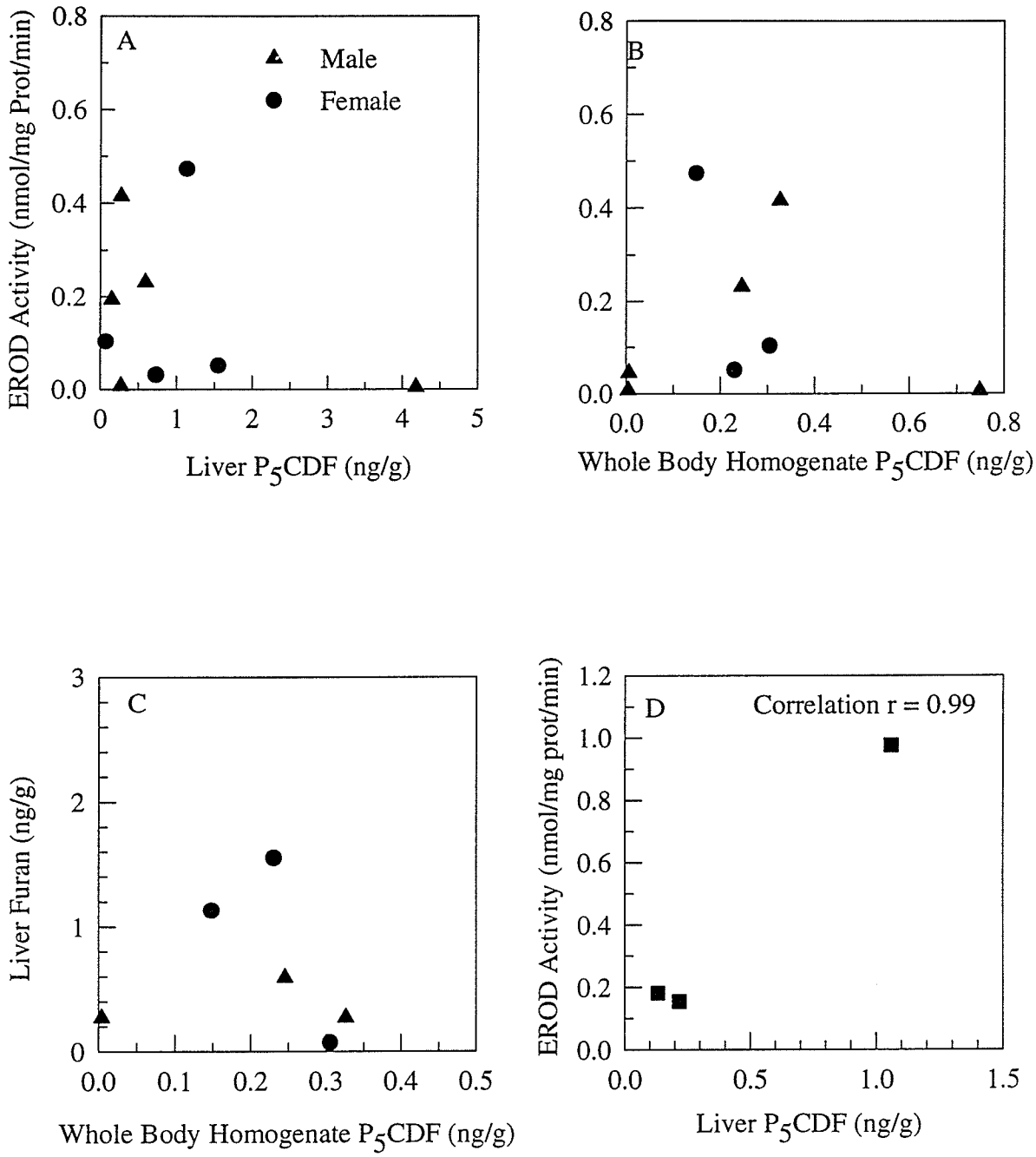


Figure 27. Relationships between (A) hepatic EROD activity and P_5CDF concentrations in liver tissue, and (B) whole body homogenates from P_5CDF-treated white sucker. Relationship between (C) P_5CDF concentrations in liver tissue and whole body homogenates and (D) relationship between mean yearly EROD activity (pooled male and female) and mean hepatic P_5CDF concentrations in P_5CDF-treated white sucker.

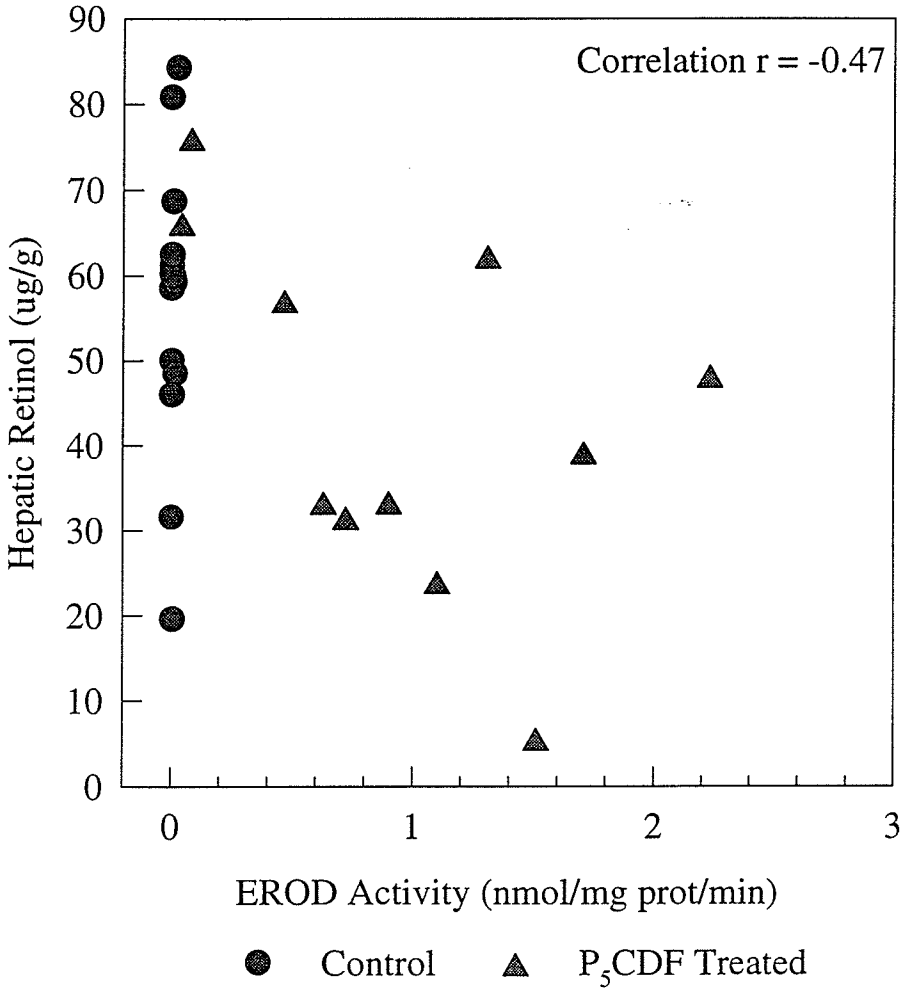


Figure 28. Relationship between hepatic EROD activity and hepatic retinol concentrations in P_5CDF-treated and control lake trout.

Discussion

Previous studies have shown that EROD activity may be increased for long periods after exposure to certain inducers ceases. Muir et al. (1990c) reported sustained induction in juvenile rainbow trout fed a diet containing the same furan congener used in the current study. They reported that EROD activity remained induced for at least 210 days during the depuration phase following exposure, and declined at a rate approximately half that of the elimination rate of the P_5CDF. Sustained increases in MFO activity has also been reported following I.P. injections of 2,3,7,8 TCDD in mammals (Poland and Glover 1974). In mammals this prolonged induction has been explained by the sustained association of TCDD with the Ah-receptor complex (Rucci and Gasiewicz 1988). Muir et al. (1990c) assumed a similar mechanism for fish in which the association of P_5CDF with the Ah-receptor remains at the same or greater concentration. Hahn and Stegeman (1994) have recently concluded that the molecular basis of the mechanism of induction appears to be conserved from fish to mammals. This study, to my knowledge, shows the longest sustained induction from a single exposure to date, with both P_5CDF-treated lake trout and white suckers exhibiting increased EROD activity for a time period in excess of 3 years (Figures 24 and 25).

The data from this study are consistent with the hypothesis that the persistence of the inducer is an important factor in the duration of the induction. Depuration of P_5CDF was extremely slow in both white sucker and lake trout with estimated mean residence times of 3 and 113 years respectively (Chapter 5). Lake trout were highly induced 1200 days after treatment (Figure 1); there is no reason to believe that this level of induction would not have continued. In white sucker, the initial depuration of P_5CDF was quicker, with slower decreases in concentration after the first year, mirroring the changes seen in EROD activity. A positive correlation between mean EROD activity and mean whole body P_5CDF concentrations was found for both species (Figure 26d and 27d). This

confirms the utility of EROD as an indicator of relative concentrations of inducing compounds.

In lake trout, the correlation of individual liver concentrations of P_5CDF with EROD activity was very good ($r = 0.84$). The relationship was not as strong between whole body concentrations and EROD ($r = 0.63$) however, this should be expected since the correlation between whole body concentrations and liver concentrations ($r = 0.61$) was not as strong (Figure 26).

In white suckers the lack of correlation of EROD activities with liver or muscle concentrations when using the raw data is somewhat puzzling, given the strong relationship between these measures in lake trout. However, a positive correlation does exist using the three pairs of mean values for each measure (Figure 27). Using means effectively removes temporal variability since means are calculated based on yearly recapture periods. These results suggest that the relationship between EROD activity and P_5CDF concentrations may also have a temporal component in white suckers. Changes in the nature of this relationship were also seen in laboratory experiments done with white suckers (See Chapter 7). In that experiment the longer the time from the exposure the more sensitive the EROD response. The reason for this is thought to be related to the time taken for uptake of the contaminant from an IP injection. The result of this is an increase in the variability of EROD activities for a given concentration of P_5CDF.

In addition to possible temporal effects, causing increased variability in the relationship between EROD activity and liver P_5CDF, at least one fish may have had an inhibited EROD response. A single male white sucker had more than 4 ng/g P_5CDF in liver but had very low EROD activity. Inhibition of catalytic activity measures of cytochrome P450 induction, such as EROD, at high concentrations of polyhalogenated aromatic hydrocarbons (PHAHs) has previously been reported for scup (*Stenotomus chrysops*) (Gooch et al. 1989), winter flounder (*Pseudopleuronectes americanus*) (Monosson and Stegeman 1991) and rainbow trout (Melancon and Lech 1983). If white

suckers are more sensitive to high concentrations than trout, this will also contribute to variability in the raw data set, contributing to the lack of correlation between EROD activity and P_5CDF concentrations in liver and whole body homogenates.

Many of the visible symptoms associated with exposure to PHAHs in mammals resemble those of Vitamin A deficiency, for example keratinizing epithelium, and impaired immune, hemopoietic and reproductive functions (Zile 1992). PHAHs have been shown to disturb Vitamin A homeostasis in mammals, but it appears that this is caused by accelerated metabolism and breakdown of vitamin A and its metabolites and a depletion of vitamin A stores from the body. The mechanisms for these events likely includes altered activities (i.e. induction and/or inhibition) of enzymes that are either directly or indirectly involved in critical metabolic pathways for vitamin A (Zile 1992).

Decreases in the levels of both circulating retinoids (dehydro-retinol and retinol) and stored retinoids (retinyl palmitate) were found in liver tissue from P_5CDF-treated lake trout. These decreases are consistent with those reported in mammalian literature for rats (Thunberg et al. 1979, Hakansson et al. 1991, Spear et al. 1988) and birds (Spear et al. 1986, 1989) exposed to PHAHs. Interest in effects of PHAHs on retinoids in fish is recent. Decreased retinoids have been reported in white suckers inhabiting a polluted area near Montreal (Spear et al. 1992), in juvenile lake trout exposed to 3,3',3,3',5-pentachlorobiphenyl (Palace and Brown 1994), in Atlantic tomcod (*Microgadus tomcod*) inhabiting areas downstream from a heavily industrialized area (Fairchild et al. 1994) and in white suckers inhabiting the Winnipeg River downstream from a pulp and paper mill (Friesen et al. 1994). A negative correlation between hepatic retinol and MFO activity has been reported in herring gulls (*Larus argentatus*) (Spear et al. 1992), similar to the negative correlation observed in P_5CDF-treated lake trout for retinol and retinyl palmitate.

Although MFO activity in fish is widely used as an indicator of exposure, the evidence linking increased with changes in other biochemical and biological performance measures is still building. Increases in MFO activity in white sucker downstream from pulp mills are coincident with changes in plasma steroid levels and reduced development of secondary sexual characteristics in males, increased age to maturity in females, decreased fecundity, increased condition factor and slower growth (McMaster et al. 1991, Munkittrick et al. 1991). Although McMaster et al. (1991) suggest that changes in circulating steroid levels may have been associated with high levels of MFO activity, no direct link could be found. Similar changes in lake whitefish (*Coregonus clupeaformis*) exposed to pulp mill effluent have also been reported by Munkittrick et al. (1992). In the area of biological performance, McMaster et al. (1992) reported that despite the increases in MFO activity, fecundity and circulating steroid levels, there were no decreases in the viability of eggs or sperm or in growth and survival of fry. This is in contrast to the negative relationship between MFO activity in spawning females and the percent of viable eggs which has been reported for starry flounder (*Platichthys stellatus*) (Spies and Rice 1988) and the results for the current study.

As described in the introduction to the thesis, the path from a change at the molecular or biochemical level, such as enzyme induction, to changes at a population level such as reproductive success, growth or survival, is long and complex with many external and internal factors influencing the outcome. The actual mechanisms of change are not likely to be directly linked to changes in biomarkers such as enzyme induction. Rather, a cascade of events occurs which are loosely linked to changes at lower levels. The linking of biochemical measures to measures of biological performance either at the individual or population level will be tenuous at best.

The sustained induction of MFO in P_5CDF-treated lake trout and white sucker and the decreases in retinoids observed in the P_5CDF-treated fish were coincident with changes in other important ecological measures. In lake trout treated with P_5CDF,

decreased survival (Chapter 1) and a suspected P_5CDF-caused disruption in oogenesis in years 2 and 3 following treatment were observed (Chapter 2). In addition changes in levels of retinyl palmitate in both males and females was correlated with increased MFO activity. In white suckers, sustained induction of MFO activity was coincident with decreased growth and decreased fertilization of eggs from treated females. Combined effects in both species have the potential to cause changes in populations. These data then indicate that as well as being a biomarker for the presence of contaminant, measurements of MFO activity may also give forewarning of population level effects.

Chapter 4. Effect of Intraperitoneal Injections of Toxaphene on Hydroxyproline, Collagen and Calcium content of Bone in Natural Populations of Lake Trout and White Sucker.

Introduction

The effect of toxaphene on bone biochemistry were first brought to light in studies on the chronic effects of toxaphene on fathead minnows (Mayer et al. 1977) and brook trout (Mehrle and Mayer 1975). In these studies brook trout fry exposed to toxaphene as eyed eggs and fathead minnows exposed as fry had decreased backbone collagen and increased calcium and phosphorous. These changes in the biochemistry of bone caused altered development and quality of the backbone, resulting in a weakened and fragile backbone. This weakened bone could result in the "broken-back" syndrome. Changes in hydroxyproline, collagen and calcium were, in effect, biochemical indicators of the syndrome.

The major fibrous protein in vertebrates is collagen. The most important function of collagen in vertebrates is as the major component of the organic matrix in connective tissues and bone. Calcification and mineralization in and around the fibrils takes place during development, resulting in mature bone as more calcium and phosphate salts are deposited (Piez and Likins in Mayer et al. 1975).

Hydroxyproline is the predominant amino acid of collagen. It is derived from the hydroxylation of the amino acid proline following incorporation into the polypeptide. This hydroxylation is catalyzed by peptidyl proline hydroxylase (Mussini et al. in Mayer et al. 1975). An important co-factor in the hydroxylation of proline is ascorbic acid (Vitamin C). The mechanism of change in the levels of collagen and associated minerals in bone is centered on the availability of vitamin C to act as a co-factor in the hydroxylation of proline. However, vitamin C is also important in the detoxification of organic contaminants by microsomal hydroxylative enzymes (Mayer et al. 1978). Mayer

et al. (1978) concluded that increased use of vitamin C in the detoxification of toxaphene by the liver in channel catfish caused a functional deficiency of vitamin C in the vertebrae of fish, leading to a decrease in collagen formation. The decreased collagen and resulting increase in the mineralization of the bone results in brittle or poorly developed bone which is more susceptible to fracture.

The current study was designed to investigate the long term effects of high levels of toxaphene in lake trout and white suckers. Part of the study included the measurement of biochemical indicators of effects of toxaphene exposure. This chapter describes the effect of treating lake trout and white suckers from a naturally occurring population with I.P. injections of toxaphene on vertebral levels of collagen, hydroxyproline and calcium. If bone biochemistry is affected it would be expected that the proportion of collagen and the concentration of hydroxyproline would decrease and the levels of calcium would increase.

Methods

Capture and Treatment of Fish

The study site, capture and treatment methods are described in Chapter 1.

Sampling of Fish and Tissues

Sub-samples of fish chosen at random for each species from each treatment group (controls and toxaphene-treated) were sacrificed with an overdose of MS-222. Fish were then placed in labeled bags and frozen. Prior to homogenization for residue determinations, 10 to 15 vertebrae were removed from each fish. Vertebrae were removed starting 2 - 3 vertebrae from the skull. At the time of removal as much tissue as possible was cleaned from the bone. Samples were then placed in small bags and re-frozen until collagen, calcium and hydroxyproline were determined.

Preparation of Bone

Prior to collagen, calcium and hydroxyproline determinations, samples had to be thoroughly cleaned of all soft tissue. This was done using a number of methods. Large tissue pieces were removed by brushing with a hand held or electric toothbrush. All bone processes (ventral ribs, spinal chord and its surrounding arch) and the dorsal aorta were removed. Samples were then placed in small beakers containing water and then sonicated for 15-20 minutes to loosen remaining connective tissue. Vertebrae were then separated with a small scalpel and the cartilage was removed. Individual vertebrae were then brushed again and any remaining tissue was scrapped off with a scalpel.

Cleaned vertebrae were placed in small aluminum weighing dishes and dried at 100 °C for a minimum of 8 h (usually overnight), to a constant weight. These were then split into two fractions, one used for collagen and hydroxyproline determinations and the other to determine calcium.

Determination of Collagen

Collagen values were determined by the method described by Flanagan and Nichols (1962). In brief, sub-samples of 5 - 15 cleaned and dried vertebrae (c.0.5 g) were place in pre-weighed 16x125-mm screw-capped culture tubes and weighed to an accuracy of 0.0001 g. Larger vertebrae which did not fit through the opening of the culture tubes were broken up. To each tube 5 mL of 0.1N NaOH was added. Tubes were then capped with Teflon-lined lids and shaken in a temperature-controlled shaking water bath at 60 rpm for 5 h, then allowed to stand overnight. The NaOH was aspirated out of each tube. To extract the ash content, 5 mL of 10% EDTA (Sigma Chemical), adjusted to pH 7.5, was added to each tube. This was shaken continuously at 2 - 5 °C for 48 h. EDTA was aspirated off and replaced every 16 h. After demineralization was complete and the last EDTA wash was aspirated off, the remaining bone (collagen) was rinsed first with deionized water and then with acetone. A final extraction was done using 5 mL of 1:1

ethanol:diethylether for 24 hours. The tubes were shaken in a room temperature water bath for the duration. The ethanol:ether was then aspirated off in a fume hood and the tubes were placed in a heating block and heated to 100 °C for 2 h to evaporate off all ether. The tubes were then placed in an oven at 100 °C for at least 3 h to take the extracted collagen to dryness. Tubes were then re-weighed to determine the amount of collagen. The proportion of collagen in each bone sample was determined as the ratio of collagen weight/dried bone weight.

Determination of Hydroxyproline

Hydroxyproline values were determined by the method of Woessner (1961). In brief, following the determination of collagen weights, the collagen samples were hydrolyzed with 5 mL of 6N HCl for 16 h at 115 °C in an aluminum heating block in a fume hood. Samples were sealed with Teflon-lined caps. Following hydrolysis, samples were filtered, under vacuum, through 5.5 cm glass microfibre filters (Whatman GF/B). Two to three drops of 0.02% methyl red indicator were added to the filtrate. The samples were then neutralized with 2.5N NaOH. Final pH adjustment to pH 6-7 were made with dilute HCl and NaOH. The filtrate was then diluted in a 2L volumetric flask with distilled water. A 2 mL sample was taken for the final hydroxyproline determination.

To each sample was added 1 mL of chloramine-T, which was prepared fresh daily. Tubes were then vortexed and allowed to stand for 20 min. Chloramine-T reagent was prepared by making a 0.05M solution of chloramine T in 20 mL of distilled water and adding 30 mL of ethylene glycol monomethyl ether (Sigma Chemical) and 50 mL of citrate buffer and stored in a glass stoppered flask. The citrate buffer was composed of 50g citric acid monohydrate, 12 mL glacial acetic acid, 120 g sodium acetate trihydrate and 34 g sodium hydroxide in 1 L of distilled water. This was adjusted to a final pH of 6 and stored refrigerated under toluene.

Next, 1 mL of perchloric acid (a dilution made by taking 27 mL of 70% perchloric acid and making it up to 100 mL with distilled water) was added to each tube and tubes were vortexed and left to stand for 5 min. The final step was the addition of 1 mL of a 20% *p*-dimethylaminobenzaldehyde solution (prepared by adding ethylene glycol monomethyl ether to 20 g *p*-dimethylaminobenzaldehyde to a final volume of 100 mL). Tubes were vortexed again and placed in a 60 °C water bath for 20 min. Samples were then cooled under tap water for 5 min. Absorbance of the solution was then determined using a Beckman DU-7 spectrophotometer at 557 nm. Final concentration was determined from a calibration curve prepared with standards of hydroxyproline.

Preparation of the hydroxyproline stock solution for the determination of the standard curve was done by dissolving 25 mg of L-hydroxyproline (Sigma Chemicals) in 250 mL of 0.004N HCl. The stock solution was diluted in small test-tubes with distilled water to produce a range of concentrations from 1 - 20 µg/tube and read at 557 nm on the spectrophotometer to produce a standard curve. Final results for hydroxyproline were standardized and expressed as µg hydroxyproline/mg collagen.

Determination of Calcium

The remaining clean and dried bone fraction was weighed precisely to 4 decimal places (0.05 -0.10 g) and then hydrolyzed in 5 mL of 6N HCl for 16 h. Samples were then diluted 200:1 with Milli-Q water. Calcium content was then determined by the method of Gitelman (1967) using a kit supplied by Sigma Chemicals (Kit #587-M). Final determinations were made using a Beckman DU-7 at 570nm. Standards of 5, 10 and 15 mg/L calcium were also obtained from Sigma Chemical and used to produce a standard curve. Final results for calcium were standardized and expressed as mg calcium/mg bone.

Statistical Analysis

Mean yearly values calculated for % collagen, hydroxyproline and calcium levels for treated and control lake trout and white suckers were tested for differences using ANOVA. Where required, data were transformed according to Taylor's power law to obtain more uniform variance (Southwood 1978). All analysis was done using SYSTAT (Wilkinson et al. 1992).

Results

Collagen

The mean proportions of collagen in the vertebrae were not different (ANOVA, $p>0.05$) between treated and control fish for both lake trout and white suckers (Table 9) in any of the years sampled.

Hydroxyproline

Mean hydroxyproline levels in backbone from treated and control lake trout and white suckers were also not different (ANOVA, $p>0.05$) in any of the years sampled (Table 10).

Calcium

Mean calcium levels in back bone were also not different (ANOVA, $p>0.05$) in any of the years sampled between treated and control fish for both lake trout and white suckers (Table 11).

Discussion

Although in this study the treatment with toxaphene had no effect on bone biochemistry as indicated by the measurement of collagen, hydroxyproline and calcium,

Table 9. Mean percentages of collagen (± S.E.(n)) in vertebrae from toxaphene-treated and control lake trout and white sucker sampled in 1989, 1990 and 1991.

	Year		
Lake Trout	1989	1990	1991
Toxaphene-Treated	54.28 ± 1.01(4)	47.30 ± 4.38(4)	51.95 ± 0.79(4)
Control	54.54 ± 0.69(3)	52.26 ± 3.29(3)	50.73 ± 3.93(4)
White Sucker			
Toxaphene-Treated	52.20 ± 1.91(3)	53.46 ± 3.12(5)	48.53 ± 3.59(5)
Control	55.67 ± 1.07(4)	49.30 ± 3.43(3)	42.82 ± 2.83(6)

Table 10. Mean hydroxyproline (µg/mg collagen ± S.E.(n)) in vertebrae from toxaphene-treated and control lake trout and white sucker sampled in 1989, 1990 and 1991.

	Year		
Lake Trout	1989	1990	1991
Toxaphene-Treated	33.62 ± 1.33(4)	39.20 ± 2.62(4)	35.48 ± 0.72(4)
Control	33.67 ± 0.69(3)	35.16 ± 1.84(3)	36.91 ± 2.92(4)
White Sucker			
Toxaphene-Treated	46.18 ± 1.96(3)	41.54 ± 1.64(5)	41.81 ± 3.89(5)
Control	44.21 ± 0.57(4)	43.78 ± 2.01(3)	48.42 ± 4.17(6)

Table 11. Mean calcium (mg/mg bone ± S.E.(n)) in vertebrae from toxaphene-treated and control lake trout and white sucker sampled in 1989, 1990 and 1991.

	Year		
Lake Trout	1989	1990	1991
Toxaphene-Treated	186.71 ± 10.01(4)	159.26 ± 21.81(4)	197.44 ± 12.78(4)
Control	195.12 ± 9.63(3)	168.38 ± 12.07(3)	182.54 ± 8.54(4)
White Sucker			
Toxaphene-Treated	191.97 ± 21.26(3)	191.59 ± 6.38(5)	238.58 ± 28.43(5)
Control	211.72 ± 13.68(4)	192.88 ± 9.80(3)	189.94 ± 3.75(6)

this should not be taken as an indication that they cannot be good indicators of effects of toxaphene. There are several factors which likely influenced the results.

The greatest influence on these results was probably the age of the fish. Mean age of lake trout and white suckers at the time of treatment was 9 - 11 years (see Table 1 & 2, Chapter 1). Growth in weight of both species in L260 is greatly reduced (i.e. reaches an asymptote) past age 12 (K.H. Mills, unpublished data). Thus there would be little formation of new bone material. Mayer et al. (1977) reported no effect of toxaphene exposures on hydroxyproline, calcium and collagen in adult channel catfish exposed to 49 to 630 ng/L of toxaphene for 100 days. Larval fathead minnows, brook trout and channel catfish have been shown to be susceptible to changes in bone biochemistry when exposed to waterborne toxaphene (Mayer et al. 1975, 1977). The early stages are likely more susceptible to changes in collagen and hydroxyproline levels because of the high rate of growth and formation of bone. If any reserves of vitamin C were utilized for the detoxification of toxaphene in the liver, then they would not be available for hydroxylation of proline in collagen.

The method of exposure may also have affected results. Because the fish were exposed by I.P. injection the distribution of the contaminant may not take the same pathways as during exposure via food or water. Although the ultimate distribution of contaminant following I.P. injection is similar to distribution following ingestion of contaminated food (Chapter 7), nothing is known of the actual pathways taken. The full dose from an IP injection may not be processed by the liver. Thus there may not be a decrease in the availability of vitamin C for use as a co-factor in the hydroxylation of proline to hydroxyproline.

The use of bone biochemistry as an indicator of exposure to toxaphene should be used with caution. No effect was seen in exposure of fish as adults in this study. However, data from literature indicate that larval and juvenile fish are more sensitive and

that chronic exposure from hatch through adult stages will cause decreases in collagen and increases in calcium.

Chapter 5. Depuration of Toxaphene, Chlordane and 2,3,4,7,8-Pentachlorodibenzofuran in Lake Trout and White Suckers in a Natural Ecosystem Following a Single I.P. dose

Introduction

This chapter describes the chemical residues of toxaphene, chlordane and P_5CDF in treated fish. As well, the fate of two particular toxaphene congeners T2 and T12 and several chlordane congeners, *cis*- and *trans*-chlordane, *cis* and *trans*-nonachlor and the metabolites oxychlordane and heptachlor epoxide are examined.

This is amoung the first studies of its kind to make use of natural populations of fish living free in their habitat to describe residue kinetics of chlorinated organics. The only other study to use naturally occuring fish is an eight year study on eels (DeBoer et al. 1994). The fish in both that study and the current study were subject to natural fluctuations of many factors such as temperature, light, food availability, competition, which cannot all be duplicated in the laboratory. Kinetic studies are normally confined to the laboratory because of the ability to control conditions, but suffer the disadvantage of using limited size classes and have a lack of realism in feeding regimes etc.

Toxaphene

Toxaphene was the most heavily used organochlorine pesticide from the late 1960's through 1982 when it was banned (Saleh 1991); it was used mostly to control cotton insects, but also as a piscicide in the early 1960's to control rough fish (Prevost 1960, Stringer and McMynn 1958, 1960). This latter use was discontinued when it was discovered that toxaphene was persistent and could prevent successful restocking of lakes (Lee et al. 1977). Although North American use was concentrated mostly in the

southeastern United States and parts of California, toxaphene has become widespread throughout freshwater and marine environments.

Toxaphene is relatively soluble in water (0.5 - 3.0 mg/L (Paris et al. 1977, Brooks 1974), but solubility is low compared to many other organic compounds. The octanol: water partition coefficient for technical toxaphene has been reported to be in the range of $10^{3.5}$ to $10^{6.4}$, which is high; therefore, toxaphene would be expected to bioaccumulate in organisms (Paris et al. 1977). The average elemental composition of toxaphene is $C_{10}H_{10}Cl_8$. Individual compounds may contain from 6 to 10 chlorine atoms on the parent molecule (Sullivan and Armstrong 1985).

Toxaphene is found in high concentrations in fish in the Great Lakes (Schmitt et al. 1985, 1990, Devault et al. 1988, Swackhammer and Hites 1988) and in the Arctic (Muir et al. 1990a, b, Bidleman et al. 1989,1993) despite the fact that these areas had little or no use of toxaphene. This widespread contamination is the result of atmospheric transport (Bidleman et al. 1989, Eisenreich et al. 1981, Rappaport and Eisenreich 1986). Toxaphene (at least some components) is considered to be extremely persistent in aquatic environments (Johnson et al. 1966, Webb 1980, Eisler and Jacknow 1985), but to date no experimental information exists on the long term effects of high body burdens in fish.

Because of the complex nature of toxaphene, being a mixture of more than 650 different compounds and congeners (Vetter 1993), it is difficult to quantify and to resolve individual peaks, except for a few which have been characterized (Saleh 1991). Differential degradation of the various compounds and congeners within the toxaphene mixture during initial deposition and in subsequent dispersal by aerial transport, bioaccumulation and biomagnification results in residues with chromatographic patterns vastly different from those in original parent material, further confusing quantification (Stern et al. 1992, Bidleman et al. 1993).

Chlordane

Chlordane is a synthetic organic pesticide used primarily as a broad spectrum insecticide. Like toxaphene, it was a replacement for DDT. It was the first of the family of chlorinated pesticides known as cyclodienes, characterized by a cyclic structure and an endomethylene bridge, to be used in agriculture (Nomeir and Hajjar,1987). It was the second most important organochlorine insecticide in 1976-77, after toxaphene, with an estimated annual U.S. production of 20 millions pounds (Tashiro and Matsumura 1977). The use of chlordane is currently restricted in Canada for the control of subterranean insects, and it has been banned in the United States.

Technical chlordane is a mixture of approximately 50 components (Parlar et al. 1979), consisting primarily of cis- and trans-chlordane (43%), cis- and trans-nonachlor (10%) chlordene isomers (25%) and heptachlor (7%) (Ribick and Zajicek 1983). As with other pesticides which are mixtures of various congeners and isomers, the residues often consist of entirely different proportions of the original components. When measuring chlordane residues, two stable metabolites, oxychlordane and heptachlor epoxide are often also detected (Ribick and Zajicek 1983). In lake trout these metabolites have been found to be more toxic than the technical mixture (Gooch et al. 1990).

Chlordane has a water solubility of 9 μg/L (USEPA 1988). The octanol:water partition coefficient for technical chlordane has been reported to be approximately $10^{5.16}$, therefore chlordane would be expected to bioaccumulate in organisms (USEPA 1988). The average elemental composition of toxaphene is $C_{10}H_6Cl_8$ with individual congeners having from 7 to 9 chlorine atoms.

Chlordane residues have been detected in fish throughout the world, including most areas of the U.S. (Schmitt et al. 1985), the Arctic (Muir et al. 1988), the Baltic (Jansson and Wideqvist 1983), the Antarctic (Kawano et al. 1986) and the Great Lakes (Gooch et al. 1990) where levels in lake Michigan lake trout fillets ranged from 255 to 596 ng/g.

2,3,4,7,8-Pentachlorodibenzofuran

Polychlorinated-dibenzofurans (PCDFs) are trace contaminants which are produced from many different sources such as through the combustion of municipal wastes, leaded automobile fuels and low temperature combustion of PCBs (Buser et al. 1978, Clement et al. 1985, Marklund et al. 1987) and deposited through aerial deposition. They are also trace contaminants in the production of various organochlorine chemicals. In Canada a major source is trace contamination of chlorophenols, which are used as wood preservatives by the forestry industry (NRCC 1984). PCDFs are also reported in effluents from pulp and paper mills (Rappe et al. 1987, Servos et al. 1994). Levels of PCDFs in Great Lakes fish are reported to range from < 15 pg/g to > 300 pg/g wet weight (Baumann and Whittle 1988).

The 2,3,4,7,8-pentachlorodibenzofuran (P_5CDF) congener used in this study has been reported to be the predominant furan congener detected in fishes from the Great Lakes (Whittle et al. 1992) Solubility of this particular congener is reported to be 0.21 µg/L (Friesen et al. 1990). The octanol:water partition coefficient is $10^{6.92}$, the highest for any Cl_5 furan or dioxin congener (Sijm et al. 1989) indicating that it will bioaccumulate and biomagnify in the food chain. This particular congener has been shown to be a potent inducer of mixed function oxygenase enzymes (Muir et al. 1990c, Parrot et al. 1995), and is considered the most potent furan in early life stage toxicity to lake trout and rainbow trout (Walker and Peterson 1991)

Materials and Methods

Chemicals

Toxaphene and chlordane from Chem Service (Westchester, Pa.) were added to corn oil (Sigma Chemical, St. Louis, Mo.) on a weight:volume basis and mixed on a wrist

action shaker for 1h. Samples of each preparation were analyzed (see below) to determine a final concentration of 7 mg/mL corn oil. The dose was chosen to be representative of the concentrations in fish from moderately to highly contaminated natural areas.

Uniformly ring labeled [^{14}C]-2,3,4,7,8-Pentachlorodibenzofuran (P_5CDF, specific activity = 2.07 x 10^{12} Bq/mol) was obtained from Chemsyn Science Laboratories Ltd. (Lexana, Kansas). The stock solution was purified to >99% by reverse phase thin-layer chromatography (Whatman RP18 plates and a solvent system of acetone:water, 80:20). A corn oil solution was prepared by evaporating an acetone solution of P_5CDF to near dryness under N_2 then mixing with corn oil. Aliquots of the corn oil solution were assayed by liquid scintillation counting (LSC) to verify a concentration of 1.0 µg/mL corn oil.

Treatment of Fish

The study site, capture and treatment methods are described in Chapter 1.

Sampling of Fish

Sub-samples of fish chosen at random for each species from each group were sacrificed with an overdose of MS-222. Fish were then placed in labeled bags and frozen.

Analysis for Toxaphene and Chlordane

Toxaphene and chlordane determinations were done on whole fish homogenates. Whole fish were homogenized by passing them through a hand operated meat grinder twice. Sub-samples (10g wet weight) were mixed with sodium sulfate and then Soxhlet extracted with dichloromethane (DCM)-hexane (1:1). The extract was then evaporated to approximately 10 mL. One mL of this was used for a gravimetric determination of lipid.

Lipid was removed from the remaining 9 mL by gel permeation chromatography (GPC) on SX-3 Biobeads using an Autoprep model 1001A with DCM-hexane as the eluant. GPC eluants were then fractionated on Florisil (1.2% deactivated with water) into three fractions (Norstrom and Won 1985). Florisil eluants were analyzed by capillary gas chromatography (GC) with ^{63}Ni-electron capture detection using a 60m x 0.25 mm DB-5 column with H_2 gas carrier. GC conditions were identical to those used in previous studies (Muir et al. 1990a, Delorme et al. 1993).

Quantification of total toxaphene was accomplished by summing the areas of 19 major peaks previously identified by GC/MS and multiplying by a response factor based on the area of the same peaks in the technical toxaphene standard. The toxaphene analytical standard was obtained from the U.S. EPA standards repository (Cincinnati, OH).

Quantification of the individual peaks T2 and T12, corresponding to *2-exo, 3-endo, 5-exo, 6-endo,* 8,8,10,10-octachlorobornane and *2-exo, 3-endo, 5-exo, 6-endo*, 8,8,9,10,10-nonachlorobornane respectively (Stern et al. 1992), was done against authentic standards developed by Hainzl et al. (1993).

Quantification of total chlordane was accomplished by summing concentrations of 16 components, including two metabolites, oxychlordane and heptachlor epoxide.

Analysis for P_5CDF

Whole fish, minus the liver, were homogenized by passing them through a hand operated meat grinder twice. Sub-samples (10g wet weight) were taken and then freeze-dried. The samples were then homogenized in toluene using a Polytron Homogenizer (Kinematica). Samples were thoroughly mixed, allowed to sit 24h, and centrifuged to separate particulate matter from the toluene. A 1 mL aliquot of toluene was counted by

liquid scintillation counting (LSC), in 6 mL of Atomlight Fluor (DuPont) on a Beckman LS7500 scintillation counter using appropriate standards and quench curves.

The amount of parent P_5CDF in the toluene was determined by HPLC analysis. Briefly, 1 mL of toluene was evaporated under N_2, then 3 mL of hexane and 1 mL of concentrated H_2SO_4 were added to destroy lipids. This was shaken vigorously and allowed to stand for 1 h. The hexane layer was removed and the H_2SO_4 extracted again with 2 mL of hexane. The hexane washings were combined and 0.5 mL of H_2SO_4 were added; the same procedure was repeated. Hexane was then evaporated under N_2 to approximately 100 µL. A volume of 40 µL was mixed with scintillation fluor (Atomlight, DuPont) and counted and 40 µl was injected into an HPLC equipped with a NovaPak C18 3.9x300mm column (Waters Scientific, Missisauga, Ont). Samples and standards were eluted isocratically with 90:10 methanol:water as mobile phase at 1.5 mL/min. The fractions (0.75 mL) were collected and counted by LSC and compared with LSC counts from an HPLC run of purified compound.

Correction for Growth Dilution

All concentrations were corrected for growth dilution. Correction was made by multiplying the final measured concentration by the ratio of final weight/initial weight. In cases where it was not possible to identify a specific individual, the mean value of the growth for other fish from the treatment group caught in that time period was used. Tables of non-corrected means with corresponding corrected means for each contaminant are contained in Appendix 2.

Depuration Modeling

Depuration of the contaminants was modeled using two methods. The first method assumed depuration to follow first order kinetics and used the formula:

$$\ln y_t = A + \lambda \ln t$$

where y is the concentration at time, t, λ is the clearance coefficient and A is a constant. The clearance coefficient (λ) is the slope of the linear regression of ln(concentration) vs time since treatment. Half-lives ($t_{0.5}$) were then calculated by the equation:

$$t_{0.5} = \frac{0.693}{\lambda}$$

Niimi (1987). All calculations were done using yearly mean concentration values.

The second method used the computer program DIMSUM (Version 3.0). This program allows the testing of the assumption that the depuration was characterized as a single pool or two pools (biphasic). The program uses a weighted least-squares non-linear regression method to fit summed exponential functions. The program was developed by J.J. DiStefano III, A. Marino, P. Waecheter and E. Landaw in the UCLA Biocybernetics Laboratory (Marino et al. 1992). This model was originally developed to model the depuration kinetics of hormones where more than one pool may exist and can result in a biphasic depuration consisting of a fast pool and a slow pool (DiStefano and Landaw 1984, Landaw and DiStefano 1984).

In the case of the biphasic depuration the form of the model is:

$$y(t) = A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t}$$

It is not possible to calculate a single half-life for the depuration of a compound. Instead a mean residence time(MRT) is calculated from the formula:

$$MRT = -\sum_{i=1}^{n} (A_i/\lambda_i^2) / \sum (A_i/\lambda_i)$$

where n is the degree of the exponential (in this case n=2), A_i is the constant coefficient of the i^{th} exponential term and λ_i is the exponent of the ith exponential term (DiStefano and Landaw 1984). An estimate of the $t_{0.5}$ can be made from MRT using the equation(Oppenheimer and Gurpide 1979): $t_{0.5} = \frac{1.44}{MRT}$

Results and Discussion

Toxaphene

Toxaphene levels in lake trout from both doses and in white suckers declined in the first year after injection (Figures 29 and 30). Total toxaphene concentrations in lake trout declined to 223 and 280 ng/g in the 7 µg/g and 3.5 µg/g doses, after 4 and 3 years respectively. These levels are still well above the mean total toxaphene concentration of 24.6 ng/g in untreated lake trout. After the initial rapid elimination from white suckers there was little further elimination of toxaphene. Concentrations in white suckers 5 years after injection were still 2300 ng/g, similar to concentrations in 1989. This is well above the mean concentration of 5.9 ng/g found in controls. Although fish were given an initial theoretical dose of 7000 ng/g and 3500 ng/g in lake trout and 7000 ng/g in white suckers, there is no way of verifying that the injections did not subsequently leak out, except for suspiciously low levels in a few fish sampled (values equal to or less than controls were excluded from calculation of mean values).

Results of depuration modeling for total toxaphene in lake trout are presented in Table 12. Depuration of toxaphene in lake trout was determined to be first order for both doses. Estimated $t_{0.5}$ from the non-linear first order model were similar to the $t_{0.5}$ calculated from the linear fit first order model. As well the MRT and half-lives estimated for the two concentrations were in very good agreement with each other.

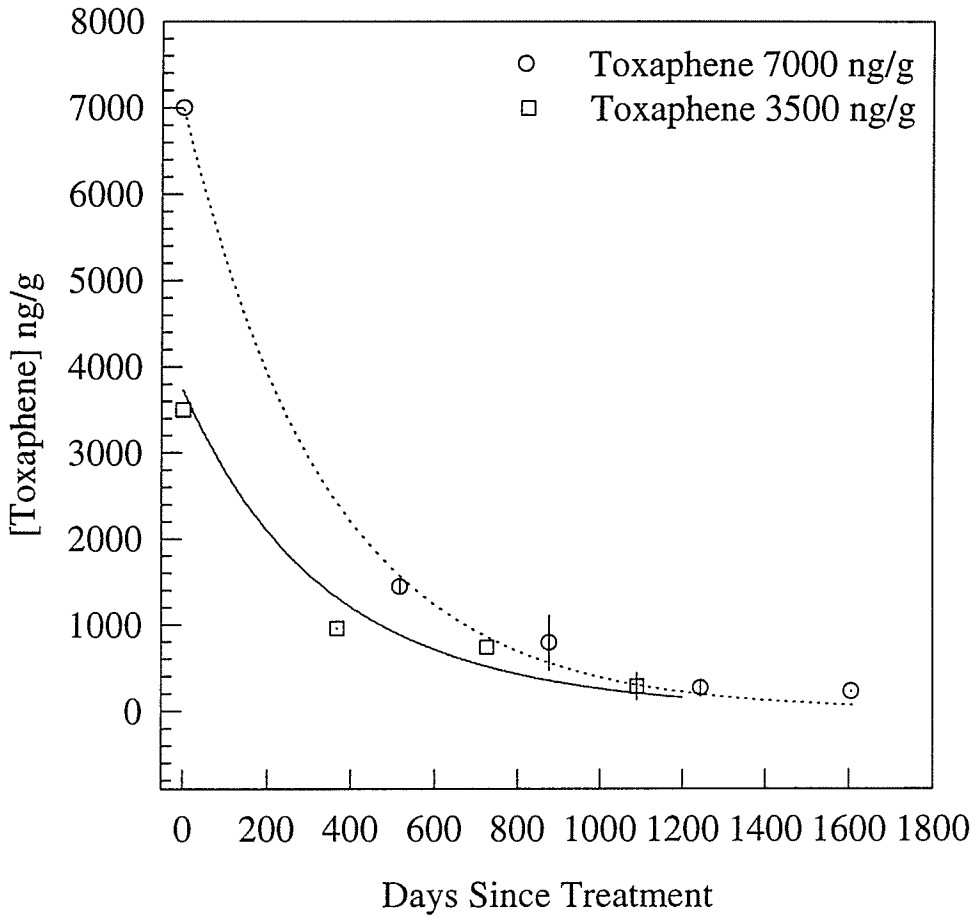


Figure 29. Mean total toxaphene concentration (ng/g± S.E) in lake trout treated with 7000 ng/g (○) or 3500 ng/g (□). Lines represent fitted depuration curves for the high (·····) and the low dose (———) from the equations generated by DIMSUM.

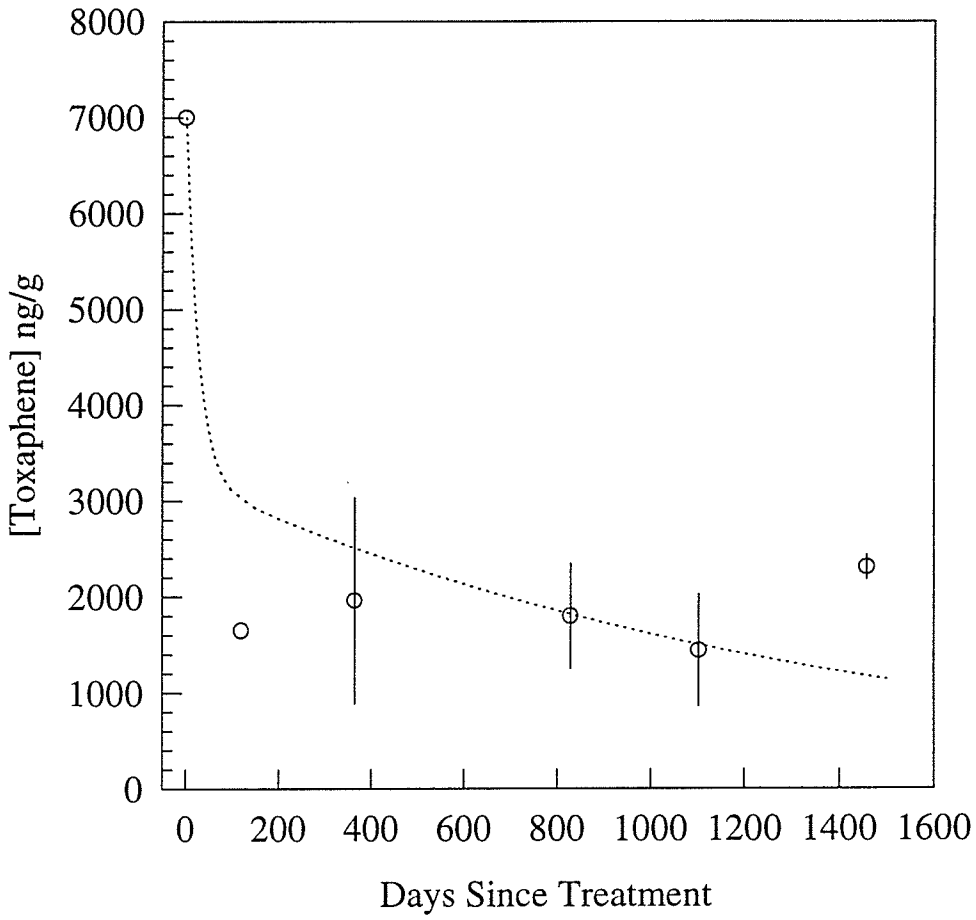


Figure 30. Mean total toxaphene concentration (ng/g±S.E) in white suckers treated with 7000 ng/g. Line (·····) represents fitted depuration from the equation generated by DIMSUM.

Table 12. Estimated Mean Residence Time (MRT) and Half-Lives ($t_{0.5}$) for total toxaphene, T2 and T12 in lake trout and white sucker and associated parameter values derived from the non-linear model DIMSUM and the linear regression model. Values in parentheses are standard deviation estimates for model parameters.

Species	Dose (ng/g)		Parameter Estimates from DIMSUM				First Phase		Overall		Parameter Estimates from Regression		
			A_1	λ_1	A_2	λ_2	MRT (d)	$t_{0.5}$ (d)	MRT (d)	$t_{0.5}$ (d)	A	λ	$t_{0.5}$ (d)
Lake Trout	7000	Total Toxaphene	6984.70	-0.002850					351	244	8.63	-0.002205	314
			(180.03)	(0.000185)									
		T2	135.85	-0.001510					662	460	5.15	-0.001913	362
			(1)	(0.000038)									
		T12	94.05	-0.003000	57.13	-0.000892	9	6	863	599	4.92	-0.001490	465
			(84)	(0.002019)	(84)	(0.000804)							
	3500	Total Toxaphene	3459.50	-0.002895					345	240	7.99	-0.002166	320
			(292.41)	(0.000557)									
		T2	88.00	-0.001678					596	414	4.61	-0.002204	315
			(1)	(0.000124)									
		T12	98.35	-0.001654					605	420	4.59	-0.001904	364
			(1)	(0.000091)									
White Sucker	7000	Total Toxaphene	3773.0	-0.036699	3227.3	-0.000696	27	19	1407	977	8.14	-0.000875	793
			(289)	(66)	(288)	(0.000093)							
		T2	133.97	-0.000743					1345	934	4.76	-0.000634	1093
			(1)	(0.000028)									
		T12	-	-					-	-	-	-	-

Two of the more recalcitrant toxaphene peaks found in aquatic biota, T2 and T12, recently identified by Stern et al. (1992) in whale blubber, were also quantified. These particular peaks have been reported as being dominant in burbot liver (Muir et al. 1990a), arctic char muscle (Muir et al. 1990b) and other arctic biota (Bidleman et al. 1993). Elimination kinetics of T2 in lake trout (Figure 31a) was similar to that of total toxaphene, whereas elimination of T12 in lake trout (Figure 31b) occurred at a slightly slower rate than for T2 (Table 12). The $t_{0.5}$ of T2 was not different from the $t_{0.5}$ calculated for total toxaphene for either dose, but the $t_{0.5}$ of T12 was approximately 40% greater in the 7.0 µg/g dose and 12% greater in the 3.5 µg/g dose in lake trout. Both T2 and T12 were still at much higher concentrations in injected fish than in control lake trout which had mean concentrations of 1.5 and 2.4 ng/g respectively indicating that these residues represent T2 and T12 retained from the treatment, not material accumulated from the environment.

Mayer et al. (1975) reported that toxaphene residues in yearling adult brook trout exposed to 502 ng/L for 161 days and then placed in clean water for 56 days were reduced by 51%, and that fish exposed to 288 ng/L had residues reduced by 32 %. Niimi (1987) has subsequently calculated $t_{0.5}$ from these data to be 63 days. These values indicate a much shorter half-life or MRT than reported here. However, there are several key differences which may account for this. In Mayer et al. (1975) the clearance study was conducted at 9°C, whereas in this study the fish were free-living. Lake trout typically inhabit the hypolimnetic region of lakes, making feeding forays into littoral zones, (Scott and Crossman 1973). In lake 260 the hypolimnetic temperature ranges from 4°C in the winter to a maximum of 8°C in late summer. Thus the temperature regimes were very different between the two studies. As well, Mayer et al. (1975) used yearling brook trout which have a much different growth and maturity pattern than do lake trout. Niimi (1987) in his review of half-life calculations stated that $t_{0.5}$ estimated from injections were longer

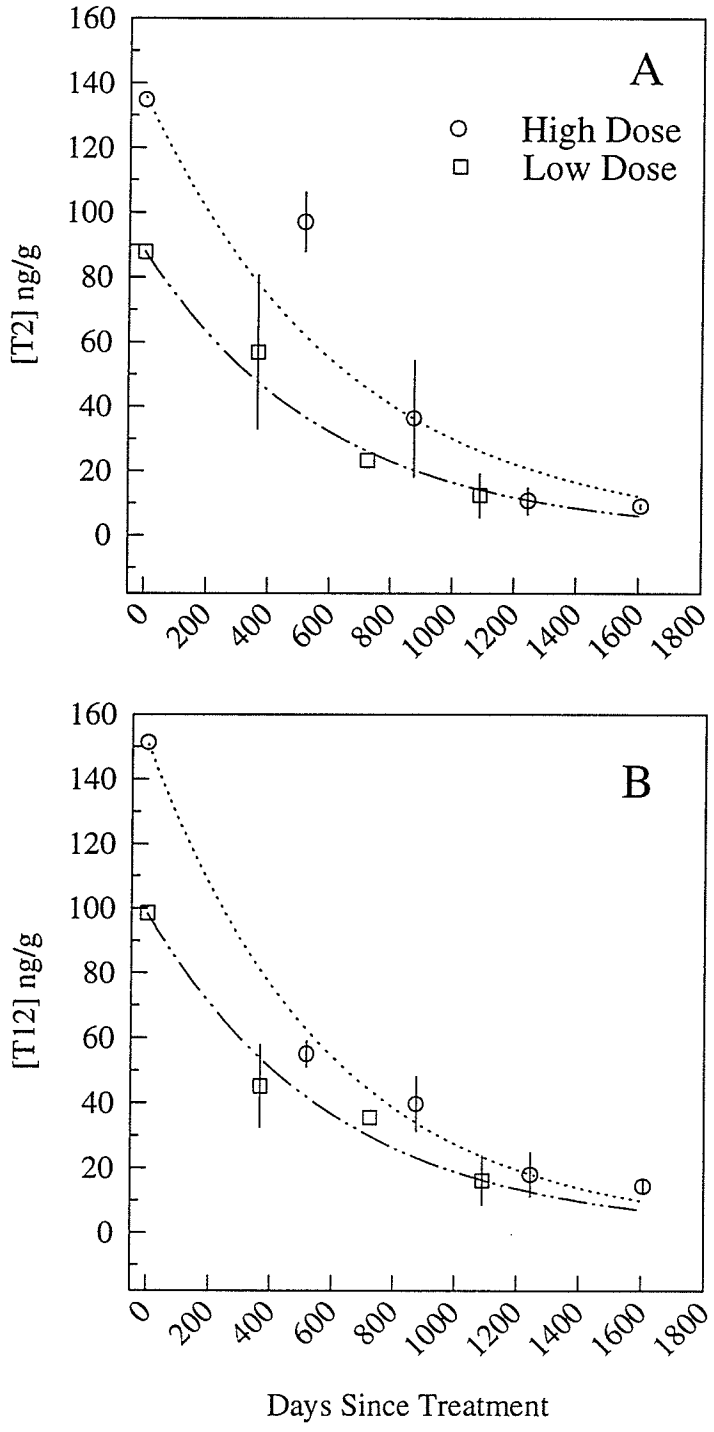


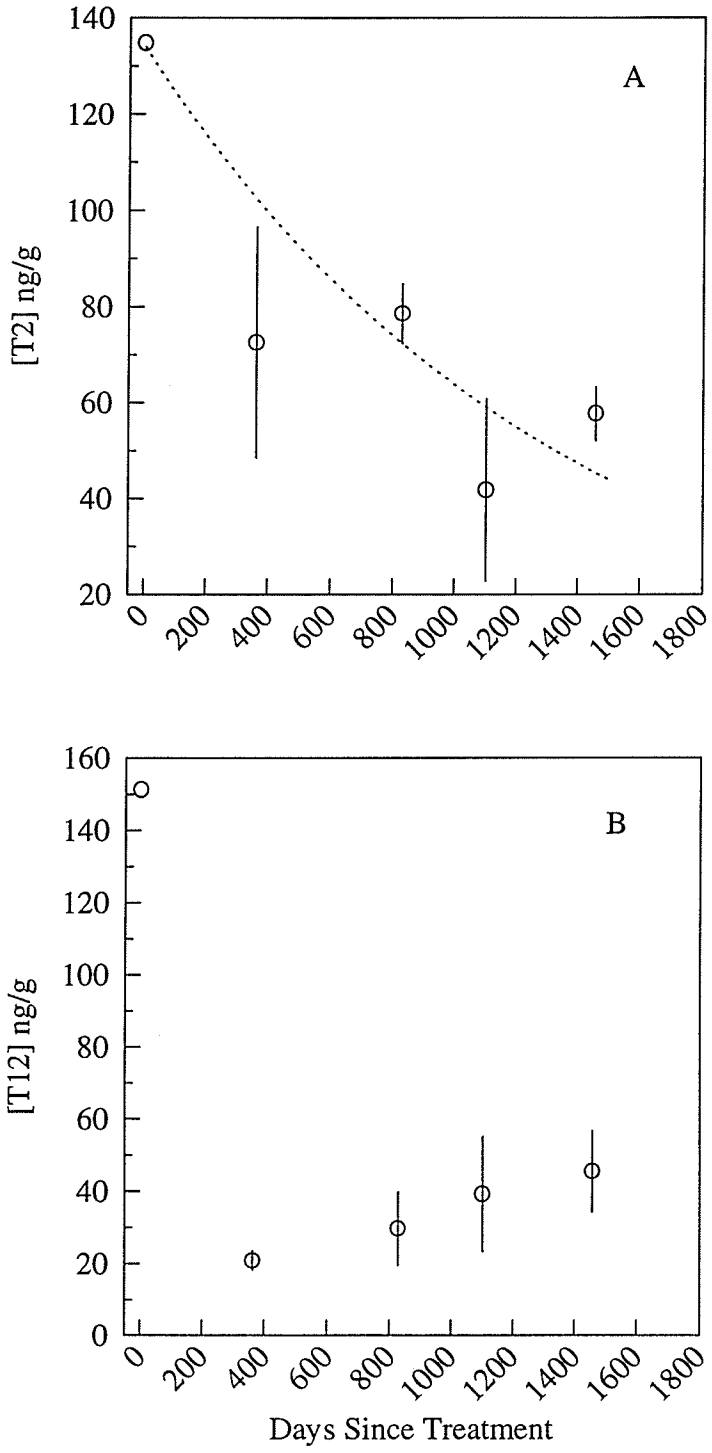
Figure 31 Mean concentrations (ng/g± S.E) of two chlorobornane components of toxaphene, T2 (A) and T12 (B) in lake trout following injection with technical toxaphene. Initial values represent initial concentrations in fish injected at the rates of 7000 ng/g (○) or 3500 ng/g (□). Lines represent fitted depuration curves for the high (·····) and the low dose (——) from the equations generated by DIMSUM

than those calculated from dietary exposures and possibly those from waterborne exposures.

Half-lives and MRT were calculated for white suckers (Table 12). Because of the apparent increase in concentrations after 1400 days (Figure 30), data from this year were excluded. Data for T12 in suckers was not modeled because of the non-linear nature of the natural log transformed data, but 50% disappearance occurred in approximately 6 months. Modeling using DIMSUM revealed that the depuration of total toxaphene in white suckers followed second order kinetics. The MRT of total toxaphene was higher in white suckers, 2710 days, than in lake trout, 351 and 345 days in the high and low treatments respectively. Depuration in white suckers was better approximated by second order kinetics, with a rapid initial phase followed by a slower second phase of depuration. In this case then the estimated $t_{0.5}$ from the linear first order model underestimated the MRT by a factor of 3.4 and the $t_{0.5}$ by a factor of 2.4.

Elimination of T2 and T12 in white suckers was difficult to evaluate. Although mean T2 concentrations decreased over time (Figure 32a), the mean concentrations of T12 actually appeared to increase (Figure 32b). Estimated half-life of T2 was similar to that of total toxaphene (Table 12). Mean residence time (MRT) for T2 was approximately half the calculated MRT for total toxaphene in white suckers. As mentioned previously the mean concentration of toxaphene did not decrease in suckers following the first year; in fact mean concentrations in fish from 1992 were slightly greater than concentrations in 1990 and 1991 for white suckers.

The proportion of total toxaphene which was accounted for by T2 and T12 was calculated for each year by dividing the concentration of T2 or T12 by the total toxaphene concentration. Mean proportions of T2 show an initial increase, followed by a slow decline in both lake trout (Figure 33a) and white suckers (Figure 33c). The mean



gure 32. Mean concentrations (ng/g± S.E) of two chlorobornane components of toxaphene, T2 (A) and T12 (B) in white suckers following injection with technical toxaphene. Lines represent fitted depuration curves from the equations generated by DIMSUM.

proportion of T2 in treated lake trout appears to be near the mean proportion in the untreated lake trout, which was roughly 5%. In white sucker controls it was determined that T2 accounted for 8% of total toxaphene, whereas in treated fish the mean proportion of T2 was 3% in 1992. T12 steadily increased in proportion in lake trout (Figure 33b), but appears to have remained at roughly the same proportion in white suckers (Figure 33d). T12 in control lake trout was slightly less than 10% of the total toxaphene, whereas in treated fish it had reached approximately 6.5%. In white suckers the mean proportion of T12 was 12% and 2% in controls and treated fish respectively. The differences in the proportions of these two toxaphene components between the control and treated fish is likely due to the different sources of toxaphene. Control fish reflect components available from atmospheric sources through water and the food chain. Bidleman et al. (1989) have shown considerable transformation in invertebrates relative to air, whereas the injected fish reflect what is available in technical toxaphene.

The initial increase of the proportion of T2 in lake trout and white suckers seen after the first year could indicate the rapid elimination of components which are more easily metabolized and eliminated within the first year after exposure or alternatively may indicate that some T2 is the result of biotransformation of other components (this metabolism would have to be dechlorination because T2 is an chlorinated aliphatic alkane). The subsequent decline reflects the elimination of this particular congener, relative to the remaining components which are equally or more persistent. In lake trout the steady increase in the proportion of T12 over time indicates that this component is eliminated more slowly than T2 or total toxaphene. Because the proportion of total toxaphene accounted for by T12 in white suckers was essentially constant over time, T12 may have a similar $t_{0.5}$ to that of total toxaphene in white suckers.

These data show that under natural living conditions lake trout and white sucker differ in their rates of elimination of toxaphene. The MRT and $t_{0.5}$ of total toxaphene

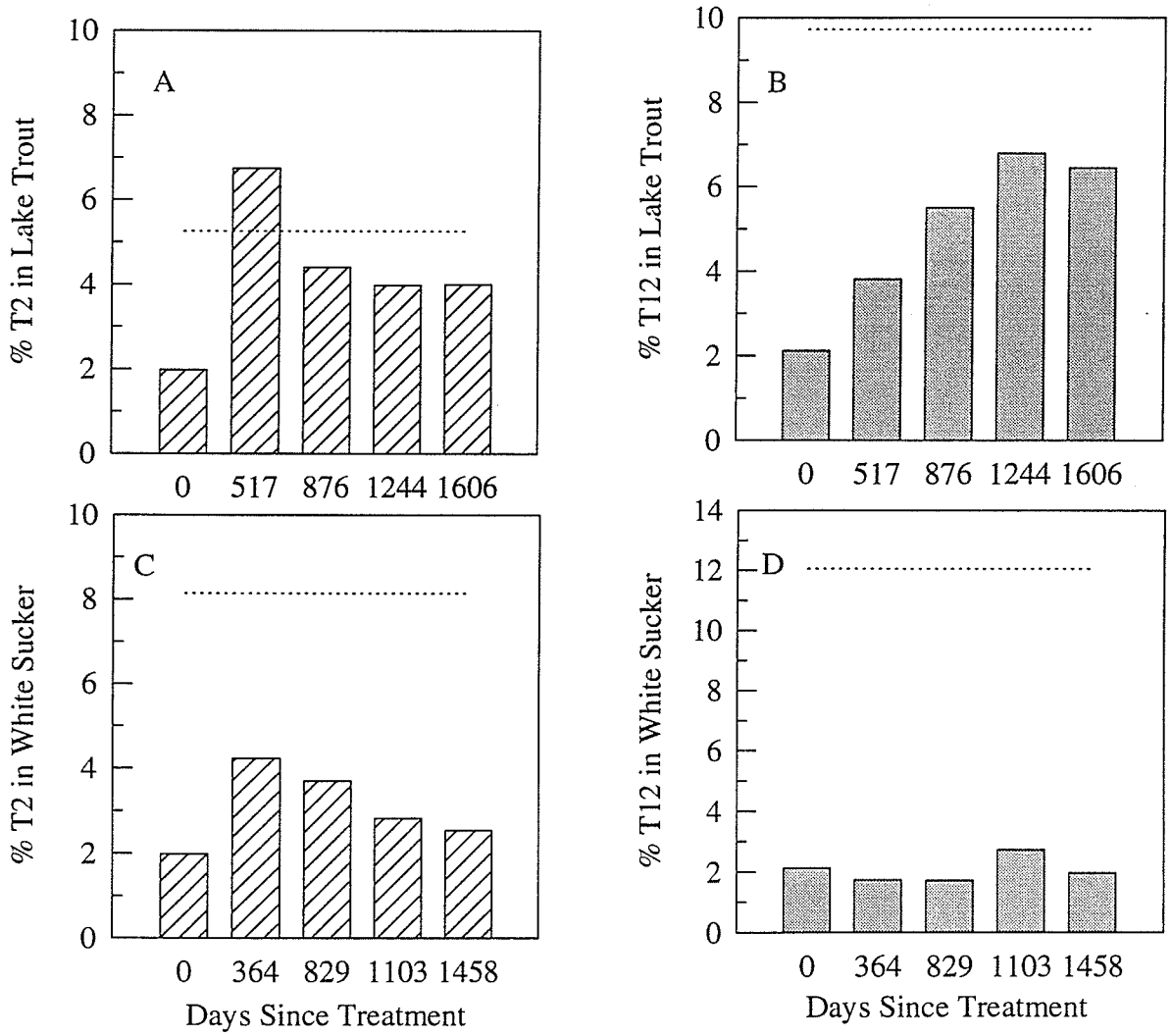


Figure 33 Proportions of total toxaphene concentrations accounted for by T2 (octachlorbornane) and T12 (nonachlorobornane) in lake trout (A & B respectively) and white sucker (C & D respectively) following I.P. injections with toxaphene. Dashed lines indicate proportions in untreated lake trout and white suckers.

measured in white suckers was approximately twice the MRT or $t_{0.5}$ in lake trout, and MRT was more than two times greater for the octachlorobornane, T2, in white suckers than in lake trout. The elimination of two different chlorobornane components (T2 and T12) of toxaphene were different within a given species. There was a 20% difference between the $t_{0.5}$ of the two chlorobornane components of toxaphene in lake trout.

Chlordane

The composition of the chlordane used was similar to the composition of technical chlordane reported by IARC (1979) and is described in Table 13. Total chlordane in untreated lake trout was relatively low with a mean concentration of 11 ± 2 ng/g as was total chlordane in untreated white suckers with a mean concentration of 8 ± 2 ng/g. The composition of chlordane in untreated lake trout and white sucker is also described in Table 13.

Depuration of total chlordane in lake trout (Figure 34) and white suckers (Figure 35) was found to follow biphasic kinetics when modeled using DIMSUM. Overall MRTs were similar between the two species (Table 14), as were half-life estimates from the first order linear regression (Table 14). Because the depuration was biphasic, the $t_{0.5}$ estimates from the first order linear regression were underestimated, by a factor of 2.2 for lake trout and 1.7 for white suckers.

Despite the heavy usage of chlordane and its wide distribution in aquatic ecosystems, few data on depuration of chlordane in fish could be found, likely because of analytical difficulties with a mixture of many different components. Half-lives have been reported for some of the major components such as *cis*-and *trans*-chlordane. Data on half-lives in other organisms include a serum half-life of 88d for clearance of ingested chlordane in humans, where depuration is thought to be relatively slow, taking days to weeks (USEPA 1983).

Table 13. Proportions (%) of major chlordane components characterized in injected chlordane, technical chlordane and untreated lake trout and white sucker.

Component	Injected Chlordane	IARC[a]	Control Lake Trout	Control White Sucker
cis-chlordane	19	19	12	18
trans-chlordane	21	24	22	25
trans-nonachlor	17	7	30	25
cis-nonachlor	4	nr	15	10
heptachlor	10	10	<1	1
heptachlor epoxide	<1	nr	3	4
oxychlordane	<1	nr	6	5
Other	28	39	12	11

[a] IARC (1979)

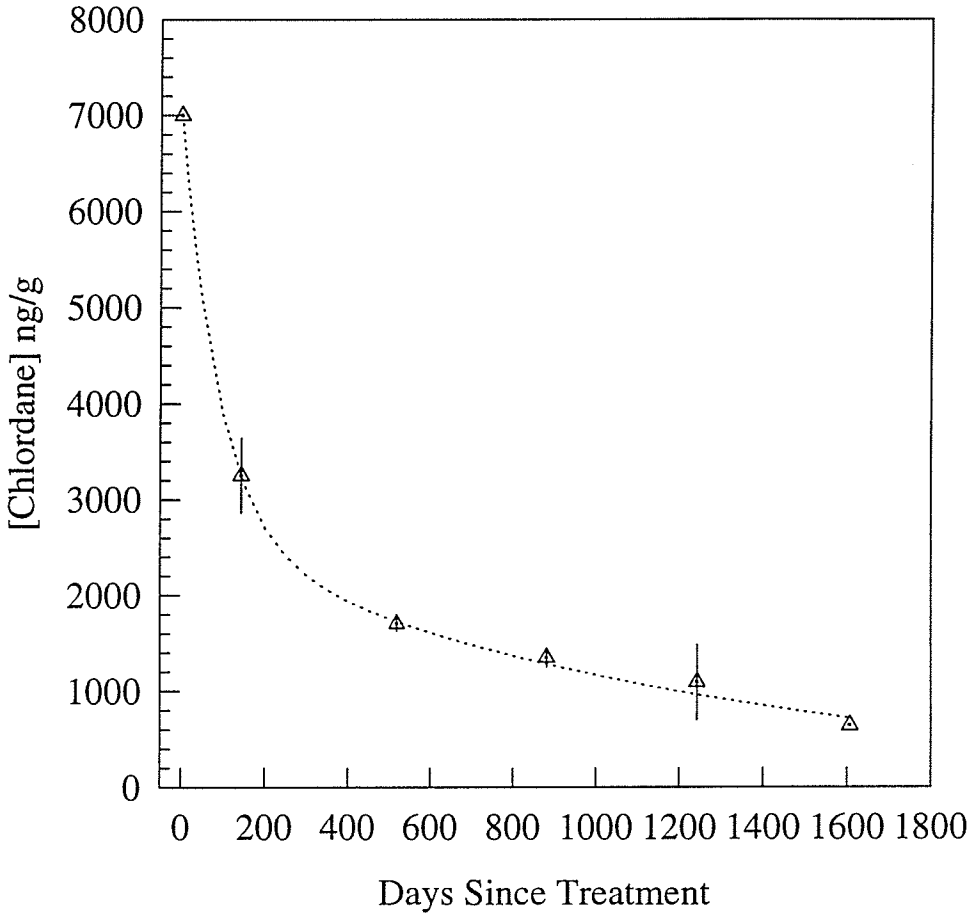


Figure 34. Mean total chlordane (ng/g±S.E) in lake trout treated with 7000 ng/g. Line represents fitted depuration from the equation generated by DIMSUM.

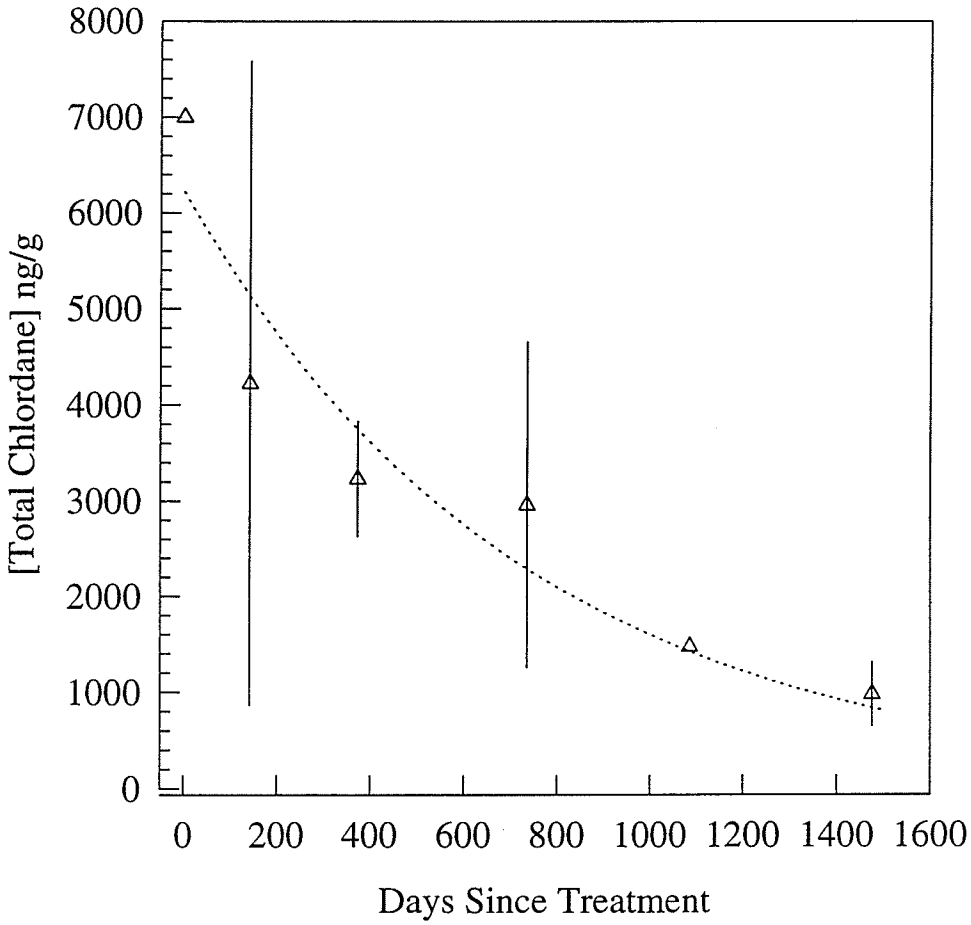


Figure 35. Mean total chlordane (ng/g±S.E) in white suckers treated with 7000 ng/g. Line represents fitted depuration from the equation generated by DIMSUM.

Table 14. Estimated Mean Residence Time (MRT) and Half-Lives ($t_{0.5}$) for total chlordane, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor in lake trout and white sucker and associated parameter values derived from the non-linear model DIMSUM and the linear regression model. Values in parentheses are standard deviations of model estimates.

Species	Dose (ng/g)		Parameter Estimates from DIMSUM				First Phase		Overall		Parameter Estimates from Regression		
			A_1	λ_1	A_2	λ_2	MRT (d)	$t_{0.5}$ (d)	MRT (d)	$t_{0.5}$ (d)	A	λ	$t_{0.5}$ (d)
Lake Trout	7000	Chlordane	4412.20	-0.010873	2587.20	-0.000773	53	37	1212	841	8.41	-0.001254	553
			(334)	(0.001612)	(316)	(0.000131)							
		cis-Chlordane	979.14	-0.009616	426.78	-0.000614	104	72	1433	995	6.77	-0.001203	576
			(39)	(0.000723)	(37)	(0.000087)							
		trans-Chlordane	1204.17	-0.010303	363.80	-0.001459	97	67	498	346	6.87	-0.002390	290
			(146)	(0.001811)	(144)	(0.000501)							
		cis-Nonachlor	275.23	-0.000365					2738	1901	5.59	-0.000322	2155
			(24)	(0.000119)									
		trans-Nonachlor	1126.82	-0.000770					1299	902	7.07	-0.000866	801
			(86)	(0.000144)									
White Sucker	7000	Chlordane	2408.0	-0.010671	4517.0	-0.000892	94	65	1077	748	8.68	-0.001219	569
			(1632)	(0.014191)	(1553)	(0.000415)							
		cis-Chlordane	1405.34	-0.001511					662	460	7.07	-0.001188	583
			(1)	(0.000019)									
		trans-Chlordane	1567.35	-0.001794					557	387	7.09	-0.001259	550
			(1)	(0.000021)									
		cis-Nonachlor	321.22	-0.000630					1587	1102	5.76	-0.000618	1121
			(1)	(0.000018)									
		trans-Nonachlor	1223.61	-0.001054					949	659	7.06	-0.000973	712
			(1)	(0.000013)									

Rats are able tc excrete chlordane at a much faster rate than humans, with 7d elimination rates of 66 - 90% having been reported (Tashiro and Matsumura 1977, Barnett and Dorough 1974). The MRTs reported for total chlordane in the current study of 3.3 and 2.6 years respectively for lake trout and white suckers are much longer than anything previously reported in the literature.

Formation of the metabolites oxychlordane and heptachlor epoxide occurred in both lake trout and white suckers. Oxychlordane accounted for 0.26% and heptachlor epoxide for 0.40% of the technical chlordane used for this experiment at the time of injection. In lake trout, oxychlordane increased steadily as a percentage of the total chlordane from the initial 0.26% to 2.0 % after 3.4 years (881 days) and remained at this level through the last sampling (Figure 36a). There was an initial increase after 4 months (143 days) in the percentage of heptachlor epoxide in lake trout from 0.40% to 1.0 % of total chlordane followed by a decrease to 0.15% in the last three years of the study (Figure 36b). Both oxychlordane and heptachlor epoxide accounted for a lower percentage of total chlordane in the treated lake trout than in the controls. However the actual concentrations were greater than controls in both cases. Mean concentration of oxychlordane in treated lake trout was 10 ng/g after 4.4 years, whereas control lake trout had a mean of 0.63 ng/g. Treated lake trout had concentrations of heptachlor epoxide of 1.0 ng/g after 4.4 years compared with mean concentrations in controls of 0.28 ng/g .

In white suckers, oxychlordane increased as a percentage of total chlordane to 2.7% after 142 days, and then stabilized at 1.7% for the remainder of the study (Figure 37a). Heptachlor epoxide remained at less than 1% of total chlordane for the duration of the experiment (Figure 37b). Although the proportion of oxychlordane was lower in treated white suckers than in controls, concentrations at the last sampling were still greater (1.51±0.65 ng/g) than in controls (0.38±0.07 ng/g).

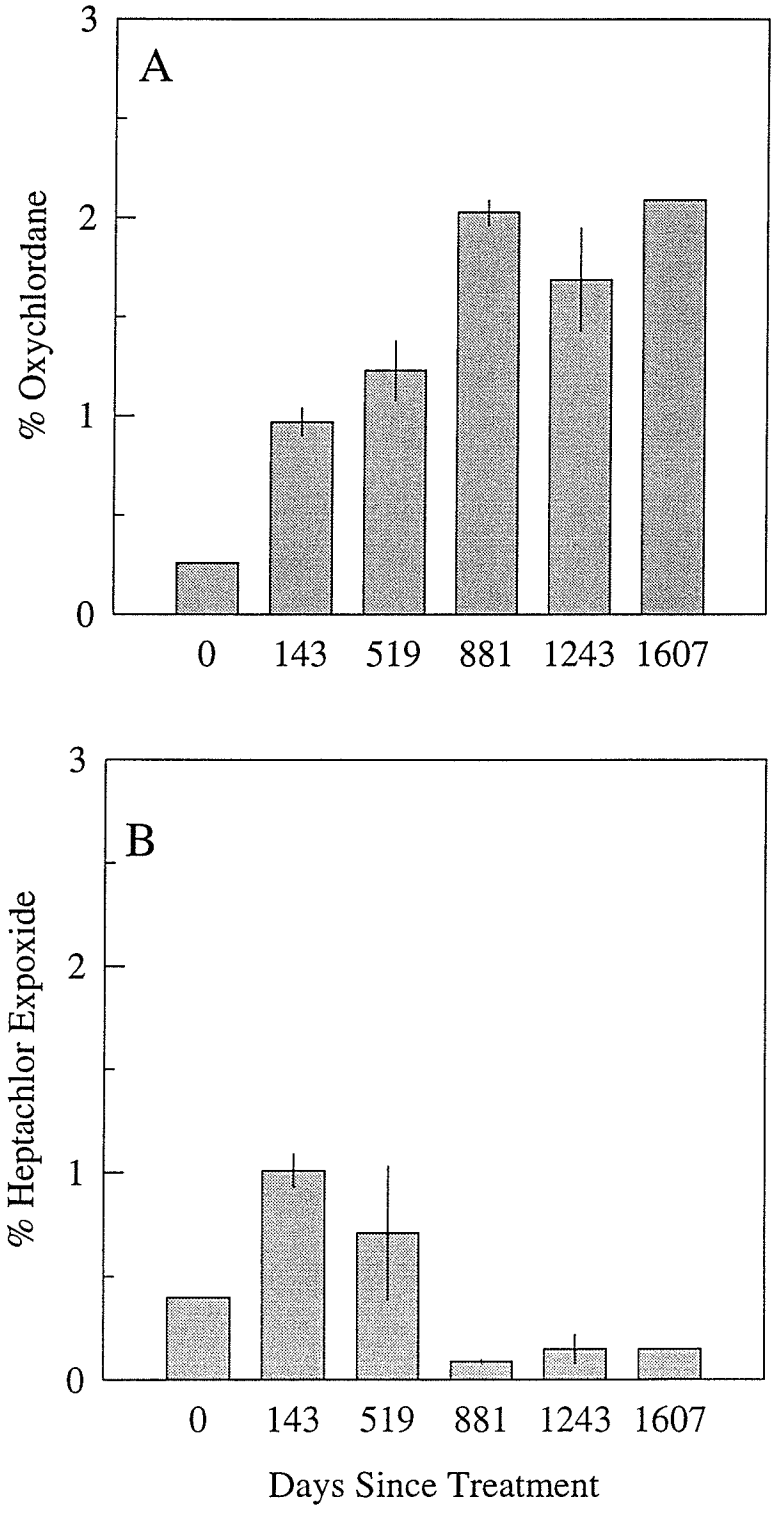


Figure 36. Mean proportions (±S.E) of total chlordane concentrations accounted for by the metabolites (A) oxychlordane and (B) heptachlor epoxide in lake trout following I.P. injections of technical chlordane.

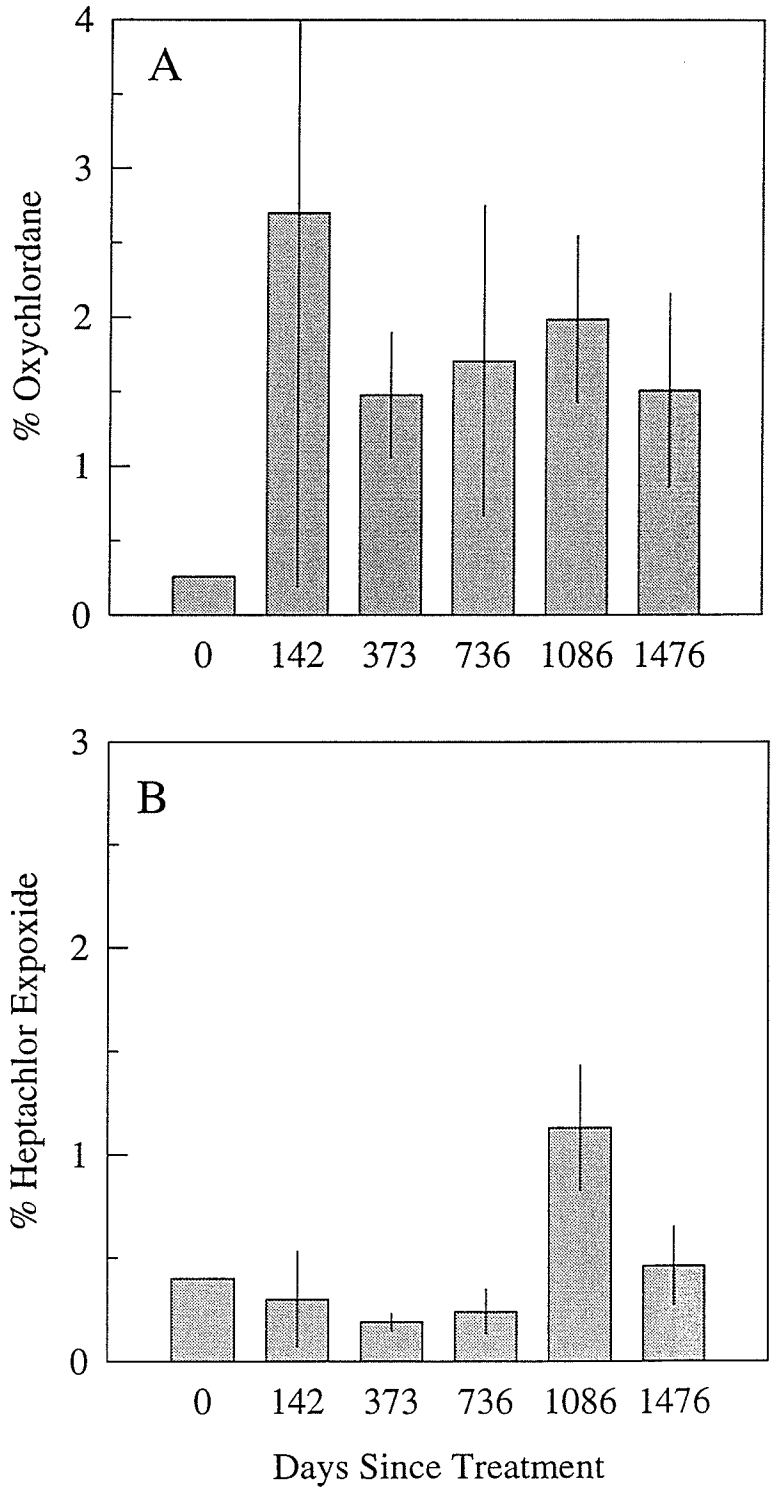


Figure 37. Mean proportions (±S.E) of total chlordane concentrations accounted for by the metabolites (A) oxychlordane and (B) heptachlor epoxide in white suckers following I.P. injections of technical chlordane.

The reason for the difference in the composition of total chlordane between control and treated fish is presumably the different sources. Control fish accumulate chlordane from atmospheric deposition, and then by bioaccumulation through the food web. The composition of chlordane is changed with each transfer between different matrices through differential degradation of the various components, resulting in a different composition from the parent material (Gooch et al. 1990, Hoff and Chan 1986).

The proportions of the major components of chlordane (*cis*- and *trans*-chlordane and *cis* and *trans* nonachlor) in lake trout and white suckers during the study are shown in Figures 38 and 39. The proportion of *trans*-chlordane showed a steady decrease in lake trout during the experiment (Figure 38a), however, in white suckers the proportion remained stable (Figure 39a). For both lake trout and white suckers *cis*-chlordane proportions remained stable over the 5 years of the experiment (Figure 38b & 39b). Higher proportions of the *cis* isomer have also been reported by Gooch et al. (1990) in lake trout from Lake Michigan and Siskiwit Lake, and in the monitoring of organochlorine contaminants in freshwater fish (Schmitt et al. 1990, 1985). The proportion of *trans*-nonachlor in lake trout and white suckers initially increased (Figure 38c & 39c) then stabilized, whereas the proportion of *cis*-nonachlor steadily increased in both lake trout and white suckers (Figures 38d & 39d), indicating a longer half-life than the other major components.

Tashiro and Matsumura (1977) have shown that in rats the conversion of *cis*-chlordane to oxychlordane is slower than the conversion of *trans*-chlordane to oxychlordane, resulting in a longer $t_{0.5}$ for *cis*-chlordane than for *trans*-chlordane. The same is true in both lake trout and white suckers. Estimated MRT and half lives for the major components of chlordane (Table 14) show the following order of depuration from fastest to slowest in lake trout: *trans*-chlordane > *trans*-nonachlor > *cis*-chlordane > *cis*-nonachlor. In white suckers the order was *trans*-chlordane > *cis*-chlordane > *trans*-nonachlor > *cis*-nonachlor.

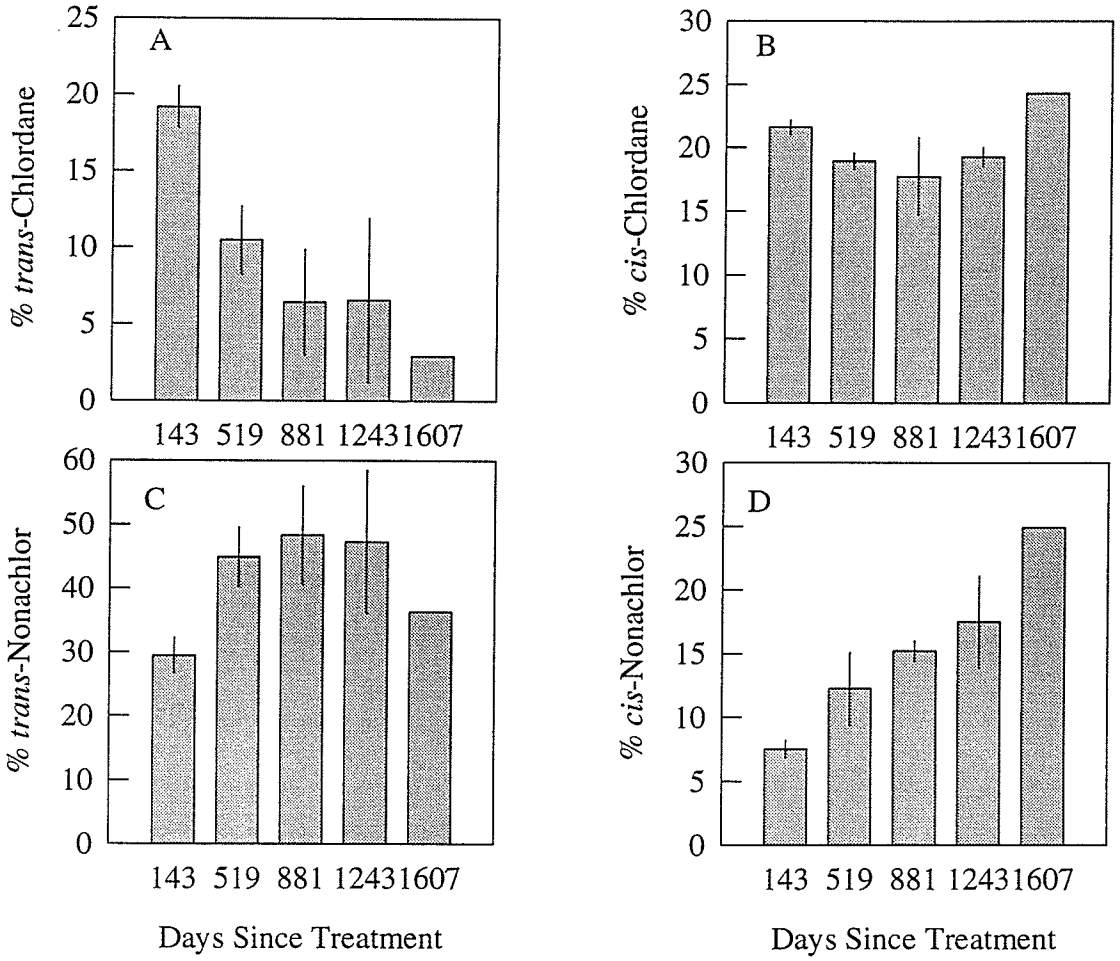


Figure 38. Mean proportions (±S.E) of total chlordane concentrations accounted for by (A) *trans*-chlordane, (B) *cis*-chlordane, (C) *trans*-nonachlor and (D) *cis*-nonachlor in lake trout following I.P. injections with technical chlordane.

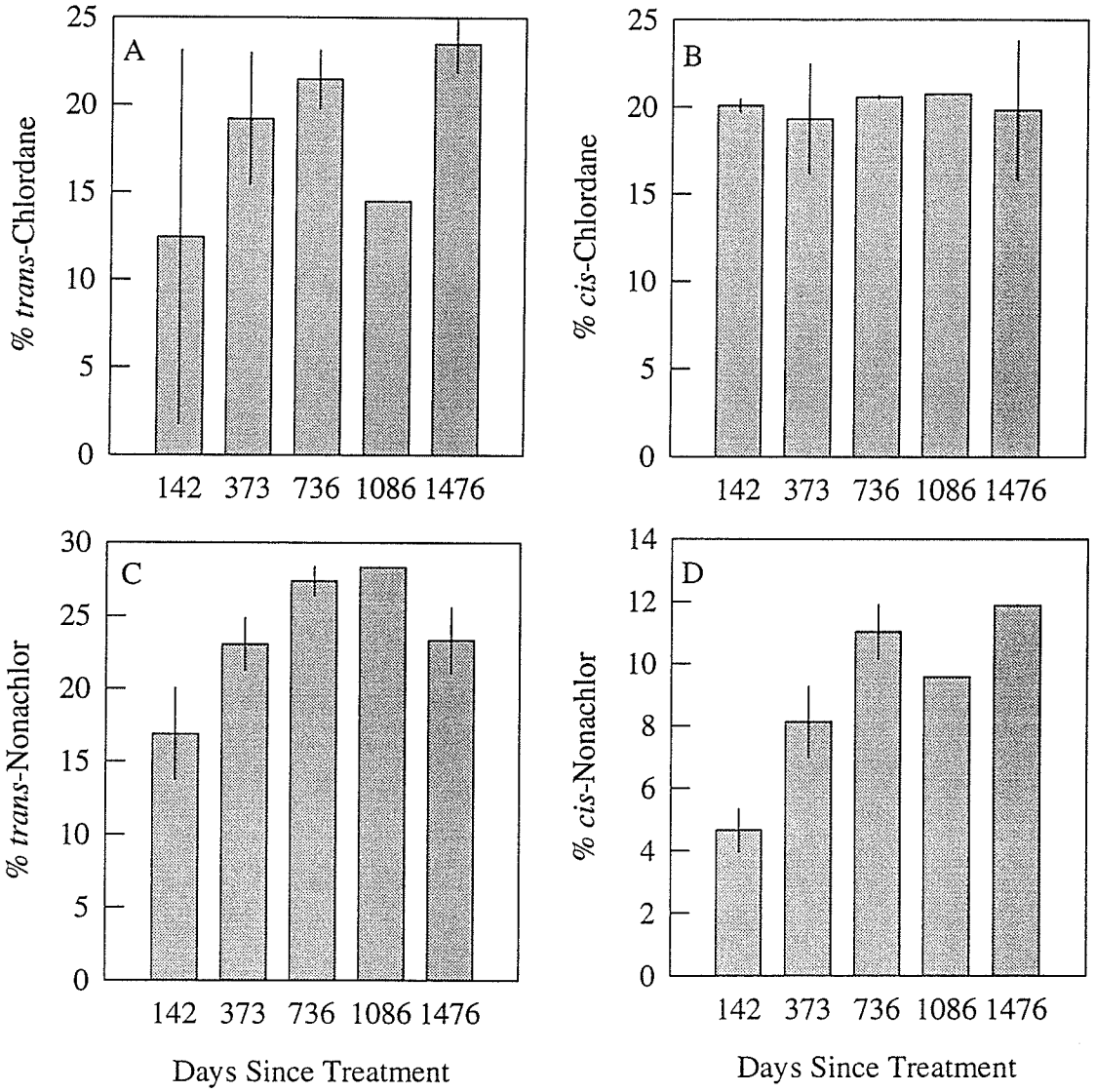


Figure 39. Mean proportions (±S.E) of total chlordane concentrations accounted for by (A) *trans*-chlordane, (B) *cis*-chlordane, (C) *trans*-nonachlor and (D) *cis*-nonachlor in white sucker following I.P. injections with technical chlordane.

This explains the decline observed in the proportion of the *trans*-chlordane, but not of the *cis*- isomer. Reported half-lives for the two isomers in northern redhorse suckers are 60 and 30 days respectively for *cis*-chlordane and *trans*-chlordane (Roberts et al. 1977), which are much less than the respective MRT-derived half-lives of 460 days (1.26 years) and 659 days (1.81 years) in white suckers and 995 days (2.73 years) and 346 days (0.95 years) in lake trout. Of the major components, the MRT of *cis*-chlordane was the closest to the MRT calculated for total chlordane, for both species.

P_5CDF

Depuration of P_5CDF in lake trout was determined to be biphasic when modelled with DIMSUM (Figure 40). The elimination of P_5CDF was almost non-existent, with an estimated MRT of 42670 days (116 years) following an initial depurative phase of about 106 days, indicating little or no depuration from the second pool (Table 15). The estimated $t_{0.5}$ from linear first order kinetics determination was 3527 days (9.6 years) (Table 15) This is a much shorter time than the estimate from the non-linear model, but is still a considerable length of time. No evidence of metabolic breakdown products was evident in HPLC analyses of toluene extracts.

Depuration of P_5CDF in white suckers was determined to follow first order kinetics when modeled with DIMSUM (Figure 41). Elimination of P_5CDF from white suckers was faster than from lake trout with an estimated MRT of 947 days (2.59 years) and a $t_{0.5}$ of 656 days (1.8 years) (Table 15). Estimated half-life from first order kinetics by the linear method was 1469 days (4.02 years) (Table 15). Unfortunately the data used to calculate these values are more variable than those used from the lake trout. As well, no data were available for white suckers from the third year following treatment because the samples were lost.

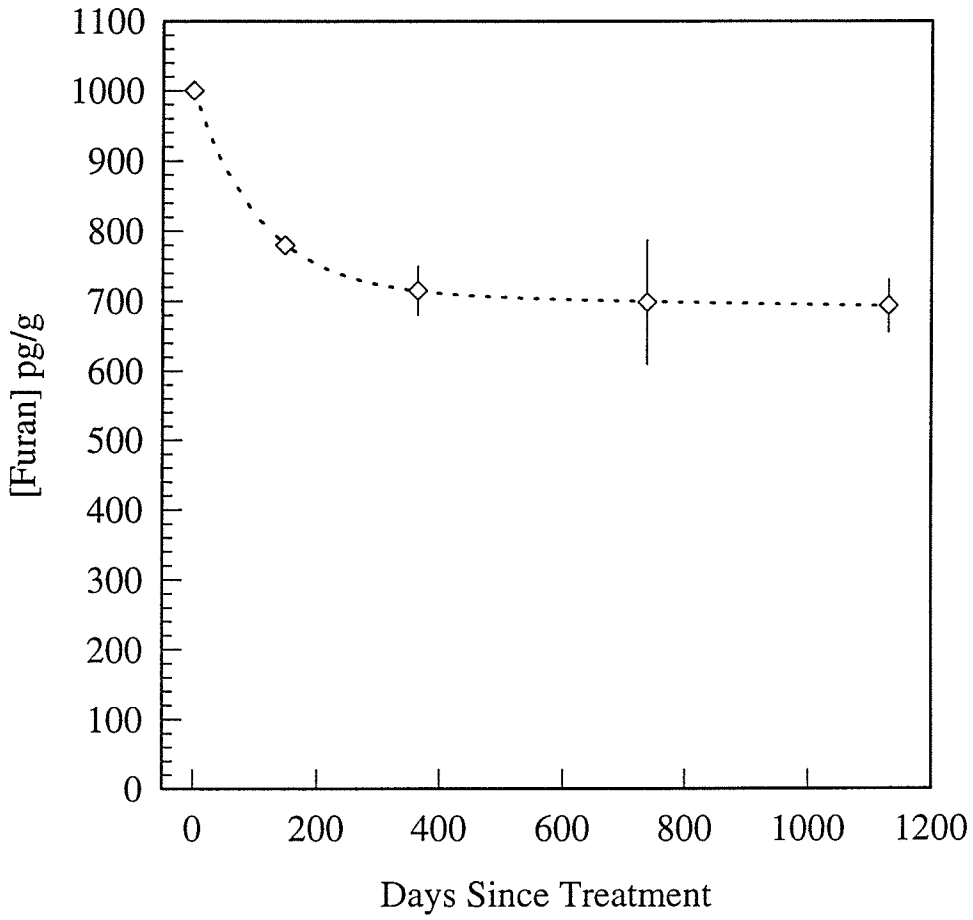


Figure 40. Mean P_5CDF concnetrations (pg/g±S.E) in lake trout treated with 1000 pg/g. Line (·····) represents fitted depuration from the equation generated by DIMSUM.

Table 15. Estimated Mean Residence Time (MRT) and Half-Lives ($t_{0.5}$) for 2,3,4,7,8-pentachlorodibenzofuran (P_5CDF) in lake trout and white sucker and associated parameter values derived from the non-linear model DIMSUM and the linear regression model. Values in parentheses are standard deviations of model estimates.

Species	Dose (pg/g)		Parameter Estimates from DIMSUM				First Phase		Overall		Parameter Estimates from Regression		
			A_1	λ_1	A_2	λ_2	MRT (d)	$t_{0.5}$ (d)	MRT (d)	$t_{0.5}$ (d)	A	λ	$t_{0.5}$ (d)
Lake Trout	1000	P_5CDF	288.62 (16)	-0.009451 (0.001411)	711.38 (16)	-0.000023 (0.000024)	106	73	42670	29632	-0.19	-0.000196	3527
White Sucker	1000	P_5CDF	999.55 (1)	-0.001056 (0.000022)					947	657	-0.52	-0.000472	1469

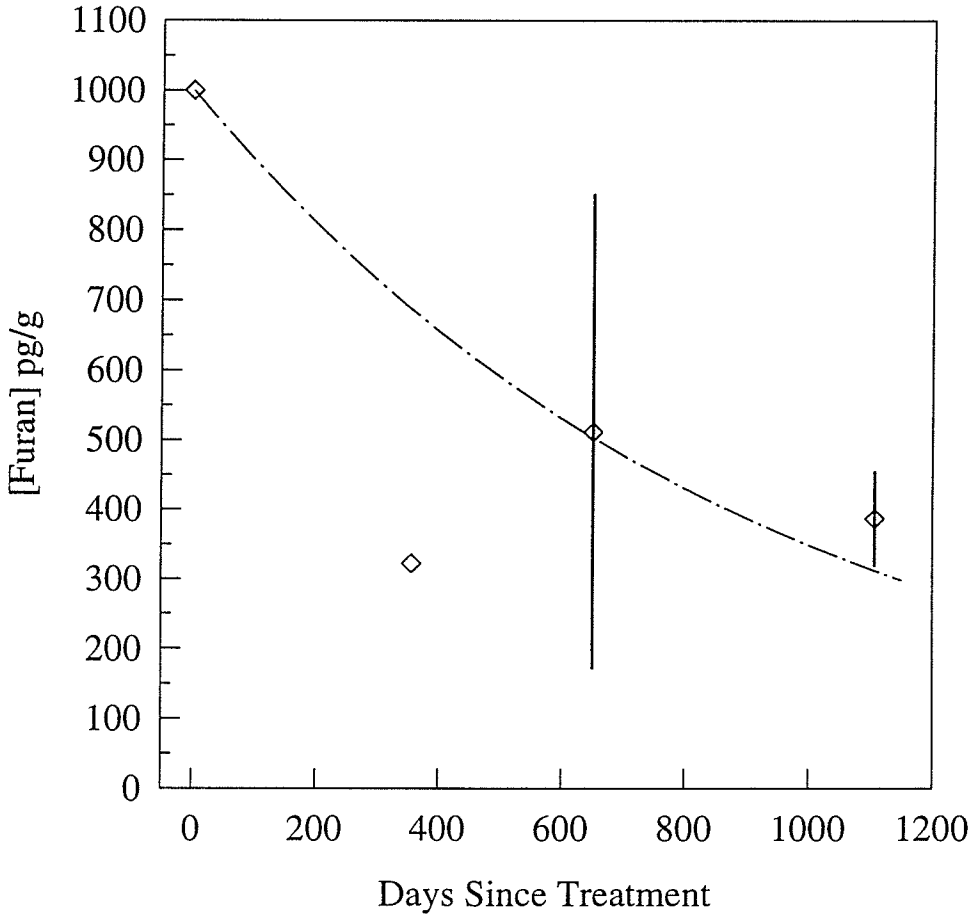


Figure 41. Mean P_5CDF concentrations (pg/g±S.E) in white sucker treated with 1000 pg/g (◇). Line (·····) represents fitted depuration from the equation generated by DIMSUM.

The longest previously reported half-life for a dioxin or furan congener is 320 days for 2,3,7,8-TCDD in carp (Kuehl et al. 1987) and 345 days in small (40g) lake trout (Cook et al. 1991). Muir et al. (1990c) reported a $t_{0.5}$ for P_5CDF of 61 - 69 days in juvenile rainbow trout. The average life span of a lake trout in L260 is 25-30 years. The MRT of P_5CDF reported from this study exceeds the average life time of a lake trout by four times, indicating that during its lifetime, no appreciable depuration would occur. The concentration can only be lowered by the process of growth dilution. Even the lowest estimate of 9.6 years is greater than a third of the life span of an average trout. Sijm et al. (1992) and Deboer et al. (1994) reported little or no elimination of several polychlorinated biphenyl congeners in guppies and eels respectively, and concluded that growth dilution was the only important process in decreasing concentrations of PCBs 153, 194 and 209. Depuration of the non-ortho substituted PCB congener 126 has also been shown to have a conservatively estimated $t_{0.5}$ of 2.27 years following exposure of lake trout to a single gavage dose (Brown et al. 1992). Interestingly, exposure via an intraperitoneal injection of similar aged lake trout to the same PCB 126 resulted in a $t_{0.5}$ greater than 6 years (S.B. Brown, personal communication).

General Discussion

The estimated half-lives from this study were substantially longer than previous estimates reported in the literature. There are several factors which may have contributed to the longer estimates. The use of an intraperitoneal injection to introduce the contaminants into the fish likely increased the estimated half-lives. Niimi (1987) reported that studies which used this method of exposure had longer $t_{0.5}$ than comparable estimates based on dietary uptake. Another important factor is temperature. The majority of studies are conducted at a constant temperature, which is well above the annual

average temperature which the natural populations of fish in this study were exposed to. Half-lives of all three contaminants were greater in lake trout than in white suckers. This may be in part due to the differences in the thermal habitat they occupy. Lake trout tend to remain in the hypolimnetic regions of the lake, especially in the summer months when thermal stratification is present, with the occasional feeding foray into epilimnetic areas (Scott and Crossman 1973). White suckers typically inhabit a variety of habitat, but are most often found in the warmer epilimnion (Scott and Crossman 1973). Niimi and Palazzo (1985) reported than depuration of mirex in rainbow trout declined by a factor of 5 when the temperature regime was changed from 18°C to 4°C

Another factor which may influence elimination is the general condition of the fish. In laboratory experiments fish are usually fed an optimum diet, containing all essential nutrients in the proper proportions, at an optimum ration. Because the lake trout and white suckers used in this study were part of natural populations, there is no way of knowing that a given fish has obtained all essential nutrients. It is clear from earlier discussions of growth (See Chapter 1) that some treated groups were not growing. However, it is unclear if this represents a failure to obtain nutrients or a failure to convert food into tissue mass. Fish in natural environments are also subject to seasonal and yearly fluctuations in food availability, and may undergo periods when little or no feeding takes place. It is unclear at this time what effects changes in nutritional status will have on the elimination of contaminants. Some research has been done on the effects of feeding on uptake (Stow and Carpenter 1994, Hilton et al. 1983), but little has been done on the effects of altered diets on elimination.

The effect of body weight on contaminant elimination has received little attention. Some studies indicate that clearance rates decrease in larger fish (Niimi 1987), but the evidence is not clear. Many of the depuration studies done in laboratories use juvenile fish such as rainbow and brook trout or alternatively use smaller species such as fathead minnows and guppies in order to overcome space and facility limitations associated with

holding large numbers of adult fish. Since juvenile fish are growing rapidly and have different metabolic requirements and conditions, the comparison of half-lives and elimination rates between juveniles and adults of the same species may not be valid.

The length of the period allowed for depuration in this study was unusual because it was years instead of months or days. This longer depuration period may have contributed to the longer estimates of MRT and/or $t_{0.5}$. Allowing a longer period of time to assess contaminant levels reduces the risk of wrongly assuming that single pool first order kinetic models are suitable when they are not. In all cases, contaminant levels appeared to level off more than one year after treatment. Much shorter half-lives would have resulted from this work if contaminant levels had only been studied over the first year after exposure.

The use of the non-linear model, DIMSUM, to characterize depuration in lake trout and white suckers, generally gave longer estimates of $t_{0.5}$ (or MRT) than did the log-linear model usually employed. The use of DIMSUM also showed that the depuration of the P_5CDF and chlordane in lake trout and toxaphene in white suckers followed biphasic depuration. It is possible that all of the contaminants followed biphasic depuation in both species studied. A major determining factor in whether or not the depuration of a contaminant follows single or two pool depuration is the number of data points available and the time over which they were taken. In the cases where kinetics were determined to be biphasic, contaminant concentrations were measured within the first year. Had sampling been more frequent, second order kinetics would likely have applied to all the contaminants in both species. In the cases where the depuration was found to be biphasic, the initial phase of depuration was short, with MRTs ranging from 9 to 106 days (with the average first phase having an MRT of 63 days Table 1,2,3). The initial decline likely represents the redistribution of the contaminants following I.P. injection. In experiments with white suckers (see Chapter 7) it was found that distribution of P_5CDF took at least 84 days following an I.P. injection for distribution to reach stability and for EROD

induction to reach a maximum. Work by Brown et al. (1992) showed similar results with I.P. injections of PCB 126 into juvenile lake trout, which required 15 to 20 weeks (105 - 140 days) for concentrations to reach maxima and be relatively constant in liver and muscle. The time course for stable distribution from the experiment by Brown et al. (1992) and the time course of distribution in white suckers presented in Chapter 7 of this work is in the some order as the estimated MRTs for contaminants following a biphasic depuration, indicating that this first phase of depuration is probably associated with redistribution of the contaminant from the IP space to muscle tissue.

The redistribution of persistent lipophilic chemicals in fish is sometimes attributed to lipid mobilization (Niimi 1987). Good correlations have been shown between lipid levels and contaminant concentrations in various tissues (Niimi 1983, Roberts et al. 1977, Mitchell et al. 1977).

Following distribution to different tissue/lipid pools, it appears that the contaminants used in this study are not readily re-mobilized, as indicated by the lengthy MRT and/or half-lives. Evidence is mounting that for certain higher chlorinated PCB congeners that there is no depuration. In a recent study DeBoer et al. (1994) transplanted eels from a contaminated site to a clean lake and monitored contaminant levels for 8 years in the transplants. They report that elimination half-lives were in the order of years for lower chlorinated PCBs and there was no elimination during the 8 years of higher chlorinated PCBs. It may be that, once sequestered within lipid, contaminants are not re-mobilized unless severe nutritional stress occurs. The dynamics of lipid movement between various tissue pools within fish is poorly understood. Further study in this area is required to understand fully how lipid dynamics affects the elimination of lipophilic contaminants.

Conclusions

The most unique aspect of these data was that they were obtained from natural populations of fish, subject to natural fluctuations in temperature, light, food availability etc., and undergoing the natural rhythms of growth and reproduction. Elimination of contaminants was slower than is seen with similar or same contaminants in laboratory studies. Lake trout generally had slower elimination than did white suckers. The P_5CDF had the longest MRT in lake trout, followed by chlordane and then toxaphene. Toxaphene was the slowest to be eliminated in white suckers, followed by P_5CDF and chlordane.

Chapter 6. Tissue Distribution and Mixed Function Oxidase Activity Following a Single Intraperitoneal Injection of 2,3,4,7,8-Pentachlorodibenzofuran in laboratory held White Sucker.

Introduction

The method of introduction of a contaminant into an organism can affect its eventual distribution in tissues. Intraperitoneal injections have been commonly used to treat fish with contaminants when studying induction of MFO enzymes (Janz and Metcalfe 1991, Melancon et al. 1989, Melancon and Lech 1983, Hahn and Stegeman 1994). This method of dosing fish is rapid and convenient, allowing the administration of a constant dose to each individual in a treatment group with a minimum of risk of contamination. However, in the environment the predominant route of uptake of hydrophobic contaminants is dietary (Muir et al. 1992). Thus it would be useful to know the time course of changes in tissue disposition of contaminant following an IP injection and how this relates to MFO activity.

This study was conducted to help understand how contaminants are distributed among tissues following intraperitoneal injection and how this distribution affects the time course of MFO induction. Knowledge of the ultimate tissue disposition following treatment will help in comparisons of studies where the predominant route of uptake is dietary, and information on the time required for an intraperitoneal dose to reach a stable distribution within the animal will aid in the design and interpretation of future experiments.

Materials and Methods

Holding and Treatment of Fish

White suckers were captured from L627 ELA using trapnets and transported back to Winnipeg in an aerated transport tank. Fish were maintained in 50 L flow-through tanks (10°C, 2L/min, City of Winnipeg dechlorinated water) located at the Freshwater Institute and fed commercial fish pellets (Martin Feed Mills) at a ration of 1.5% body weight 3 times a week.

Each fish was tagged using individually coded VI® tags (Northwest Marine Technology, Shaw Island, Washington). Sixteen fish, randomly selected, were injected intraperitoneally with [^{14}C]-2,3,4,7,8-pentachlorodibenzofuran (P_5CDF) in corn oil at the rate of 1 ng/g using the same stock solution as was used in treatment of fish in the field study (see Chapter 1 for a full description of the preparation of the P_5CDF). An additional 16 fish were injected as controls with corn oil.

Sampling

Fish were sampled at 1,3,7 and 12 weeks after injection. Fish were anesthetized in MS-222, weighed and measured. Blood was taken from the caudal vein (c. 5 ml), centrifuged and then separated into plasma and red blood cells and frozen. Livers were removed and immediately frozen on dry ice and stored at -80°C until analyzed for MFO activity. The remaining tissues (spleen, kidney, intestine and muscle) were removed and placed in labelled Whirl-paks and then frozen at -40°C until analyzed for residues.

Residue Determinations

Determination of residue levels in individual tissues was done using the method described for P_5CDF in Chapter 5 of this thesis. Estimation of depuration parameters,

mean residence times (MRT) and half-lives ($t_{0.5}$) for the various tissue pools was done using DIMSUM (see Chapter 5 for description). Modelling of depuration was done using mean concentrations from each tissue for each sampling period.

EROD Determinations

Preparation of microsomes and determination of EROD activities and protein levels were done according to the methods described in Chapter 3 of this thesis.

Results and Discussion

Tissue Concentrations

Tissue concentrations were highest in the visceral organs (kidney, liver, intestine and spleen), with concentrations declining during the 84 days of the experiment (Figure 42a-f). Although concentrations were measured in gonads, too few fish of either sex were available to determine trends for either sexes. Muscle tissue showed an initial decline followed by a slow increase in P_5CDF levels (Figure 42b). Slow increases in muscle concentrations have also been shown in rainbow trout following IP injections of a PCB mixture (Melancon et al. 1989). Decreases in other tissue compartments occurred concurrently with increases in the muscle compartment, indicating a redistribution of the contaminant within the animal.

Mean residence times (MRT) were estimated for all tissues except muscle. Values ranged from 30 days for blood to 198 days for kidney (Table 16). These estimated MRT values for the visceral organs may be of limited meaningfulness since visceral tissues would have initially been bathed in the corn oil containing the contaminant. Initial concentrations may include a large amount of contaminant which was adsorbed to surfaces rather than being integrated into the tissues. In the case of blood and muscle, these tissues were not in direct contact

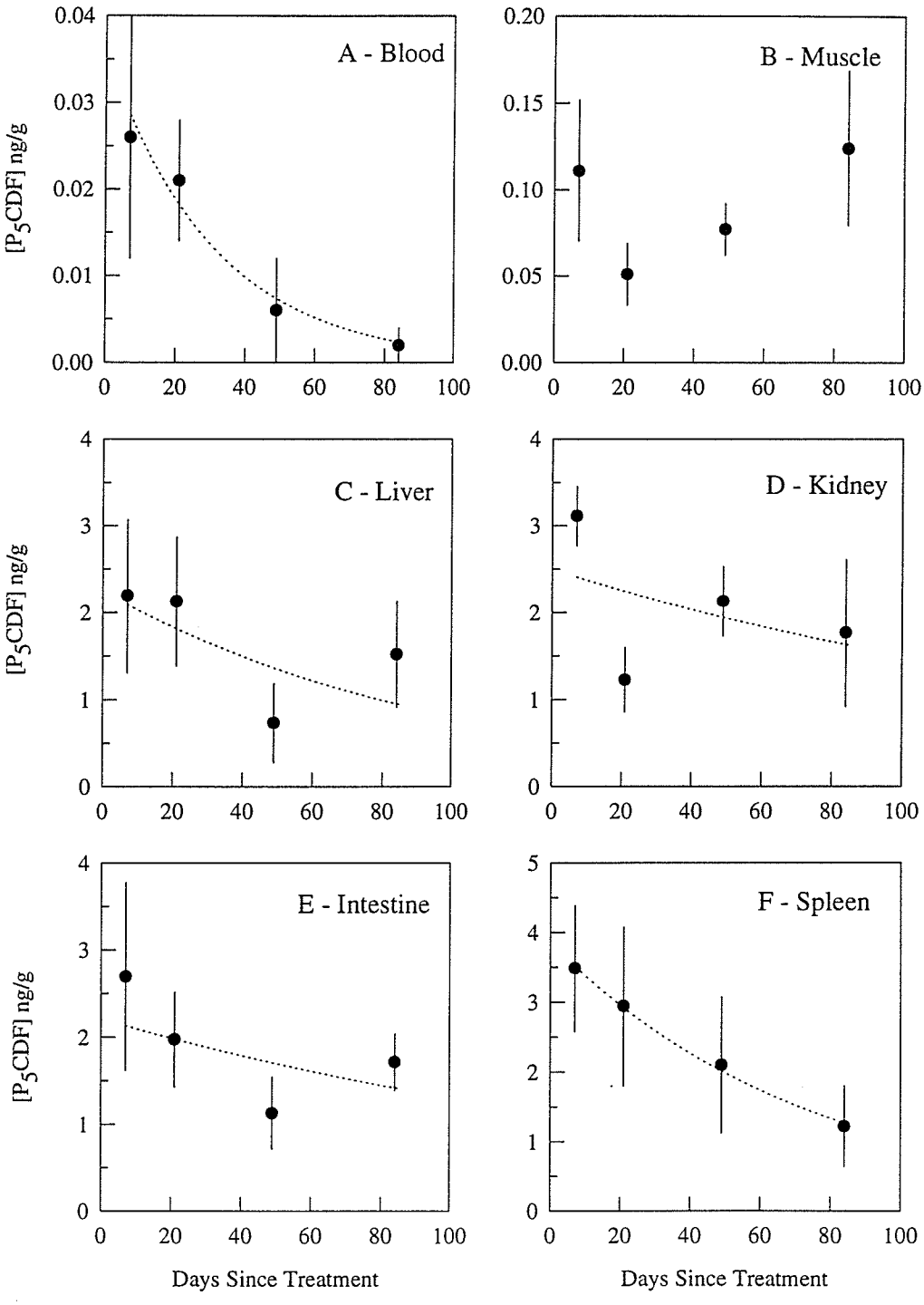


Figure 42. Mean P_5CDF concentrations in tissues of treated white suckers.

Table 16. Estimated mean residence time (MRT) and half life ($t_{0.5}$) for P_5CDF in visceral tissues and blood of treated white suckers. Values in parentheses are standard deviations of the parameter estimates.

Tissue	A_1	λ_1	MRT (d)	$t_{0.5}$ (d)
Blood	0.04 (0.16)	-0.032 (0.19)	31	21
Spleen	3.85 (1.03)	-0.013 (0.008)	75	52
Liver	2.25 (0.93)	-0.010 (0.011)	98	68
Intestine	2.21 (0.80)	-0.005 (0.007)	189	131
Kidney	2.49 (0.83)	-0.005 (0.008)	198	137

with the contaminant and are therefore good indicators of redistribution. Using blood concentrations as an indicator of redistribution it appears that the movement of contaminant is starting to level off by 84 days. In a similar study where PCB 126 was injected into juvenile lake trout, Brown et al. (1992) reported that muscle concentrations required 20 weeks (140 days) to equilibrate. In contrast to IP-injected fish, Brown et al. (1992) reported that a stable distribution of contaminant was achieved in 1 to 3 weeks in juvenile lake trout given a gavage dose of the same PCB 126.

On a mass balance basis, the majority of the P_5CDF (60 - 80 %) was found associated with muscle and intestine (Table 17). During the experiment, the proportion of P_5CDF declined in tissues other than muscle (Table 17), where proportions increased. This increase in proportion is consistent with the increasing concentration and the theory that the dose was being redistributed within the animals from visceral organs to muscle.

From these results it appears that the corn oil and associated P_5CDF acted as a separate compartment in the intraperitoneal space of the animals. The corn oil and associated contaminant was then slowly redistributed to other tissue compartments. Concentrations in visceral organs (liver, intestine, kidney and spleen) were generally 2 to 3 times higher than the nominal 1 ng/g dose. These initally high concentrations likely represent contaminant which is sorbed to tissue surfaces following the I.P. injection.

The tissue distribution from this single I.P. injection after 84 days was similar to those found by Sijm et al. (1990) in rainbow trout given a single oral gavage dose of 2,3,4,7,8-P_5CDF. Sijm et al. (1990) reported that 25 - 55% of the dose of P_5CDF was found in the intestine, muscle and skin, which is similar to the proportions found in this study.

Table 17. Mean percentages (± SE) of P_5CDF in tissues of white suckers following IP injection with P_5CDF.

	Days Since Treatment			
Tissue	7	21	49	84
Liver	11.46 ± 3.84	14.56 ± 1.10	7.06 ± 4.02	8.81 ± 1.52
Spleen	2.41 ± 0.58	2.16 ± 0.34	1.97 ± 0.56	0.87 ± 0.29
Gonad	7.61 ± 4.39	16.11 ± 5.03	9.90 ± 5.89	9.20 ± 2.35
Kidney	2.41 ± 1.57	2.47 ± 0.78	7.20 ± 1.68	3.29 ± 0.89
Intestine	37.04 ± 12.36	39.70 ± 6.07	28.41 ± 6.37	31.76 ± 9.51
Muscle	36.87 ± 11.74	22.14 ± 3.60	44.86 ± 3.91	45.93 ± 9.92
Blood	2.19 ± 1.34	2.86 ± 0.89	0.60 ± 0.60	0.13 ± 0.13

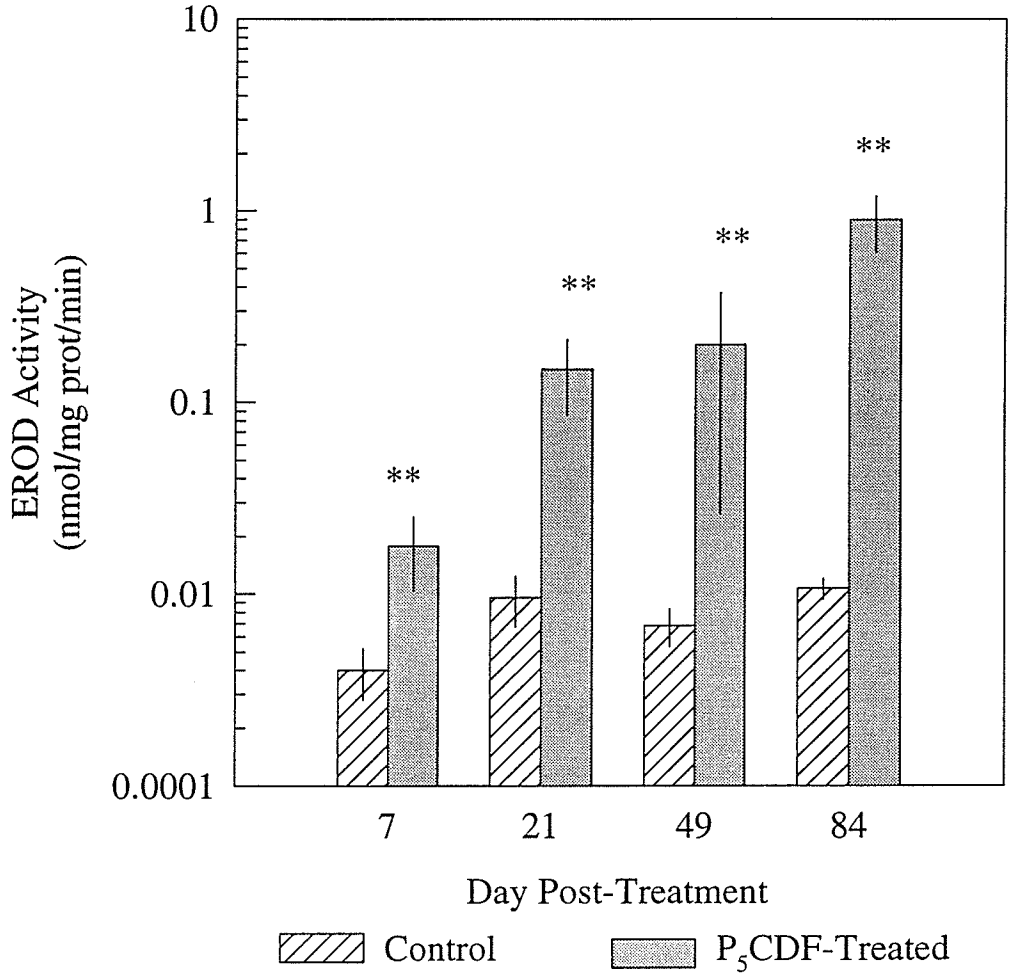


Figure 43. Mean EROD activity in control and P_5CDF-treated white suckers sampled at 7, 21, 49 and 84 days after treatment. ** indicates significant difference ($p<0.05$) between treated and control groups.

EROD Activity

EROD activities were shown to be significantly increased in treated fish on days 21 (15x), 49 (35x) and 84 (82x). Treated fish from day 7 were induced (4.5x) compared to controls (Figure 43). The level of induction increased each week with a maximum being reached by day 84. It is not known if induction levels would have been higher had the experiment been carried on longer. Results reported by Brown et al. (1992) showed steadily increasing EROD activity for 6 weeks following I.P. injections of PCB 126 in juvenile lake trout and then a period of leveling off. These results are contrary to results reported by others. Hahn and Stegeman (1994) report maximal EROD induction (51 fold) in scup eight days after IP injection of 2,3,7,8 tetrachlorodibenzofuran (10 nmol/kg), Janz and Metcalfe (1991) reported maximal induction of AHH activity 3 days following injection of rainbow trout with PCB 126 (1.64 μmol/kg). The reason for the differences between these studies, including this one is not known. Although it is tempting to suggest that the differences are due to species differences, this cannot be totally supported in light of the differeing results of Brown et al. (1992) and Janz and Metcalfe (1991) who both used PCB 126, corn oil and a salmonid species.

EROD activity was positively correlated ($p<0.05$) with inidividual liver concentrations of P_5CDF. However, the nature of this relationship changed from week to week. Regression of P_5CDF concentrations on EROD activity for each week revealed that the slope from each week increased with increasing time, that is for a given liver concentration of P_5CDF present in the liver the level of induction was higher (Figure 44) following longer exposure times. This is likely an indication of the redistribution of the P_5CDF from the intraperitoneal space into tissues as previously discussed. Initial concentrations determined for liver tissue may be biased by a large amount of contaminant associated with the surface of the tissue. As the corn oil and associated contaminant was redistributed to various tissue pools the concentration in whole liver samples decreased (Figure 42c) but EROD activity increased, offering further support to

the conclusion that contaminant was initially sorbed to external surfaces and over time was taken up and redistributed within the fish.

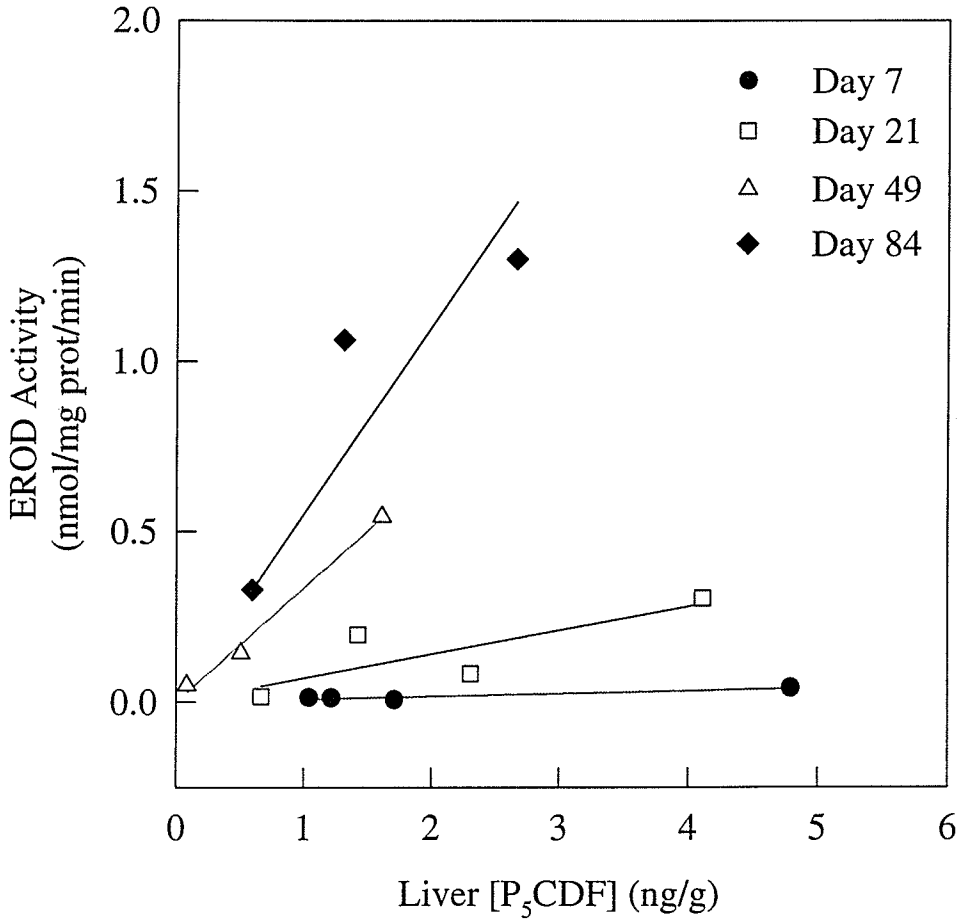


Figure 44. Relationship between individual EROD activity and concentrations of P_5CDF in liver for each sampling date. Lines indicate fitted regressions.

Chapter 7. Effects of a Single Intraperitoneal Injection of 2,3,4,7,8-Pentachlorodibenzo-furan on Adult Rainbow Trout and their Offspring.

Introduction

Polychlorinated dibenzofurans (PCDFs) have become widely dispersed contaminants in the aquatic environment. PCDFs are formed as byproducts in the production of chlorinated phenols, chlorinated phenol-derived products and commercial PCBs and during combustion of diverse materials (Safe 1990). PCDFs are also associated with releases from bleached kraft pulp and paper mills using molecular chlorine and are found in some paper products (Servos et al. 1994, Rappe and Buser 1989). 2,3,4,7,8-Pentachlorodibenzofuran (P_5CDF) is a predominant furan congener detected in fishes from the Great Lakes (Whittle et al. 1992) the Baltic Sea (Wiberg et al. 1992) and the Netherlands (van den Berg et al. 1987). Among the chlorinated dioxin and furan congeners, P_5CDF, has been reported to be one of the most potent inducers of mixed function oxygenase enzyme activity in rainbow trout (Clemons et al. 1994, Parrot et al. 1995). Of furan congers, P_5CDF has the most potent biological effects in rodents (Safe 1990) and on early life stage mortality in rainbow trout (Walker and Peterson 1991). Although the exact mechanism of toxicity of PCDFs, polychlorinated dibenzo-*p*-dioxins, (PCDDs) and related compounds (non-ortho substituted PCBs & methylcholanthrene) is still unknown, they interact with a specific cellular receptor, the **Ah** receptor. Furthermore, they all produce similar characteristic patterns of toxicity that correlate with their affinity for the **Ah** receptor (Safe 1990).

Laboratory (Muir et al. 1990c) and environmental studies (Rogers et al. 1989) reported that accumulation of PCDFs and PCDDs in fish is associated with prolonged induction of hepatic monooxygenase enzyme activities (MFO). Although this enzyme activity provides a sensitive indicator of exposure of fish to these compounds, the

toxicological relevance of MFO activity remains undefined. It has been hypothesized that elevated MFO activity may be related to impaired reproduction in fish exposed to PCDF-like contaminants (Lee 1988). MFO activity is thought to compromise reproductive hormone function and/or metabolism, but, definitive experiments are lacking.

There is a need to determine whether toxic responses occur following parental transfer of contaminants. There is limited information on the dynamics of parental transfer of contaminants. Questions remain as to what proportion of the contaminant burden in parental tissues will be passed on to potential offspring. These data are necessary to assess ecological risk of the effects of existing body burdens of PCDDs, PCDFs and non-ortho-substituted PCBs. Walker (Walker and Peterson 1991, Walker et al. 1992) recently reported an egg injection method for assessing early life stage mortality in rainbow trout. The LD50s reported from these studies were similar to those reported from waterborne exposure studies (Spitsbergen et al. 1991, Walker et al. 1990). All these studies show similar responses to TCDD or 2,3,7,8-substituted stereoisomers on early life history stages. Giesy et al. (1986) pointed out that direct exposure of eggs or fry to contaminants may be inappropriate because the internal and external effects, the absorption and disposition of the toxicant may not be similar to what happens in a natural exposure. However, it is difficult to conduct long-term life cycle experiments on long-lived species. Whether these responses occur following parental transfer of contaminant needs to be determined. As well, limited attention has been given to the effects contaminants might have on gamete viability. Egg incubation and injection techniques do not address this question. Reduced fertility of eggs and/or sperm is another possible source of reproductive impairment.

Most recent data on the sensitivity of early life history stages of fish to planar chlorinated aromatic hydrocarbons are on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Reported effects of TCDD on early life history stages include developmental and growth retardation, a lethal generalized edema (blue sac), higher

incidence of skeletal malformations (Helder 1980, 1981), and hemorrhages 1 week prior to hatch (Spitsbergen et al. 1991). These are accompanied by higher mortality at hatch and reduced hatchability (Spitsbergen et al. 1991) .

In this study, the effects and distribution of a single, sublethal dose of P_5CDF were investigated in broodstock rainbow trout maintained throughout a reproductive cycle. In addition, the effects of parental transfer of P_5CDF on the viability of gametes and on the subsequent development of offspring were examined. Brood stock rainbow trout (*Oncorhynchus mykiss*) were given intraperitoneal injections of P_5CDF. These fish were then allowed to undergo gonadal development and were spawned 10 months later. Fish were sacrificed after spawning for examination of tissue P_5CDF levels, hepatic ethoxyresorufin-o-deethylase (EROD) activity and liver vitamins (retinoids, tocopherol).

Materials and Methods

Fish maintenance

Broodstock rainbow trout, *Oncorhynchus mykiss*, (2-4 yrs; weight, 967 ± 51 g; ± SE, N = 28) were obtained just before spawning from the Department of Fisheries and Oceans' Rockwood Experimental Fish Hatchery, Gunton, Manitoba. Female fish were stripped of eggs in late January and all fish received two visual implant tags (Northwest Marine Technology, Shaw Island, WA). Fish were held at the Freshwater Institute in 900-L fibreglass tanks with flowing, aerated, dechlorinated Winnipeg city water. The tanks had translucent tops and each tank received at least 2 L of aerated water per gram of fish each day. Temperature ranged seasonally from 11.5 to 14.1 °C but during the experiment varied less than 2 °C. Fish were fed Martin Feeds trout food at a ration of 1% wet body weight every second day. The experiment was conducted between February and December. A foreshortened seasonal light-cycle (Pohl-Bransheid and Holtz 1990) was

used to regulate reproductive development. The light was initially at 8 h (8L:16D) and was automatically incremented to 17 h (17L:7D) and then back to 8 h (8L:16D) over a 9 month period using an AMF Paragon Suntracker. Fish were spawned 10 months after the beginning of the light regime.

Chemicals

Uniformly labelled [^{14}C]-2,3,4,7,8-Pentachlorodibenzofuran (P_5CDF, specific activity = 2.07 x 10^{12} Bq/mol) was obtained from Chemsyn Science Laboratories Ltd. (Lexana, Kansas). The stock solution was purified to >99% by reverse phase thin-layer chromatography (Whatman RP18 plates and a solvent system of acetone:water, 80:20). A corn-oil solution was prepared by evaporating an acetone solution of P_5CDF to near dryness under N_2 and then mixing with corn oil. Aliquots of the corn oil solution were assayed by liquid scintillation counting (LSC).

Treatment of fish

Fish were allowed 2 weeks to recover from handling and stripping before being dosed. Fish were lightly anesthetized in water containing tricaine methanesulphonate (TMS, 0.38 mmol/L) solution neutralized to tank pH with ammonium hydroxide and made approximately iso-osmotic with fish plasma with NaCl (150 mmol/L). When the fish lost equilibrium, they were blotted weighed and an intial blood sample was taken. Each fish was injected intraperitoneally (1 mL/kg) with corn oil (controls) or corn oil containing the prepared dose of P_5CDF (3 ng/g). Thus, the volume injected was proportional to the size of each fish. Disposable syringes (1 or 3-mL) and needles (21 gauge) were used to deliver each dose. Fish recovered from the procedure in anesthetic-free water within 3 min.

Spawning of Fish

Fish were spawned 10 months after injection, over a period of 3 weeks. Ripe fish were lightly anesthetized using MS-222, rinsed in fresh clean water to remove any traces of anesthetic and blotted to decrease the chances of mucous mixing with the eggs. Eggs from individual females were expressed into 1L stainless steel bowls, then divided into two roughly equal lots. One lot was fertilized using a treated male, the other using a control male. Eggs were gently mixed immediately following the addition of milt. Water (500-700 mL) was added to each bowl approximately 3 minutes after milt was added. Clean water was added approximately every 10 minutes while the eggs water-hardened for 45 - 60 min.

Sampling

Fish were sacrificed by anesthetization in pH-neutralized TMS (0.76 mmol/L) solution 2 weeks after spawning. Blood was removed and tissues were quickly dissected out, weighed and processed as required. For gonad weights the residual gonad and any spawned amounts were combined.

Relative fecundity was estimated using the number of eggs spawned plus the number estimated to be remaining in the body cavity (calculated as the weight of eggs remaining/mean egg weight for that fish).

Maintenance of Eggs and Fry

After water-hardening, eggs were transferred to incubation trays (Heath Techna Corp.). Each incubation tray was divided into two sides with a piece of plexiglas, with one lot of eggs being placed on each side. Fertilized eggs were maintained in the flow through incubation trays (10^{o}C, 2L/min., dechlorinated water) from fertilization through

yolk sac resorption. Dead eggs or fry were removed every 2-3 days and fixed in Davidson's fluid for counting.

To decrease the amount of handling, a photograph of each tray was taken and eggs were counted using projected negatives. When approximately 50% of the fry were swimming in the water column, a sample of 400 from each $F_C \times M_C$ and $F_t \times M_t$ cross were transferred to 40-L flow through glass aquaria. These were fed a commercial trout starter daily for 21 days post swim-up. After six weeks of rearing in aquaria, sub-samples of crosses were transferred to 150-L fiber glass tanks where they were held for the next 10 months.

Eggs in which there was no visible embryo were examined with a dissecting microscope. If no evidence of development was present around the germinal disc, the egg was categorized as unfertilized. Fertilization success was calculated as the total number of fertilized eggs/ total number of eggs from the cross. Mortalities were calculated as follows:

$$\% \text{ PreHatch Mortality} = \frac{\text{\# Dead Embryos}}{\text{\# Fertilized Eggs}} \text{x} 100$$

$$\% \text{ PostHatch Mortality} = \frac{\text{\# Dying PostHatch}}{\text{\# Fertilized Eggs}} \text{x } 100$$

$$\% \text{ Survival} = \frac{\text{\# alive at 1st Feeding}}{\text{\# Fertilized Eggs}} \text{x } 100$$

$$\% \text{ TotalSurvival} = \frac{\text{\# alive at 1st Feeding}}{\text{\#Eggs Spawned}} \text{x } 100$$

Determination of P_5CDF Concentrations

Samples from each $F_C \times M_C$ and $F_t \times M_C$ cross were taken after water hardening (70 - 150 eggs), 24h posthatch, 1 week posthatch and just prior to initiation of feeding (about 3 weeks posthatch). Testes, muscle, liver, intestine and kidney samples were taken from adults, and water-hardened and ovarian eggs were taken from the same individuals. Samples were weighed, and then freeze dried. P_5CDF concentrations and the amount of parent P_5CDF were determined using the methods described in Chapter 5.

To determine the amount of P_5CDF in embryos and fry, samples of 15 - 20 embryos or posthatch fry were taken from each $F_C \times M_C$ and $F_t \times M_C$ cross, blotted dry and weighed individually. Five samples of 2 - 4 fry from each cross were then oxidized using a Packard Model 306D oxidizer and then counted using LSC.

EROD Determinations

Preparation of microsomes and determination of EROD activities and protein levels from adult livers were done according to the methods described in Chapter 3 of this thesis.

Retinoid and Tocopherol assay

Retinol, dehydroretinol, retinyl palmitate and tocopherol were measured in liver samples from adults as described in Chapter 3.

Statistics

Bartlett's test for homogeneity of variance was applied to the data. Where required, data were transformed according to Taylor's power law to obtain more uniform variance (Southwood 1978). For clarity of presentation, arithmetic means and standard

errors are given in results. Two-way ANOVA was used to test for group differences in parameters at the end of the experiment. Differences between weights at injection and final weights were compared using paired *t*-tests. The Pearson product moment correlation was used to test the relationships between EROD and the different P_5CDF levels found in liver tissue. Probabilities of < 0.05 were considered significant.

Differences in measures of survival and mortality between the different cross groups were tested using t-tests. Analysis of correlation between P_5CDF concentrations in eggs and mortality was done using Pearson's correlation coefficient. All statistical analyses were done using SYSTAT (Wilkinson et al. 1992).

Results and Discussion

Parental Tissue P_5CDF Concentrations

The fraction of the initially injected P_5CDF that was found in dorsal muscle, liver, gonads, intestine, and posterior kidney averaged 34.8 ± 7.5% and 53.3 ± 7.0% in females and males respectively. Proportions for each tissue are listed in Table 18. P_5CDF was more persistent in these adult fish than in juvenile rainbow trout reported by Muir et al. (1990c) who found that the elimination half-life of dietary P_5CDF ranged between 60 and 70 d. In this experiment the elimination half-life of P_5CDF exceeded 300 d. This long residence time is similar to that reported for 2,3,7,8-TCDD in carp averaging 1.5 kg and 19% lipid (Cook et al. 1991), but not as long as for lake trout in the field study (see Chapter 5). Differences in P_5CDF levels between male and female fish may have been partly related to lipid content. Male fish contained higher lipid levels in liver and intestine (Table 18) and had proportionally greater P_5CDF concentrations. The differences in lipid content could not completely account for higher gonad or muscle

P_5CDF levels of male fish. The reason for more rapid elimination of P_5CDF by female fish is unknown.

The toluene procedure for extracting tissue P_5CDF was 90 ± 1% efficient. Analysis of the tissue extracts by HPLC showed that 86.2 ± 6.9 % of the radioactivity found in the toluene represented P_5CDF. In both sexes, the highest P_5CDF concentrations occurred in liver and gut (Table 18). P_5CDF concentrations were lower in muscle but because of this tissues large mass, it contained about 80% of the total amount of contaminant in each fish.

The injected dose of 3 ng/g was about 80 times higher than congener specific levels of P_5CDF recently reported for salmonids from Lake Ontario (Whittle et al. 1992) and the Baltic (Wiberg et al. 1992). If 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TCDD-TEQ) (Safe 1990) are considered, our dose represents about 1.5 ng TCDD-TEQ/g. Recent reports show that 0.2 to 1.8 ng TCDD-TEQ/g have been detected in fishes from Lake Ontario (Niimi and Oliver 1989, Huestis et al. 1993) and Lake Michigan (Smith et al. 1990). In spawned females, the injected dose produced TCDD-TEQ levels in muscle (0.34 ng/g) and eggs (0.13 ng/g) (calculated from values in Table 18) comparable to those found in Lake Michigan salmon (Williams et al. 1992, Williams and Giesy 1992). On a TEQ basis then, the dose used here falls within the range of contamination found in Great Lakes fish.

Because dietary uptake predominates in the environment (Muir et al. 1992), intraperitoneal injection represents an unnatural dosing route for fish to obtain P_5CDF or TCDD-like contaminants. However, intraperitioneal injection does provide a rapid and convenient method to deliver a uniform dose to each individual. The P_5CDF tissue disposition in the post-spawned brood stock rainbow trout (Table 18) approximated that reported by Sijm et al. (1990) 2 to 3 weeks after dietary gavage of P_5CDF to 75-175 g rainbow trout.

Table 18. Mean concentrations (wet weight), percent lipid and percent of the initial dose of P_5CDF in liver, intestine, posterior kidney, dorsal muscle and gonad of rainbow trout 322d after I.P. injection with 3 ng/g of P_5CDF.

	Female			Male		
Tissue	Concentration (ng/g)	% Lipid	% Initial Dose	Concentration (ng/g)	% Lipid	% Initial Dose
Liver	1.679 ± 0.624	1.9 ± 0.12	1.16 ± 0.4	2.533 ± 1.279	3.46 ± 0.26	1.86 ± 0.89
Intestine	1.565 ± 0.374	19.62 ± 6.03	2.79 ± 0.93	3.252 ± 0.712	34.9 ± 8.28	4.43 ± 0.66
Kidney	0.595 ± 0.217	nd*	0.18 ± 0.06	1.194 ± 0.676	nd*	0.48 ± 0.21
Muscle	0.604 ± 0.184	7.7 ± 1.18	28.4 ± 8.08	0.868 ± 0.116	5.72 ± 0.85	43.51 ± 5.97
Gonad	0.422 ± 0.200	3.83 ± 0.32	2.28 ± 0.52	2.248 ± 1.300	11.3 ± 0.26	3.05 ± 1.47

* Not determined

MFO activity

More than 10 months after P_5CDF injection, hepatic EROD levels were strikingly elevated, with male EROD activity being 340-fold greater than controls and female induction being 160-fold greater than controls (Table 19). This degree of induction is even greater than the 100-fold induction reported in lake trout from the field study (Chapter 3), a possible indication that rainbow trout are more sensitive. Enzyme induction was concentration-dependent as EROD activity and liver P_5CDF level were significantly correlated (Figure 45). However, the relationship was different for males and females reflecting the differences in liver concentrations of P_5CDF. Sustained dose-related MFO induction following P_5CDF exposure was previously reported in juvenile rainbow trout by Muir et al. (1990c), and is reported for both lake trout and white suckers in this thesis.

Hepatic retinoids and tocopherol

Retinoids (vitamin A) are essential for vision, reproduction and cellular immune function (Fox 1993). Tocopherol (vitamin E) is important as an antioxidant in protecting cellular and subcellular membranes (Burton and Traber 1990).

P_5CDF treatment reduced hepatic concentrations of dehydroretinol, retinol and retinyl palmitate in male fish but did not affect tocopherol levels (Table 19). Liver retinoid stores also decline with exposure to TCDD-like contaminants in mammals (Brouwer 1991) and birds (Spear and Moon 1986). Induction of phase I (MFO) and the phase II conjugating enzymes are thought to stimulate hepatic metabolism of retinoids (Zile 1992). While these mechanisms may account for the low retinoids observed in male fish, female fish displayed 160-fold induction of EROD without significant change in

Table 19. Biochemical parameters, liver somatic index (LSI), gonadosomatic index (GSI) and instantaneous growth rate in post-spawned rainbow trout exposed to P_5CDF for 322d. Values represent means ± S.E. of 6-9 fish per group.

	Female		Male	
Parameter	Control	Treated	Control	Treated
EROD (pmol/mg/min)	3.9 ±1.1	624 ± 142*	3.4 ±0.6	1153 ±210*
Dehydroretinol (nmol/g)	169 ±55	122 ± 23	151 ±16	25 ±3*
Retinol (nmol/g)	54.4 ±15.1	53.1 ± 5.2	42.2 ±4.6	15.9 ±1.8*
Retinyl Palmitate (nmol/g)	448 ±99	411 ± 64	658 ±67	144 ±28*
Tocopherol (nmol/g)	314 ±61	279 ± 30	480 ±67	286 ±74
LSI (%)	1.07 ±0.11	1.28 ± 0.11	0.92 ±0.07	1.35 ±0.06*
GSI (%)	34.8 ±5.3	31.2 ± 2	3.54 ±0.47	3.07 ±0.27
Growth Rate (% wt/d)	0.16 ±0.02	0.16 ± 0.01	0.18 ±0.03	0.15 ±0.03

*Mean values significantly different from controls of same sex, $P<0.05$

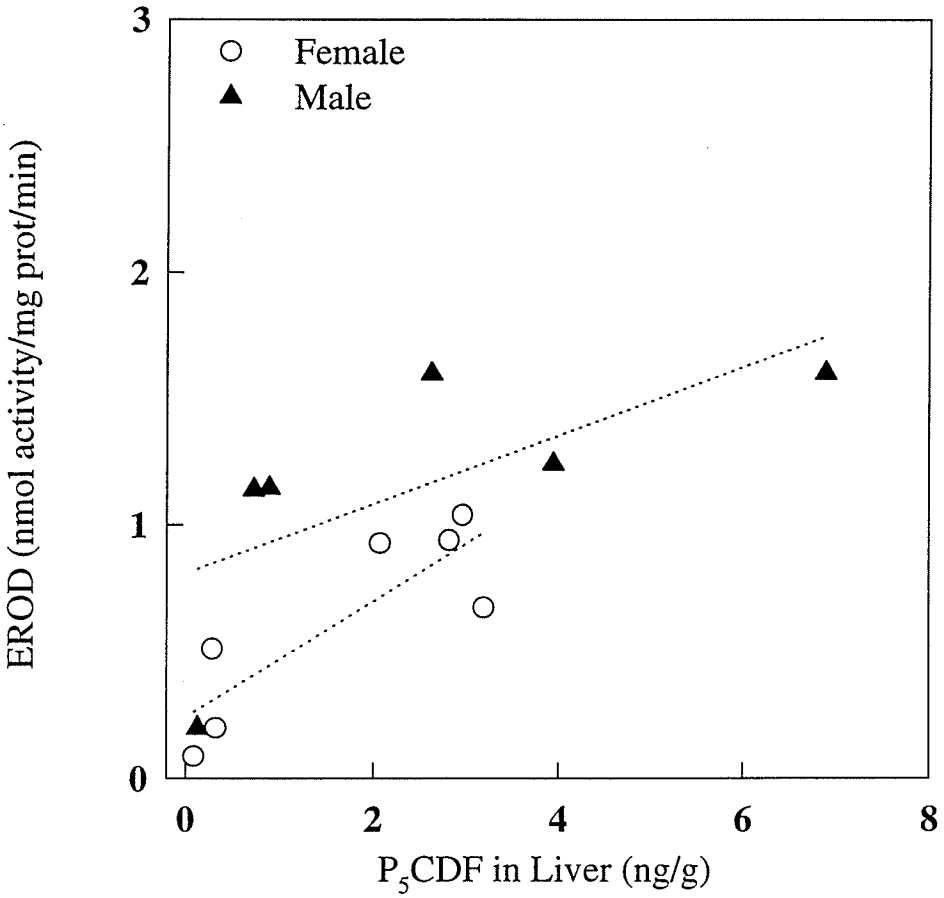


Figure 45. Relationships between EROD and contaminant concentrations in livers of male and female rainbow trout treated with 3.0 ng/g P_5CDF.

retinoid levels. Furthermore, both retinol and tocopherol may act as antioxidants following exposure to compounds that cause oxidative damage (Ribera et al. 1991). TCDD produces oxidative stress in mammals (Stohs 1990) and thus, the lower retinoids in male fish may imply that P_5CDF was exerting oxidative stress.

Growth rate and gonadosomatic index

Specific growth rates (Table 19) did not differ between male and female fish and were unaffected by sublethal P_5CDF exposure. Gonadosomatic indexes (Table 19) showed expected differences between male and female fish but were also unmodified by treatment.

Fecundity and Fertilization Success

Production of eggs as measured by relative fecundity (eggs/g carcass wt ±SE) was not different between treated females, 4.52±0.27, and controls, 5.06±0.64. No differences were seen in mean fertilization success of eggs spawned from treated or control females, or in fertilization success using sperm from treated or control males (Table 20), although there was a trend for more variability and lower fertilization success in crosses using treated females.

Contaminant Levels in Offspring

Concentrations of P_5CDF in eggs ranged from 55 to 398 pg/g with a mean of 229±45 pg/g (N=7, ±SE). Mean concentrations remained relatively constant through 26 days posthatch, when the data are corrected for growth of the fry (Figure 46). The slow elimination of P_5CDF posthatch, is similar to results reported by Zabel and Peterson (1992). For individual crosses in the $F_t x M_t$ group, the concentration of P_5CDF in eggs was highly correlated with the proportion of unfertilized eggs (r=0.909), prehatch

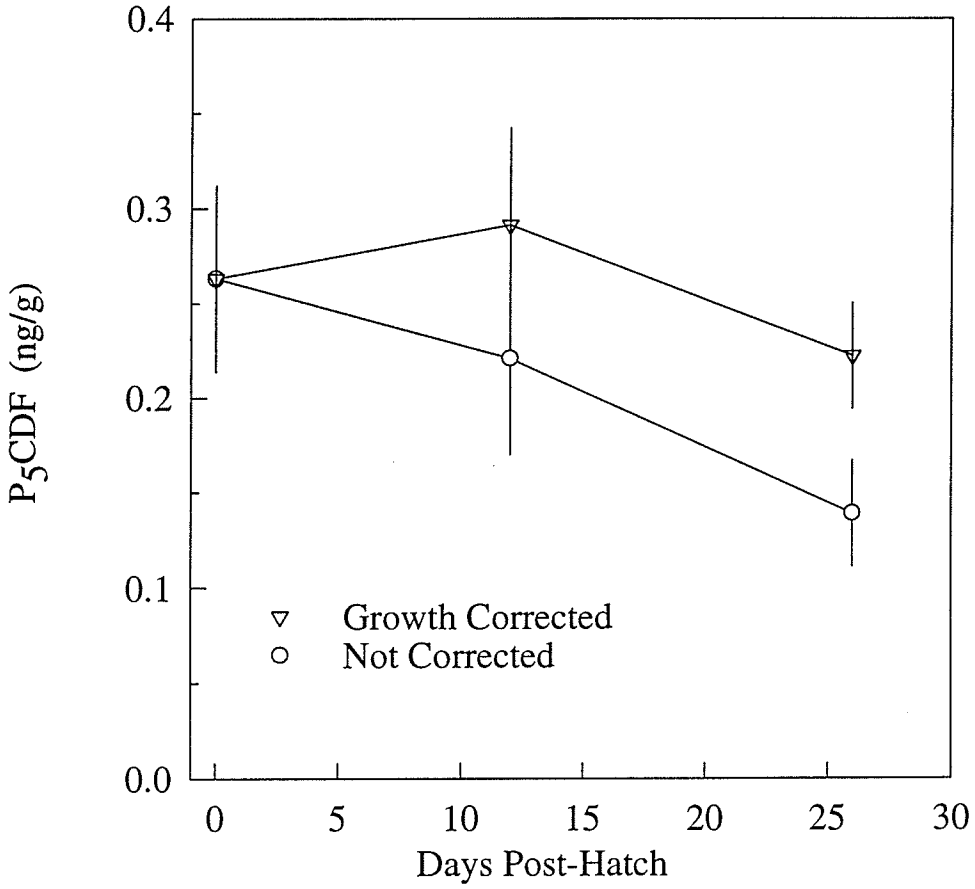


Figure 46. Mean concentrations of P_5CDF (ng/g) in rainbow trout eggs and sac-fry larvae from P_5CDF-treated females. Each point represents a mean from 7 crosses. Error bars represent SE.

Table 20. Mean fertilization success, mortality of fertilized eggs, embryos and sac fry larvae and survival from crosses done with control and P_5CDF-treated rainbow trout parents. All values ± SE.

Cross Type (Female x Male)	N	% Fertilization	Mortality of Fertilized Eggs: % Pre-Hatch	% Hatching	% Post-Hatch	Survival of Fertilized Eggs	Total Survival
Control x Control	5	97.89 ± 1.37	2.06 ± 0.83	0.93 ± 0.61	0.74 ± 0.07[a]	96.27 ± 1.39	94.20 ± 1.36
Control x Treated	6	96.10 ± 1.33	2.90 ± 1.00	1.70 ± 0.83	1.10 ± 0.18[a]	94.30 ± 1.73	90.72 ± 2.72
Treated x Control	7	90.70 ± 6.69	4.14 ± 3.19	1.05 ± 0.41	1.73 ± 0.41[ab]	93.08 ± 3.72	85.79 ± 8.17
Treated x Treated	6	89.63 ± 7.06	9.01 ± 4.73	1.25 ± 0.58	2.71 ± 0.57[b]	87.02 ± 5.26	79.65 ± 9.42

* Values with different letters significantly different ($p<0.05$).

mortality of fertilized eggs(r=0.881), total mortality of fertilized egg (r=0.884) and total mortality of eggs spawned (r=0.930). No correlation was found between P_5CDF concentrations in eggs and posthatch mortality or mortality from blue-sac.

Survival of Offspring

A total of 24 crosses were performed, using 7 treated females (F_t) and 6 control females (F_C). Eggs from twelve of the thirteen females were fertilized with both treated (M_t) and control males (M_C). One of the 7 treated females had only enough ripe eggs to be fertilized with a control male. Five of the six measures of reproductive success shown in Table 20 showed a trend for offspring of crosses with one treated parent to do less well than controls, and those with two treated parents to do even less well. However, only one of these measures (posthatch mortality) was statistically significant. Total survival of of offspring from crosses with untreated parents was 94%, while that of $F_c \times M_t$ and $F_t \times M_c$ were 91% and 86% respectively and only 80% for $F_t \times M_t$ (Table 20). Mortality in both cross groups using treated females was greater before hatch than at hatch or posthatch. However, there was also an increase in the variability of all measures with two treated parents. Post-hatch mortality was greater in $F_t \times M_t$ than in $F_C \times M_C$ and $F_C \times M_t$ groups. The proportion of mortality attributable to blue sac was higher in both groups of crosses using eggs from treated females (Figure 47). The overall mortality from blue sac was less than 3% of the total mortality (Figure 47). There was also no difference in the proportion of hemorrhages in embryos from treated females.

In this experiment most mortality was associated with fertilization and pre-hatch development. The mean concentration of P_5CDF in eggs and fry was slightly less than the LD_{50} range (305 - 882 pg/g) determined for this furan congener by egg injection (Walker and Peterson 1991). The P_5CDF concentrations in 2 of 7 crosses fell within this range but mortality was greater than 50% in only one of these crosses. Fertilization

success fell with increasing P_5CDF in eggs and pre-hatch mortality in the $F_t \times M_t$ crosses increased with P_5CDF in eggs. Results of experiments done using egg injections or incubation with TCDD and other TCDD analogues have demonstrated mortality just prior to and at hatch, attributed to half hatching, and also in the posthatch sac fry stage when it is caused by subcutaneous yolk sac edema resembling blue-sac disease (Helder 1980, 1981, Spitsbergen et al. 1991, Walker and Peterson 1991, Walker et al. 1990, 1992, Wisk and Cooper 1990a, 1990b).

Although the mortality due to blue sac was higher in eggs from treated females, it was not a major source of mortality in this experiment. It is notable that there was a trend to greater incidence of blue sac in crosses which used treated males (Figure 47). It is not clear if this trend was caused by P_5CDF in seminal fluid or some other mechanism. Given that male gonad levels of P_5CDF were very high (Table 18), it is possible that there was transfer of P_5CDF from treated males to eggs. Our results differ from those with egg injection or incubation in contaminated water (Helder 1980, 1981, Spitsbergen et al. 1991, Walker and Peterson 1991, Walker et al. 1990, 1992, Wisk and Cooper 1990a, 1990b) with respect to timing and etiology of mortality. The results imply that the route of exposure may be important in determining the ultimate effects on reproductive and embryological success, and that successful fertilization of eggs may be affected by exposure to P_5CDF or other dioxin analogues.

There are several possible explanations for our results in light of previously published results (Walker and Peterson 1991). The dynamics of uptake of contaminant from yolk into tissue may be one factor to explain differences. We must also consider the route of exposure. Egg injection techniques of exposure involve a mechanical disruption of the chorionic membrane, 24 - 50 h after fertilization. This time corresponds roughly to the beginning of the formation of the blastoderm with 100 to 150 cells (Knight 1963). Because little differentiation into specific tissue types has occurred by this stage, effects on any cell may potentially affect further developmental processes. It is unknown if

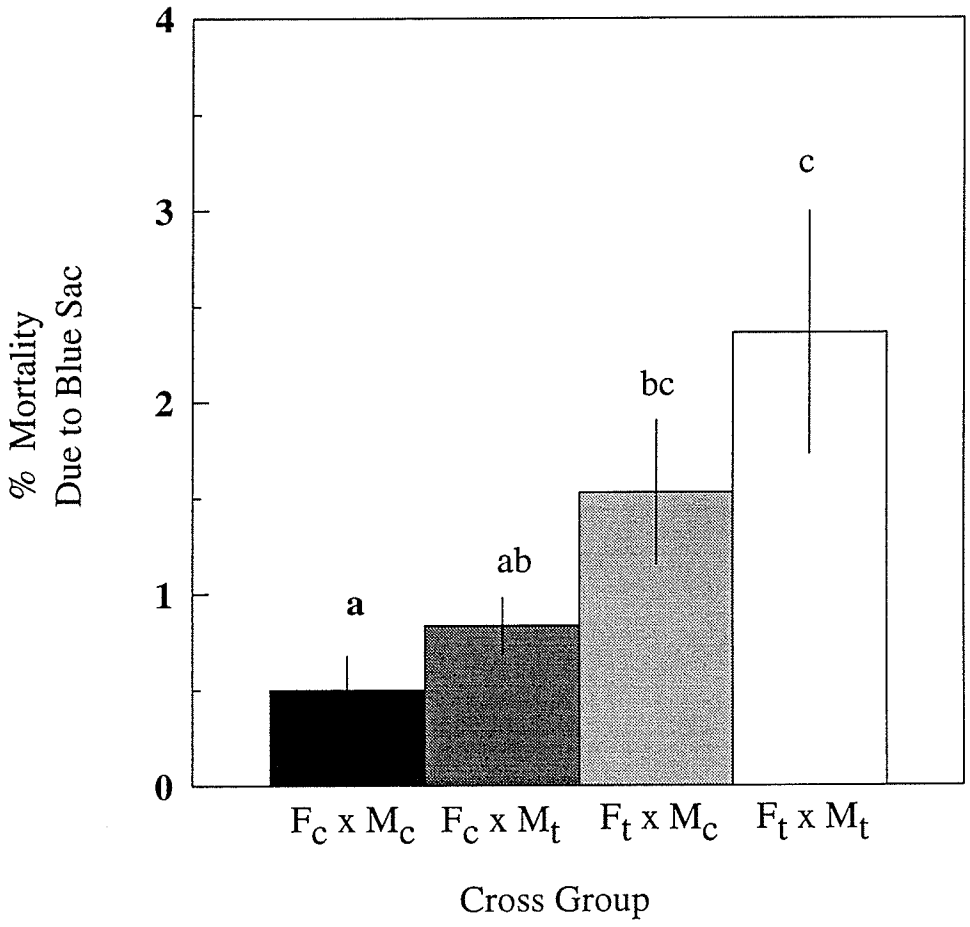


Figure 47. Mean proportions of total mortality accounted for by "blue-sac like" disease, for the four different types of crosses. F and M refer to sex, subscripts refer to control and treated. Bars with different letter are significantly different at $p < 0.05$.

contaminants injected into eggs or those taken up from water are sorbed only to the surface of the yolk or if they penetrate into the yolk itself. If the former is the case then the potential exists for the developing cell mass to receive a greater dose than calculated from total egg weight. For parentally transferred contaminants we assume that the contaminant is continuously added during formation of the yolk and therefore occurs more uniformly throughout the yolk mass. For 2,5,2',5'-tetrachlorobiphenyl (PCB 52), residues in yolk remain constant through the first stages of development (egg stage) and then are steadily transferred to the larva after hatching (sac-fry stage) (Guiney et al. 1980). In this case it would mean that the actual dose mobilized at any one time would be less than the total concentration in the egg. It has been shown that medaka exposed to TCDD after formation of the liver did not exhibit hemorrhages and edema, indicating stage specific toxicity (Wisk and Cooper 1990b).

Growth of Fry

Growth in fry from treated parents was not different from control crosses for a period of 10 months post-hatch (Figure 48). Although P_5CDF caused no decrease in post-hatch growth, it is unknown what the effects of maternally transferred P_5CDF might be on the still developing systems (e.g.. nervous, endocrine and reproductive) of fry and juvenile fish. The possible effects that impairment or altered development of these systems might have on the subsequent (F_1) generation requires additional study. Holdway and Dixon (1986) have reported decreased hatch and greater numbers of abnormalities in offspring from parents of flagfish (*Jordanella floridae*) exposed to methoxychlor as juveniles.

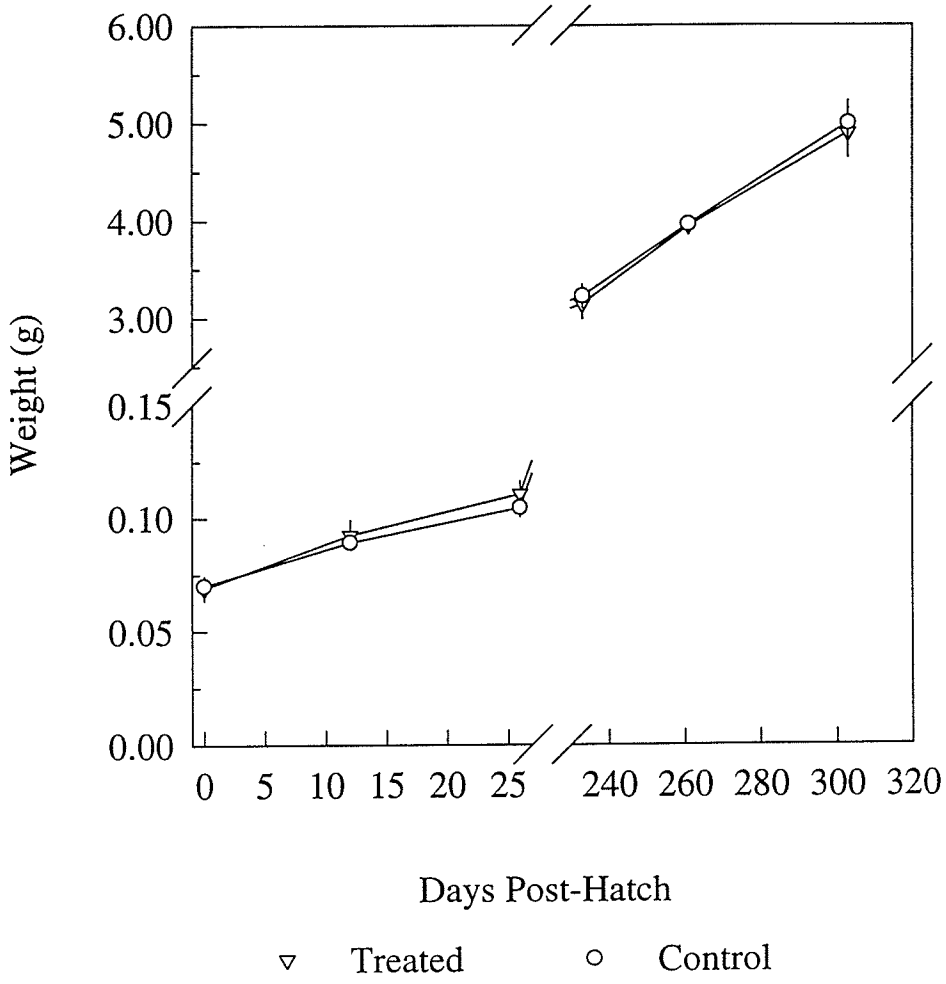


Figure 48. Mean weights of fry from crosses using two control parents or two P_5CDF-treated parents over a period of 10 months posthatch. Error bars represent SE.

General Discussion

The interaction and movement of contaminants between body compartments where contaminants are stored (muscle, visceral fat) and compartments where potential effects occur (e.g.. gonad, liver) has received limited attention. In this study muscle concentrations of P_5CDF correlated well with egg concentrations (r=0.922) (Figure 49a) and were negatively correlated with total survival (r=-0.759) but only at the 90% significance level (Figure 49b). Establishing a relationship between dorsal muscle concentrations, egg concentrations and effects on the viability of the resulting offspring is necessary to understanding the resulting effects of parental transfer on potential offspring. More work is necessary to determine the origin of lipids (and associated contaminants) used in egg formation. Do they originate from the lipid stores within the body or are they derived directly from dietary lipids at the time of egg formation, or some combination of these two? These types of data are very important for assessing the potential reproductive impacts of these types of contaminants in feral fish.

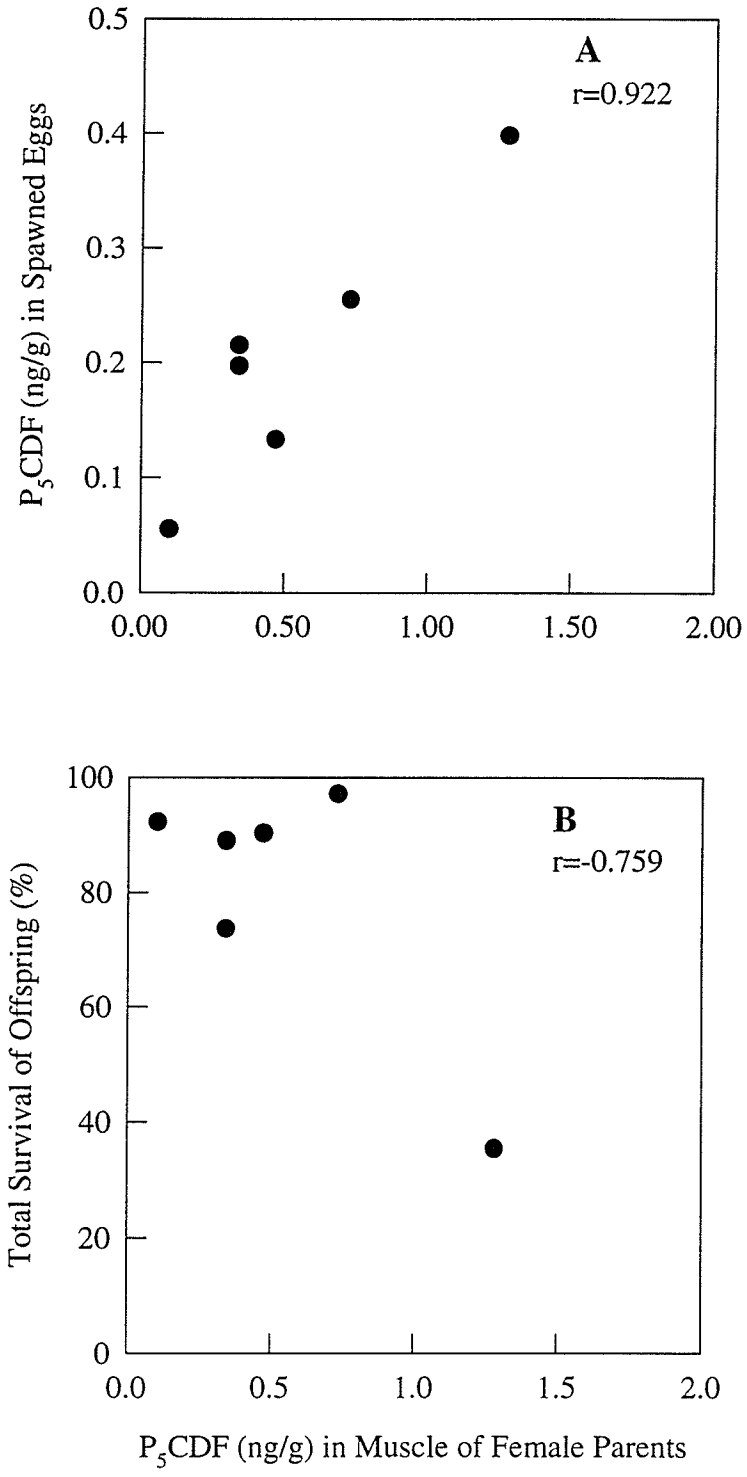


Figure 49. Relationship between (A) muscle concentrations of P_5CDF in treated female rainbow trout and egg concentrations of P_5CDF and (B) muscle concentrations and total survival of offspring to first feeding.

Chapter 8. Summary of Results and Assessment of the Theoretical and Potential Population Effects Caused by treatment with Toxaphene, Chlordane or 2,3,4,7,8-Pentachlorodibenzofuran in Lake Trout and White Sucker.

Introduction

This chapter provides a summary of the effects reported in the previous chapters. It also takes a broader look at the implications of effects and offers an assessment of the potential overall effects of the three contaminants, toxaphene, chlordane and P_5CDF at a population level.

Summary of Field Study

The overall effects of the treatment of lake trout and white suckers with toxaphene, chlordane or P_5CDF have been summarized in a simplified format in two tables, one for lake trout (Table 21) and one for white sucker (Table 22). Each table is divided into effects in the areas of survival, growth, reproductive success and biomarkers. The category of reproductive success is further subdivided into male and female sections and biomarkers are further sub-divided into contaminant-specific areas, namely hydroxyproline for toxaphene treated fish and MFO activity and retinoids for the P_5CDF treated fish.

Decreases in survival of adult fish were seen in lake trout and white sucker adults exposed to toxaphene and chlordane; exposure to P_5CDF caused decreased survival only in lake trout. Effects on growth were much less pronounced, with only the P_5CDF-treated white suckers showing any clear consistent decrease in growth. Effects on reproductive success were most pronounced in females. All contaminant-treated groups exhibited decreased success of female gametes or failure to spawn. Effects of contaminants on males, in the form of decreased fertilization success were found only in white sucker.

Table 21. Simplified summary of effects on lake trout from single IP injections of toxaphene, chlordane or P_5CDF.

Contaminant	Survival	Growth	Reproductive Success[1]		MFO	Retinoids	Hydroxyproline
			Females	Males			
Toxaphene	Decreased	Unchanged	Decreased[2]	Unchanged	-	-	Unchanged
Chlordane	Decreased	Decreased Year 2	Slightly Decreased	Unchanged	-	-	-
P_5CDF	Decreased	Unchanged	Decreased	Unchanged	Increased	Decreased	-

[1] Includes effects on adults, eggs, embryos and larvae
[2] Failed to spawn 5 months after injection

Table 22. Simplified summary of effects on white sucker from single IP injections of toxaphene, chlordane or P_5CDF.

Contaminant	Survival	Growth	Reproductive Success[1]		MFO	Retinoids	Hydroxyproline
			Females	Males			
Toxaphene	Decreased	Slightly Decreased	Decreased	Decreased	-	-	Unchanged
Chlordane	Decreased	Unchanged	Decreased	Decreased	-	-	-
P_5CDF	Unchanged	Decreased	Decreased	Decreased	Increased	Unchanged	-

[1] Includes effects on adults, eggs, embryos and larvae

Conclusions from the Field Study

1. Under the experimental conditions used, environmentally relevant levels of toxaphene, chlordane, and P_5CDF caused changes in lake trout and white sucker populations. These changes were caused by decreased survival and/or decreased reproductive success.

2. Exposure of lake trout and white sucker to P_5CDF caused changes in biochemical biomarkers (MFO activity and retinoid concentrations). These changes were coincident with decreased adult survival and reproductive success, indicating that they may be early indicators of effects at higher levels of biological organization.

3. Exposure of lake trout and white suckers to toxaphene did not alter calcium, collagen or hydroxyproline content in bone. This result likely indicates that adult fish are at less risk than younger, fast growing life stages.

4. Depuration of toxphene, chlordane and P_5CDF in these naturally occuring populations of fish were longer than measurments from laboratory studies.

Summary of Laboratory Studies

In the first laboratory study white suckers were injected with P_5CDF at the rate of 1 ng/g. Analyses of tissues showed that all tissues except muscle showed decreasing concentrations over duration (12 weeks) of the experiment indicating a redistribution of the contaminant. Muscle and intestine accounted for 60 to 80 % of total P_5CDF in each fish after 84 days. These tissue distributions were similar to those reported from feeding studies.

EROD activity was significantly induced at days 7, 21, 49 and 84. Induction increased over time, with greatest induction seen 84 days after injection. EROD activity was correlated with liver concentrations of P_5CDF, but this relationship changed from week to week, probably indicating a redistribution of contaminant from the intraperitoneal space to tissues.

The results of this study show that caution should be used when interpreting and comparing results from studies which use different routes of exposure. Care must be

taken to ensure that the injected dose has reached an equilibrium distribution before tissue residues are related to effects. Under the conditions used in this experiment, distribution of the contaminant required at least 84 days and data from Brown et al. (1992) suggests that a period of longer than 100 days may be necessary. The ultimate tissue disposition of the contaminant appears to be similar to that from dietary uptake. In addition it also shows that IP injections of contaminants can be a useful treatment method for simulation of body burdens of contaminants, provided the study extends beyond the time necessary for distribution within the animal.

In the second laboratory study P_5CDF treatment of brood stock rainbow trout with 3 ng/kg produced changes in liver EROD in both sexes. In male fish, liver size and retinoid stores were also altered. However, somatic growth and gonad development appeared normal during the first reproductive cycle following treatment. Only minor effects on the viability of eggs and fry from parents treated with P_5CDF were found, despite highly induced mixed function oxidase enzymes, decreased retinyl palmitate and other changes in parental fish . The type of mortality and the time when it occurred was different from early life stage (ELS) assays (Walker and Peterson 1991) in which exposures were made via water or by egg injections

It must be emphasized that the current experiment was limited to a single reproductive cycle. This does not imply that subsequent cycles will be unaffected. In fact evidence from the long term field experiment using lake trout indicated that subsequent reproductive efforts may be impaired. The long term consequences of chronically elevated liver MFO or depleted vitamin stores over multiple reproductive cycles requires additional study. Additional studies are also needed to assess the effects on successive generations of fish arising from contaminanted parents.

Conclusions from Laboratory Studies

1. Following a single IP injection of P_5CDF the distribution of P_5CDF to tissues of white suckers required at least 12 weeks to reach equilibrium levels.

2. Maximum EROD activity in white sucker was reached 12 weeks after a single IP injection of P_5CDF.

3. Treatment of broodstock rainbow trout with IP injections of P_5CDF resulted in changes in liver EROD activity and retinoid concentrations but not in hepatic tocopherol concentrations.

4. Treatment of broodstock rainbow trout with IP injections of P_5CDF did not cause any changes in gonadosomatic index (GSI) or in fecundity.

5. Fertilization success and total survival of offspring from treated parents decreased with increasing P_5CDF concentrations in eggs.

Assessment of the Theoretical and Potential Population Effects

In order to understand fully the potential effects of contaminants on a population one must first understand the basic structure of a population. As a simplified model, a fish population can be divided into three compartments. These are: 1. eggs, embryos and larvae or early life stages (ELS); 2. juveniles and sub-adults; and 3. adults (Figure 50). The adults responsible for the contribution of gametes (eggs and sperm) to the next year class. It is assumed for the purposes of this discussion that there is only 1 year class produced each year. For this reason the first compartment comprises a single age class and the other two comprise several age classes. The total numbers of individuals in the population will be a function of the carrying capacity of the ecosystem, which will determine the population's equilibrium density (Krebs 1978). If a population exists at its carrying capacity then a balance will exist among the 3 compartments, with relatively stable recruitment and mortality rates in each compartment (Shuter 1990).

In general, mortality and recruitment in fish populations act in a density-dependent manner. That is as abundance increases mortality increases and recruitment

decreases and as abundance decreases mortality decreases, and recruitment increases. This ability of a population to respond in a density dependent fashion allows a population to be buffered, within a range, to changes in the environment such as changes in the availability of food, predation rates, mortality from disease, alterations in habitat etc. The actual response of a population to changes in the environment can be acheived by altering one or more of the three processes which govern population dynamics, reproduction, mortality or growth (Shuter 1990). Reproduction adds individuals, mortality removes individuals and growth is the process which allows new individuals to reproduce. Growth, condition and fecundity are all linked (Munkittrick and Dixon 1989), so that changes in growth can alter the reproductive potential of an animal. These processes ultimately control the rate at which individuals move from one life stage to the next (Shuter 1990).

Some of the potential contaminant effects which may alter a fish population include decreased survival of adults and/or sub-adults, reproductive dysfunction in adults, decreased viability of gametes, increased embryo/larval mortality, and decreases in growth. There are several implications for populations associated with each type of contaminant-caused effect. These are discussed below.

The death of reproducing adults or juveniles and sub-adults, especially females, will result in an immediate decrease in the reproductive reserve, defined by Shuter (1990) as the innate capacity of a typical population member to survive and reproduce. The death of individuals will reduce the reproductive potential of the population (i.e. the total number of eggs spawned in a year) and affecting the potential number of offspring for the next year class. Depending on the severity of the mortality involved, the population may or may not have the ability to recover through density-dependent increases in survival of offspring and recruitment of individuals into the reproducing population. A chronic mortality of adults and/or sub-adults would result in a reduced abundance of adult fish and hence a reduction in the potential future population (Munkittrick and Dixon 1989),

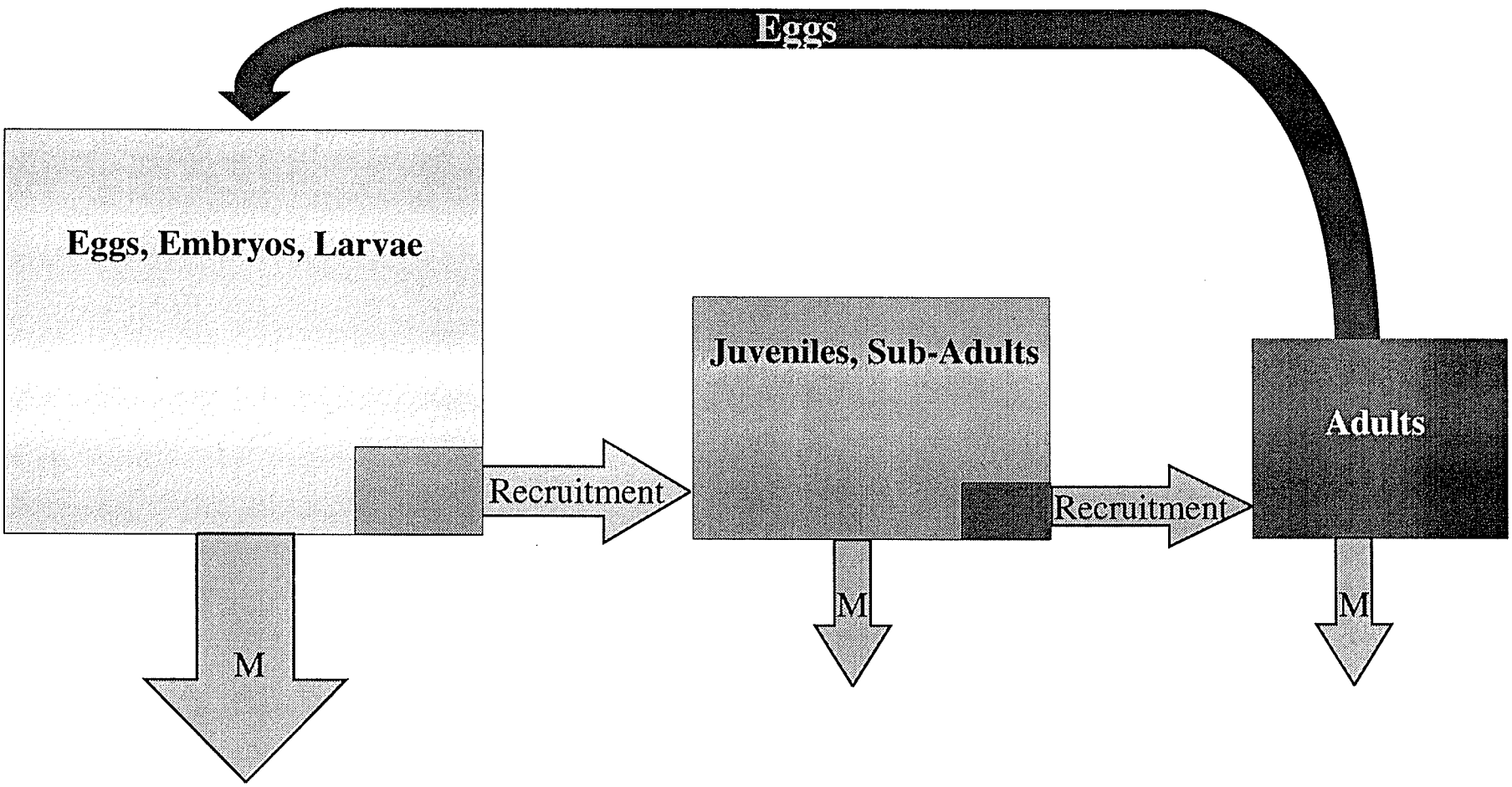


Figure 50. Diagrammatic representation of three compartment model of a fish population.

although initially there may be some compensation in the form of increased growth of remaining individuals and the resulting increases in fecundity (Shuter 1990).

Effects on reproductive function in adult fish would also cause a similar decrease in the numbers of eggs (offspring) for the next year class. For example, in this field study both toxaphene- and furan-treated female lake trout failed to spawn during at least one spawning period. Other effects of contaminants on reproductive function in adults would include effects such as toxicant-induced decreases in fecundity or delays in age of maturation. Such effects would also decrease the reproductive potential of the population. However, these types of effects may be temporary since the individual is still alive and may still contribute in the future.

Decreases in the viability of gametes, either male or female, could result in decreased abundance of young fish. The loss of viable gametes could directly reduce the strength of an emerging year class. Similarly, decreases in year class strength would also occur when embryos and/or larval fish die from contaminants. The degree of any effect will depend on the proportion of mortality caused by contaminants. Mortality rates greater than "normal" would significantly impact on future year class strength, altering the overall structure of the population.

Decreases in growth, although not a direct threat to the population, may also alter the structure of a population. Reduced growth may cause delayed onset of sexual maturity in sub-adults, since for many fish sexual maturity is a function of size (Weatherly and Gill 1987). In reproducing fish, decreased growth will cause a decrease in an individual's reproductive potential since fecundity is related to size (Bagenal 1978). Thus an individual's fecundity would be less than expected if growth is reduced.

Assessment of Effects

The degree of risk that a population faced when exposed to contaminants would be dependent on a number of factors including the number of different effects caused by the contaminant, the magnitude of each effect and the ecology of the species. The ecology of the species being assessed is important because it will be a determining factor in how large an effect is necessary to cause change in the population. For example comparing lake trout and white sucker, lake trout fecundity is much lower and maturity occurs at a larger size than in white sucker (Scott and Crossman 1973). As well, because they are top predators, lake trout are less abundant (in a given system) than prey such as white sucker. Thus similar reductions in adult numbers, fecundity, or survival of offspring would probably be more detrimental in lake trout populations.

Toxaphene

Treatment of lake trout with toxaphene caused decreased survival of adults and was the suspected cause of a failure to spawn in the first year following treatment with 7.0 µg/g toxaphene. Although the effects on lake trout were mainly in the first year after treatment, in a real situation where a contaminant is continually being taken up by the fish it could reasonably be expected for effects to continue as long as the concentrations of toxaphene were high. White suckers treated with toxaphene also had decreased survival of adults and although the remaining fish spawned, gamete viability was reduced in both males and females, as was survival of embryos and sac-fry from treated females, resulting in a reduced number of viable offspring. Since viability of eggs and sperm were assessed in separate crosses, one can only speculate that the effects would have been additive, resulting in even lower fertilization and reproductive success in pairings where both male and female were treated. Given these results, the combination of decreased success in

reproduction and decreasing numbers of adults would be cause for concern, at the very least the structure of the populations would be changed.

Chlordane

Treatment of lake trout with chlordane caused decreased survival of adults, a slight decrease in growth and a suspected decrease in reproductive success following treatment with 7.0 μg/g chlordane. White suckers treated with chlordane also had decreased survival of adults and although the remaining fish spawned, gamete viability was reduced in both males and females, as was survival of embryos and sac-fry from treated females, resulting in a reduced number of viable offspring. As with the results from toxaphene, one must assume that effects on egg and sperm viability would have been additive, resulting in even lower fertilization and reproductive success in pairings where both male and female were treated. These effects would be cause for concern since they have the potential to alter the structure of each species population.

P_5CDF

Effects of P_5CDF in lake trout were the most likely to cause irreparable damage to a population. The interruption of normal oogenesis in females, at a time when concentrations had already declined, coupled with the decreased survival of adults would likely lead to the demise or very severe reduction of the population. The delay in the disruption of oogenesis is of particular concern since there is an almost total lack of information for most contaminants on effects through multiple reproductive cycles. The effects of P_5CDF in white sucker were not as dramatic as in lake trout. Effects included decreased growth and reductions in reproductive success of both males and females. These effects would likely still result in an altered population.

The induction of MFO enzymes was coincident with the effects in both lake trout and white suckers. Although at this time a direct cause-effect linkage cannot be made, the coincident nature of induction with decreases in adult survival and reproduction advances the case that biochemical indicators, apart from indicating altered physiology, may be early indicators of effects at higher levels of biological organization.

Limitations of the Field Study

Although this attempt may have lacked some refinements, future studies can utilize the knowledge gained to help design more robust and comprehensive experiments. There were several limitations in the design used in the field experiment. The simultaneous testing of three contaminants (as mandated by the funding) only allowed a single concentration for each of the contaminants. Thus, although it can be stated that there were effects, it is not possible to determine threshold concentrations are for the various responses. Nor can it be determined from the data how much higher or lower concentrations would have to be in order to cause or not cause a response. A dose response type experiment in which treatments were made at low, medium and high concentrations for a single contaminant would be the best design.

A second limitation was the numbers of fish in each treatment group. Although statistically significant effects were found in some of the measures, an increase in the sampling size would improve the power of statistics to detect differences and in some cases would allow more certain conclusions. The original design was a conservative approach because good estimates of population sizes for the two species were not available and it was uncertain what effect the additional handling required for treatment would have on survival. For future studies of this nature I would recommend treatment groups of at least 75 individuals.

The third possible limitation was the method of exposure. As mentioned previously, the exposure of fish to contaminants by intraperitoneal injections is an unnatural exposure because the contaminants do not go through the "normal" pathway of uptake and distribution through the digestive and circulatory systems. The most natural method of exposure would be a continual feeding of contaminated food. However this would not be possible in a natural ecosystem except as a whole lake contaminants study. Another alternative to IP injections is a single oral dose as has been used by Sijm et al. (1990) and Brown et al. (1992) in laboratory experiments. Although allowing "normal" uptake and distribution, the concentration necessary to acheive reasonable body burdens at equilibrium may require lethal concentrations. A variant of this method would be a series of oral doses.

Future Directions

The completion of the field study for this thesis demonstrated that it is possible to treat fish from a natural population with contaminants and monitor responses at the population and biochemical levels of organization and to determine depuration rates of contaminants. The next experiment of this type should be a dose-response with three of four doses of a single contaminant. The focus of such an experiment should be on the linking of responses, such as changes in biochemical measures, (for example endocrine system measures, EROD, vitamin stores) with alterations in measures of preformance such as growth, survival and reproductive success. Survival and reproductive success measures should include the survival of offspring from contaminated parents and the ability of these offspring to reproduce successfully. As was shown with the P_5CDF-treated lake trout in thus field study it is also important to monitor the reproductive success of treated adults for several reproductive cycles. Further research is required in both the laboratory and in field settings on effects of contaminants on F1 generations.

Summary

The approach taken in conducting the field experiment was unique for its use of natural populations and the duration of the study. From this study it can be said that effects of environmentally meaningful concentrations of toxaphene, chlordane and 2,3,4,7,8-pentachlorodibenzofuran, although sometimes subtle, do occur at the higher levels of biological organization, i.e. population and that it is possible to study and characterize the responses of populations to such contaminants. The depuration of these contaminants was shown to be slower under natural conditions than under laboratory conditions, indicating that caution should be used when extrapolating laboratory results to real world situations.

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Appendix 1: Summarized Mark-Recapture Data and Jolly-Seber Death-Only Model output for Treated and Control Lake Trout and White Sucker.

The following tables are summaries of the mark-recapture data from the control and contaminant treated groups of lake trout and white suckers. Notation for variables is that of Jolly (1965). Tables also include input and output data from a Jolly-Seber Death-Only model.

Table A1.1 (A) Summarized mark-recapture data for corn-oil treated lake trout, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

A

Date	Newly Marked (s_i)	Fish Caught (n_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in s1988	f1988	f1989	f1990	f1991	Total (m_i)	Z
s1988	30	30	0	0	0	0	0	0	0	
f1988	8	8	0	8	0	0	0	0	8	16
f1989	13	15	2	10	5	0	0	0	15	7
f1990	13	13	0	4	1	8	0	0	13	3
f1991	10	10	0	0	0	1	9	0	10	4
f1992	12	12	0	2	0	0	2	8	12	
Total (R_i)				24	6	9	11	8		

B

DATE	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
s1988	1	30	0	0	30	24	0	0
f1988	2	8	8	0	8	6	16	16
f1989	3	15	15	2	13	9	7	7
f1990	4	13	13	0	13	11	3	3
f1991	5	10	10	0	10	8	4	4
f1992	6	12	12	0	12	0	0	0

C

Date	i	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
s1988	1	0.03	0	30	0	0.95	0.17
f1988	2	1	28.57	28.57	5.08	0.87	0.19
f1989	3	1	24.8	24.8	3.46	0.72	0.13
f1990	4	1	16.5	16.5	2.87	0.9	0.12
f1991	5	1	14.89	14.89	3.05	0	0
f1992	6	0	0	0	0	0	0

Table A1.2 (A) Summarized mark-recapture data for chlordane treated lake trout, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

A

Date	Newly Marked (s_i)	Fish Caught (n_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in s1988	f1988	f1989	f1990	f1991	Total (m_i)	Z
s1988	33	33	0	0	0	0	0	0	0	
f1988	5	7	2	7	0	0	0	0	7	13
f1989	9	12	3	8	4	0	0	0	12	5
f1990	7	10	3	4	0	6	0	0	10	1
f1991	1	3	2	1	0	0	2	0	3	2
f1992	0	2	2	0	0	0	2	0	2	
Total (R_i)				20	4	6	4	0		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
s1988	1	33	0	0	33	20	0	0
f1988	2	7	7	2	5	4	13	13
f1989	3	12	12	3	9	6	5	5
f1990	4	10	10	3	7	4	1	1
f1991	5	3	3	2	1	0	2	2
f1992	6	2	2	2	0	0	0	0

C

Date	i	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
s1988	1	0.03	0	33	0	0.68	0.15
f1988	2	1	22.6	22.6	4.82	0.93	0.23
f1989	3	1	19.14	19.14	3.8	0.72	0.18
f1990	4	1	11.6	11.6	3	0.81	
f1991	5	1	7	7			
f1992	6	0	0	0	0	0	0

Table A1.3 (A) Summarized mark-recapture data for P_5CDF treated lake trout, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

A

Date	Newly Marked (s_i)	Fish Caught (n_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1989	1990	1991	1992	Total (m_i)	Z
1989	23	24	1	0	0	0	0	0	
1990	5	9	4	10	0	0	0	10	4
1991	3	6	3	4	2	0	0	6	1
1992	0	4	4	0	1	3	0	4	
Total (R_i)				14	3	3	0		

B

DATE	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1989	*i*	24	0	1	23	14	0	-1
1990	2	9	10	4	5	3	4	4
1991	3	6	6	3	3	3	1	1
1992	4	4	4	4	0	0	0	0

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1989	1	0.04	0	22.4	0	0.7	0.17
1990	2	1.1	16	15	3.71	0.64	0.22
1991	3	1	7	7	2.19	0	0
1992	4	0	0	0	0	0	0

Table A1.4 (A) Summarized mark-recapture data for toxaphene treated lake trout (7 µg/g), (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

A

Date	Newly Marked (s_i)	Fish Caught (n_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in s1988	f1988	f1989	f1990	f1991	Total (m_i)	Z
s1988	30	30	0	0	0	0	0	0	0	
f1988	1	1	0	1	0	0	0	0	1	13
f1989	5	8	3	7	1	0	0	0	8	6
f1990	4	6	2	4	0	2	0	0	6	4
f1991	2	5	3	1	0	2	2	0	5	1
f1992	1	3	2	1	0	0	0	2	3	
Total (R_i)				14	1	4	2	2		

B

DATE	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
S1988	1	30	0	0	30	14	0	0
F1988	2	1	1	0	1	1	13	13
F1989	3	8	8	3	5	4	6	6
F1990	4	6	6	2	4	2	4	4
F1991	5	5	5	3	2	2	1	1
F1992	6	3	3	2	1	0	0	0

C

Date	i	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
S1988	1	0.03	0	30	0	0.47	0.09
F1988	2	1	14	14	2.73	1	0.13
F1989	3	1	15.2	15.2	3.45	1	0.38
F1990	4	1	12.67	12.67	5.01	0.56	0.27
F1991	5	1	6	6	2.19	0	0
F1992	6	0	0	0	0	0	0

Table A1.5 (A) Summarized mark-recapture data for toxaphene treated lake trout (3.5 µg/g), (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

A

Date	Newly Marked (s_i)	Fish Caught (n_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1989	1990	1991	1992	Total (m_i)	Z
1989	20	20	0	0	0	0	0	0	
1990	11	13	2	13	0	0	0	13	7
1991	12	14	2	4	10	0	0	14	5
1992	9	14	5	3	2	9	0	14	
Total (R_i)				20	12	9	0		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1989	*i*	20	0	0	20	20	0	0
1990	2	13	13	2	11	12	7	7
1991	3	14	14	2	12	9	5	5
1992	4	14	14	5	9	0	0	0

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1989	1	0.05	0	20	0	0.97	0.17
1990	2	1	19.46	19.46	3.71	1	0.22
1991	3	1	20.5	20.5	1.68	0	0
1992	4	0	0	0	0	0	0

Table A1.6 (A) Summarized mark-recapture data for corn oil treated white sucker, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

Time	Newly Marked (S_i)	Fish Caught (N_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1988	1989	1990	1991	1992	Total (m_i)	Z
1988	69	69	0	0	0	0	0	0	0	
1989	38	39	1	39	0	0	0	0	39	13
1990	42	43	1	10	33	0	0	0	43	4
1991	32	32	0	3	0	29	0	0	32	6
1992	16	18	2	0	1	5	12	0	18	2
1993	12	12	0	0	0	0	2	10	12	
Total (Ri)				52	34	34	14	10		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1988	1	69	0	0	69	52	0	0
1989	2	39	39	1	38	34	13	13
1990	3	43	43	1	42	34	4	4
1991	4	32	32	0	32	14	6	6
1992	5	18	18	2	16	10	2	2
1993	6	12	12	0	12	0	0	0

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1988	1	0.01	0	69	0	0.78	0.05
1989	2	1	53.49	53.49	3.79	0.91	0.05
1990	3	1	47.91	47.91	3.99	0.96	0.11
1991	4	1	45.2	45.2	6.29	0.47	0.1
1992	5	1	21.09	21.09	4.11	0	0
1993	6	0	0	0	0	0	0

Table A1.7 (A) Summarized mark-recapture data for chlordane treated white sucker, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

Time	Newly Marked (S_i)	Fish Caught (N_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1988	1989	1990	1991	1992	Total (m_i)	Z
1988	68	68	0	0	0	0	0	0	0	
1989	29	37	8	37	0	0	0	0	37	8
1990	27	32	5	6	26	0	0	0	32	4
1991	22	25	3	1	1	23	0	0	25	5
1992	13	16	3	0	1	3	12	0	16	1
1993	8	8	0	1	0	0	0	7	8	
Total (Ri)				45	28	26	12	7		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1988	1	68	0	0	68	45	0	0
1989	2	32	32	8	24	26	13	13
1990	3	35	35	5	30	27	4	4
1991	4	26	26	3	23	14	5	5
1992	5	18	18	3	15	7	1	1
1993	6	8	8	0	8	0	0	0

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1988	1	0.01	0	68	0	0.65	0.06
1989	2	1	44.04	44.04	3.76	1	0.08
1990	3	1	39.43	39.43	4.14	0.99	0.08
1991	4	1	34	34	4.89	0.65	0.11
1992	5	1	20	20	4.09	0	0
1993	6	0	0	0	0	0	0

Table A1.8 (A) Summarized mark-recapture data for P_5CDF treated white sucker, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

Time	Newly Marked (S_i)	Fish Caught (N_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1989	1990	1991	1992	1993	Total (m_i)	Z
1989	35	35	0	0	0	0	0	0	0	
1990	23	29	6	29	0	0	0	0	29	3
1991	16	20	4	2	18	0	0	0	20	1
1992	6	10	4	1	0	9	0	0	10	0
1993	3	3	0	0	0	0	3	0	3	
Total (Ri)				32	18	9	3	0		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1989	1	35	0	0	35	32	0	0
1990	2	29	29	6	23	19	3	3
1991	3	20	20	4	16	9	2	2
1992	4	10	10	4	6	3	1	1
1993	5	4	4	0	4	0	0	1

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1989	1	0.03	0	35	0	0.93	0.05
1990	2	1	32.6	32.6	1.76	0.88	0.1
1991	3	1	23.4	23.4	3.31	0.61	0.15
1992	4	1	11.75	11.75	3.17	0.65	0.11
1993	5	1	20	20	4.09	0	0

Table A1.9 (A) Summarized mark-recapture data for toxaphene treated white sucker, (B) Jolly-Seber Death-Only model inputs and (C) Jolly-Seber Death-Only Model outputs.

Time	Newly Marked (S_i)	Fish Caught (N_i)	Fish Sacrificed (l_i)	Recaptures of Fish marked in 1988	1989	1990	1991	1992	Total (m_i)	Z
1988	74	74	0	0	0	0	0	0	0	
1989	35	40	5	40	0	0	0	0	40	12
1990	29	34	5	10	24	0	0	0	34	2
1991	19	23	4	1	0	22	0	0	23	1
1992	7	11	4	1	0	0	10	0	11	0
1993	2	2	0	0	0	0	0	2	2	
Total (Ri)				52	24	22	10	2		

B

Date	i	n_i	m_i	l_i	s_i	R_i	Z_i	ZP_i
1988	1	74	0	0	74	52	0	0
1989	2	40	40	5	35	24	12	12
1990	3	34	34	5	29	22	2	2
1991	4	23	23	4	19	10	1	1
1992	5	11	11	4	7	2	0	0
1993	6	2	2	0	2	0	0	0

C

Date	*i*	A_i	M_i	N_i	Var N_i	Φ_i	Var Φ_i
1988	1	0.01	0	74	0	0.77	0.07
1989	2	1	57.28	57.28	4.98	0.7	0.08
1990	3	1	36.61	36.61	4.4	0.79	0.09
1991	4	1	24.82	24.82	4.28	0.53	0.11
1992	5	1	11	11	3.06	0	0
1993	6	0	0	0	0	0	0

Appendix 2: Residue Concentrations: Measured and Growth Corrected Yearly Mean Values for Lake Trout and White Sucker

This appendix contains tables of mean measured residue concentrations and mean growth corrected residue concentrations. Data for lake trout are contained in Table A2.1 and those for white sucker in Table A2.2.

Concentrations reported are yearly means ± standard errors, for both measured and growth corrected values. Each measured value was individually growth corrected by multiplying it by a correction factor. The correction factor was calculated as the ratio of w_r/w_i, where w_r was the weight at the time of sacrifice and w_i was the weight at the time of injection. Mean correction factors (± SE) are also reported for each year to give an idea of the magnitude of change. The number of days is the average number of days exposed.

Table A2.1 Means of measured and growth corrected contaminant concentrations in lake trout treated with single IP injections of toxaphene, chlordane or P_5CDF. Day 0 values indicate treatment level. Concentrations reported as ng/g for toxaphene and chlordane and as pg/g for P_5CDF.

Contaminant	Day	n	Measured Concentration	Growth Corrected Concentration	Mean Correction factor
Toxaphene	0		7000	7000	
(High)	517	3	1342 ± 100	1438 ± 132	1.08 ± 0.10
	877	2	486 ± 242	785 ± 445	1.72 ± 0.21
	1245	3	210 ± 86	263 ± 167	1.29 ± 0.05
	1607	2	188 ± 32	223 ± 8	1.22 ± 0.18
Toxaphene	0		3500	3500	
(Low)	367	2	922 ± 3	956 ± 8	1.04 ± 0.01
	723	1	635 ±	733 ±	1.16 ±
	1090	5	191 ± 100	280 ± 334	1.38 ± 0.08
Chlordane	0		7000	7000	
	143	3	2558 ± 612	2953 ± 671	1.22 ± 0.03
	519	3	1406 ± 35	1706 ± 138	1.21 ± 0.06
	881	3	1281 ± 101	1347 ± 167	1.05 ± 0.04
	1243	3	691 ± 384	1089 ± 678	1.75 ± 0.55
	1607	1	463 ±	639 ±	1.38 ±
P_5CDF	0		1000	1000	
	148	1	734 ±	780 ±	1.06 ±
	364	3	688 ± 119	715 ± 38	1.04 ± 0.02
	738	2	562 ± 63	698 ± 125	1.24 ± 0.02
	1131	5	469 ± 34	688 ± 32	1.48 ± 0.17

Table A2.2 Means of measured and growth corrected contaminant concentrations in white sucker treated with single IP injections of toxaphene, chlordane or P_5CDF. Day 0 values indicate treatment level. Concentrations reported as ng/g for toxaphene and chlordane and as pg/g for P_5CDF.

Contaminant	Day	n	Measured Concentration	Growth Corrected Concentration	Mean Correction factor
Toxaphene	0		7000	7000	
	120	1	1247 ± 0	1648 ±	1.32 0.00
	364	2	1609 ± 885	1960 ± 1518	1.22 ± 0.01
	829	3	1306 ± 463	1798 ± 944	1.42 ± 0.08
	1103	2	1195 ± 507	1441 ± 822	1.22 ± 0.03
	1458	2	812 ± 552	2299 ± 179	1.63 ± 0.63
Chlordane	0		7000	7000	
	142	2	4246 ± 2646	4225 ± 4745	0.98 ± 0.02
	373	4	2601 ± 495	2987 ± 1337	1.25 ± 0.05
	736	2	2447 ± 786	2953 ± 2399	1.71 ± 0.17
	1086	1	1292 ± 484	1468 ± 1011	1.14 ± 0.08
	1476	2	393 ± 137	976 ± 566	2.60 ± 0.49
P_5CDF	0		1000	1000	
	356	1	245 ±	322 ±	1.31 ±
	652	3	448 ± 300	511 ± 339	1.15 ± 0.02
	1106	3	287 ± 71	386 ± 68	1.32 ± 0.13

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