EFFECTS OF ENVIRONMENTAL AND PHYSIOLOGICAL VARIABLES ON THE UPTAKE OF HYDROPHOBIC CONTAMINANTS BY THE GILLS OF RAINBOW TROUT, Salmo gairdneri (Richardson)

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To the Graduate Council:

I am submitting herewith a dissertation written by Marsha Carolyn Black entitled "Effects of Environmental and Physiological Variables on the Uptake of Hydrophobic Contaminants by the Gills of Rainbow Trout, Salmo gairdneri (Richardson)." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology.

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ABSTRACT

The uptake of contaminants by diffusion across fish gills may be altered by changes in the contaminant's physicochemical form or changes in fish respiration. This study investigated the effects of changes in contaminant uptake due to sorption to humic acid and changes in fish respiration, induced by altering respiratory demand via an acute change in temperature and by altering the diffusional capacity of the gill membrane by exposure to chlorine. In each experimental design the uptake of selected polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl congeners (PCBs) by the gills of rainbow trout, Salmo gairdneri (Richardson) were measured simultaneously with measurements of fish respiratory functions using a fish metabolic chamber.

Binding to humic acid (HA) reduced the bioavailability of benzo[a]pyrene (BaP) and 2,2',5,5'-tetrachlorobiphenyl (TCB). A 1:1 correlation was found between reductions in the percentage of BaP or TCB that was freely dissolved (not bound to HA) and reductions in uptake of the contaminant by trout gills. These results imply that only the freely dissolved BaP or TCB was available for uptake.

Eight to 24-h exposure to chlorine resulted in damage to gill lamellae. Reductions in the uptake efficiencies of oxygen and three PCB congeners in chlorine-exposed trout were hypothesized to result from the chlorine-induced damage to the gill membrane. However, increases in ventilatory functions compensated for the lowered uptake efficiencies and resulted in no net change in either oxygen or PCB uptake.

An acute decrease in temperature resulted in reduced respiration and uptake of oxygen and BaP, TCB, and naphthalene (NAP) by trout. Reductions in uptake efficiencies were hypothesized to result from temperature-induced reductions in the permeability of the gill membrane. Reductions in ventilatory functions and oxygen consumption were attributed to a reduced metabolic demand at the lower temperature.

These results indicate that physiological and environmental factors can have a direct modulating effect on the diffusion of contaminants across the gill membrane. The quantitative relationships between sorption and bioavailability and fish respiration and contaminant uptake found in these experiments can be used to improve models of contaminant uptake in the natural environment.

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CHAPTER I

GENERAL INTRODUCTION

Aquatic organisms exposed to xenobiotics in the natural environment accumulate these substances primarily by two routes, via the food chain and through direct uptake from respiratory water. For hydrophobic chemicals, including many of the higher molecular weight polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) having octanol-water partition coefficients (K_{ow}) ranging from 10^3 to 10^6, uptake from water by diffusion across respiratory surfaces is considered to be the most important exposure route (Hunn and Allen, 1974; Norstrom et al., 1976; Neely, 1979; Bruggeman et al., 1981). These compounds will preferentially partition in lipids and will readily diffuse across the gill membranes of fish.

Predictive models of accumulation of hydrophobic toxicants by fish have related laboratory determinations of steady-state bioaccumulation factors (BCF) to measurements of compound hydrophobicity, such as the compound's K_{ow} (Neely et al., 1974; Metcalf, 1977; Veith et al., 1979; Kenaga and Goring, 1980; Bruggeman et al., 1984) and water solubility (Chiou et al., 1977; Metcalf, 1977; Kenaga and Goring, 1980). These relationships allow for the prediction of toxicant accumulation based on an easily measured chemical property of the toxicant, and have been shown to be useful for compounds within a wide range of hydrophobicities.

Bioaccumulation is the net effect of the offsetting processes of contaminant uptake and elimination. Additional physiological

processes are also involved that may alter either or both processes. To account for some of these effects on uptake or elimination bioaccumulation models have been adjusted to include factors such as fish growth (Nimii and Cho, 1981; Thomann, 1981), compound metabolism (Southworth et al., 1980), and accumulation through other routes, including cutaneous accumulation (Metcalf, 1977) and uptake via the food chain (Roberts et al., 1979; Bruggeman et al, 1981; Thomann, 1981). These additions to the steady-state BCF approach provide additional specificity for modelled predictions of contaminant accumulation, and enable models to be tailored to include additional physiological properties of the exposed biota and physicochemical properties of the contaminant.

Availability of contaminants for uptake by diffusion across gill membranes can be affected by the physicochemical form of the diffusing contaminant. Binding of contaminants to dissolved sorbents will alter the physicochemical nature of the contaminant and may inhibit its diffusion across the gill membrane due to the increased size of the contaminant-sorbent complex and/or the hydrophilic properties of the sorbent. By binding to sorbents, diffusion of the contaminant would be predicted to be altered in the absence of physiological changes in respiration or reductions in gill diffusional capacity.

Fish ventilatory functions control the amount of contaminant-laden water that comes in contact with the gill membrane. Gill ventilation will vary to meet the fish's demand for oxygen and will also change in response to irritation of the gills by chemical irritants. For both metabolic and chemically induced changes in fish

respiration, the resulting alterations in contact time and effective exposure of ventilatory water with the gill membrane may affect the diffusion of water-borne contaminants.

In addition to affecting fish ventilation, chemical irritants may also alter the structure and integrity of the gill membrane. Damage to the gill membrane may result in reductions in the functional surface area of the gill and an increase in the gill membrane diffusion distance. Each of these effects would be expected to cause a reduced capacity for diffusion of contaminants and oxygen by damaged gills.

The primary objective of this study was to quantify the effect of changes in compound bioavailability and changes in fish respiration on the diffusion of selected hydrophobic contaminants across the gills of rainbow trout, Salmo gairdneri (Richardson). The compounds used in the study include two PAHs; benzo[a]pyrene (BaP) and naphthalene (NAP), and three PCB congeners; 2,2'4,4'-tetrachlorobiphenyl (22'44'-TCB), 2,2',5,5'-tetrachlorobiphenyl (22'55'-TCB), and 2,2',3,3',5,5'-hexachlorobiphenyl (22'33'55'-HCB). Using a fish metabolic chamber (McKim and Goeden, 1982), simultaneous measurements of fish ventilation volume, ventilation rate, and the uptake of oxygen and compound were made under three different experimental designs.

The environmental and physiological variables manipulated in these experiments were chosen to include three methods of altering contaminant uptake by fish: alteration of fish ventilatory functions through changes in metabolic demand, reductions in the diffusional capacity of the respiratory surface by exposure to a gill tissue

irritant, and changes in the physicochemical form of the toxicant through sorption to a dissolved sorbent. These changes in respiratory functions and compound bioavailability are predicted to affect vital components of the process by which fish accumulate water-borne toxicants via the gill, and may result in changes in the efficiency of contaminant uptake by trout.

In the first series of experiments (Chapter II) the bioavailability of hydrophobic contaminants to rainbow trout in the presence of humic acid (HA) (Aldrich Chemical Corporation, Milwaukee, WI), a commercially-available source of dissolved organic macromolecules (DOM), was investigated (Black and McCarthy, 1988). Benzo[a]pyrene and 22'55'-TCB, two compounds having different affinities for binding to DOM, were used in this study. By directly measuring gill uptake of these contaminants using the fish metabolic chamber, the effect of binding to dissolved sorbents on contaminant uptake by fish was determined. Using this approach reductions in toxicant uptake can be compared with reductions in the freely dissolved contaminant measured in separate binding experiments. Comparisons were made to test the hypothesis that only the freely dissolved contaminant can diffuse across respiratory membranes and be accumulated by trout gills. Validation of this hypothesis would make it possible to develop relationships for estimating the effect of sorbents such as HA on contaminant uptake by fish based on determinations of the affinity of the contaminant for binding the dissolved sorbent (K_D).

Increased accumulation of DDT, organic mercuric chloride, and BaP by fish has been measured at elevated temperatures (Murphy and Murphy, 1971; MacLeod and Pessah, 1973; Reinert et al., 1974; Jimenez et al., 1987). Although fish ventilation was not measured in any of these experiments, increased contaminant accumulation was attributed to increases in fish ventilatory functions, thought to result from an increased metabolic demand at higher temperatures. In experiments described in Chapters III and IV, the influence of changes in trout respiratory functions on the uptake of toxicants by fish gills was examined. Trout respiration was altered by two approaches, using an invasive and a noninvasive method of altering trout respiration. Chapter III describes experiments in which trout were exposed to PCB congeners concomitantly with exposure to chlorine, a gill irritant that is a common additive to industrial process wastewaters and municipal sewage discharges. Changes in the gill diffusional capacity were also monitored through histopathological analysis of gill tissue. In research described in Chapter IV, trout experiencing an acute decrease in temperature were simultaneously exposed to BaP, 22'55'-TCB, or NAP. Each series of experiments was designed to cause specific changes in trout respiration, so that the effect of both increases and decreases in respiration on contaminant uptake could be investigated.

In all three experimental approaches, the underlying general hypothesis was that the uptake of hydrophobic toxicants by fish gills can be affected by environmental and physiological processes that alter either the physicochemical properties of the diffusing molecule,

the diffusional capacity of the respiratory surface, or fish respiration, the physiological process which exposes the contaminant molecules to the respiratory surface. These experiments have been designed to identify and quantitate predicted changes in the contaminant's physical form or the respiratory physiology of the exposed organism and to relate them to changes in contaminant uptake. The general relationships found between changes in respiration and physicochemical form of the contaminant and changes in contaminant uptake have potential use in fine-tuning predictive models of contaminant uptake to account for similar physiological and environmental changes that occur during contaminant exposures in the natural environment.

CHAPTER II

INFLUENCE OF HUMIC ACID ON THE UPTAKE OF BENZO[A]PYRENE AND 2,2',5,5'-TETRACHLOROBIPHENYL BY RAINBOW TROUT, *Salmo gairdneri* (Richardson)

Introduction

Aquatic organisms are exposed to numerous hydrophobic organic contaminants (HOCs) in their natural environment as a result of pollution inputs from industrial and municipal sources. Although some HOCs can be accumulated via dietary exposure, a major route of accumulation for hydrophobic compounds by fish and aquatic invertebrates is via passive diffusion across respiratory membranes (Hunn and Allen, 1974; Norstrom et al., 1976; Neely, 1979; Bruggeman et al., 1981; and McCarthy, 1983). Contaminants bound to sediments and particles cannot traverse this membrane barrier (McCarthy, 1983; Adams et al., 1985). Binding of contaminants to naturally occurring dissolved organic macromolecules (DOM) will also alter the availability of these compounds for uptake by aquatic organisms. Numerous studies have demonstrated reductions in the accumulation of HOCs, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and DDT, by fish and aquatic invertebrates in the presence of naturally occurring and commercially available DOM sources (Leversee et al., 1983; Spacie et al., 1983; Landrum et al., 1985; McCarthy and Jimenez, 1985; McCarthy et al., 1985; Landrum et al., 1987). Using steady-state and kinetic analyses, a quantitative relationship was found between reductions in PAH uptake

rates and bioaccumulation by Daphnia magna and reductions in the freely dissolved PAH in the presence of a commercially prepared humic acid (HA). Since D. magna do not readily metabolize PAHs and depuration rates were unaffected by the presence of HA, reductions in PAH accumulation were concluded to result from the reduced bioavailability of the PAH bound to humic acid (McCarthy et al., 1985).

Reductions in the bioavailability of PAHs were seen with the amphipod Pontoporeia hoyi in the presence of naturally occurring DOM from sediment interstitial waters (Landrum et al., 1987). A biological partition coefficient (K_b) was calculated based on the assumption that only the freely dissolved PAH is bioavailable. The concentration of freely dissolved PAH in the exposure water was estimated from the uptake kinetics of control exposures with no DOM added. The concentration of PAH bound to DOM was calculated by subtracting the estimated freely dissolved PAH concentration from the total PAH concentration for each exposure with DOM. The K_b was calculated as the ratio of the bound concentration per gram of DOM divided by the freely dissolved concentration. This kinetically determined biological partition coefficient (K_b) agreed very well with physicochemical determinations of the binding of the PAHs to DOM (Landrum et al., 1987).

These studies indicate a quantitative relationship between reductions in bioavailable HOC due to binding to DOM and reductions in accumulation of these HOCs by invertebrates. However, previous results from bioaccumulation studies with aquatic vertebrates, such as

fish, have been less conclusive. In a study in which bluegill sunfish (Lepomis machrochirus) were exposed to benzo[a]pyrene (BaP) with and without humic acid (20 mg C/L), humic acid reduced the accumulation of BaP by sunfish by 90%, compared to control exposures with no humic acid present. However, this study was unable to confirm that BaP bound to humic acid was not bioavailable, because estimates of the uptake rate coefficient for BaP bound to humic acid were not zero, but were approximately 10% of those estimated for dissolved BaP (McCarthy and Jimenez, 1985).

In this study one aspect of the accumulation process, uptake of contaminants by fish gills, was isolated in order to investigate the effect of humic acid on the availability of HOCs to fish. By making direct measurements of contaminant uptake efficiency (percentage uptake), potential errors due to rapid biotransformation of the compound or pharmacodynamics within the organism can be eliminated. Direct comparisons were made between the changes in uptake efficiencies and changes in the concentration of the freely dissolved contaminant due to the presence of humic acid. Using this approach the hypothesis that only the freely dissolved BaP or TCB is available for uptake by fish gills was tested. Verification of this hypothesis would justify the use of physicochemical determinations of contaminant binding to DOM to predict contaminant bioavailability in the presence of DOM in the natural environment.

Materials and Methods

Fish

Adult rainbow trout, Salmo gairdneri (Richardson), obtained from the Cross-eyed Cricket hatchery (Lenoir City, Tennessee) and Smoky Mountain Trout Farm (Hampton, Tennessee), were acclimated in large flow-through tanks at 18 °C for at least one month prior to experimentation. The mean trout weight (±STD) was 523 g (±331, n = 18). Trout were fed daily with trout chow (Purina Mills, Inc., St. Louis, MO) until 24 h prior to experimentation. Mean water chemistry parameters for the experimental dechlorinated tap water were: pH, 8.04; conductivity, 246 μmhos/cm; alkalinity, 104.1 mg $CaCO_3$/L; and hardness, 131 mg $CaCO_3$/L.

Chemicals

Benzo[a]pyrene (BaP) ([7-^{14}C]benzo[a]pyrene, 11.7 mCi/mmol) was obtained from California Bionuclear, Sun Valley, California, and 2,2',5,5'-tetrachlorobiphenyl (TCB) ([ring-UL-^{14}C]-2,2',5,5'-tetrachlorobiphenyl, 12.6 mCi/mmol) was obtained from Pathfinder Laboratories, St.Louis, Missouri.

Exposure solutions were prepared by recirculating filtered, UV sterilized, dechlorinated tap water through a 8- by 20-cm glass generator column filled with 3-mm glass beads coated with ^{14}C-BaP or ^{14}C-TCB by roto-evaporation. BaP solution concentrations ranged from 0.5 to 3 μg/L. TCB solution concentrations ranged from 0.2 to 2.5 μg/L. The generated BaP and TCB solution concentrations were below

published water solubility values for each compound; 4 μg/L for BaP (Mackay and Shiu, 1977) and 41 μg/L for TCB (Mackay et al, 1980).

Stock humic acid solutions (1 mg/L, dry weight basis) were prepared by dissolving humic acid (Aldrich Chemical Corporation, Milwaukee, Wisconsin) in distilled water followed by filtering the solution through precombusted glass fiber filters (Type A-E, nominal cutoff of 3 μm, Gelman Sciences, Inc.). The organic carbon content of the humic acid solutions was determined using a total carbon analyzer (OI Corporation, College Station, Texas). The total carbon analyzer quantifies the carbon dioxide released from persulfate oxidation of the organic carbon contained in the sample using a non-dispersive infrared detector. Humic acid concentrations are expressed as milligrams of carbon per liter of solution (mg C/L).

Stock humic acid solutions were added to 50 L of the radiolabeled BaP or TCB solution and allowed to equilibrate for 30 min (BaP) or 24 h (TCB). This solution was mixed with equal volumes of chilled, UV sterilized, filtered dechlorinated tap water, yielding an exposure solution with the desired humic acid concentration at 18 °C flowing into the metabolic chamber. Exposure water was pumped into the metabolic chamber by a peristaltic pump with flow rates adjusted to be in excess of each fish's ventilation volume. Oxygen concentrations of the inflowing water exceeded 80% saturation.

Trout were exposed to radiolabeled BaP solutions with humic acid concentrations of 0, 0.1, 0.5, 1, 3, and 5 mg C/L and to radiolabeled TCB solutions with humic acid concentrations of 0, 3.4, and 11.8 mg C/L. Different humic acid concentrations were chosen for each

compound because BaP and TCB have different affinities for binding to DOM. Average log K_p values for BaP and TCB are 6.3 and 4.8, respectively (Hassett and Milicic; McCarthy and Jimenez,1985; Landrum et al, 1987; McCarthy and Black, 1987). Humic acid concentrations were estimated to yield comparable percentages of freely dissolved compound for both contaminants (see Environmental Implications, Eq. 2-5, pp. 22-23). Each exposure lasted 3 h and contaminant uptake and respiratory functions were measured at 2 and 3 h after the beginning of the exposure. Contaminant exposures (with humic acid added) were compared with control contaminant exposures (no humic acid added) for each fish. The order of all exposures (with or without humic acid) for each fish were selected at random.

Surgical Procedures

Trout were anesthetized with MS-222 (tricaine methanesulfonate, Crescent Research Chemicals, Paradise Valley, Arizona) and placed on a fish surgical table (McKim and Syrett, 1982). Each trout was immobilized by spinal transection, and a latex membrane was sutured to the trout's mouth. The anesthetized trout was then transferred to a fish metabolic chamber (McKim and Goeden, 1982) where it was allowed to recover from the anesthesia and surgical procedures for at least 18 h.

Metabolic Chamber

The fish metabolic chamber is a modified respirometer-metabolism chamber previously described in detail (McKim and Goeden, 1982). The metabolic chamber is divided into three chambers (A, B, and C), each

separated by a latex membrane (Fig. II-1). To test for leaks between chambers A, B, and C, a dye was added to chamber B. Chambers A and C were analyzed spectrophotometrically for the presence of dye, indicating leakage between chambers. No leaks were detected into either chamber.

Exposure water containing oxygen and the ^{14}C-labeled test chemical flowed into chamber A and was pumped across the gills into chamber B by the ventilatory activity of the fish. Excess water from chambers A and B flowed out of standpipes within each chamber. Ventilation volume (milliliters of water passing over the gills per minute) was determined by measuring the volume of water that overflowed the standpipe in chamber B as a function of time. Ventilation rate (ventilatory strokes per minute) was measured by a physiograph (Narcotrace 40, Narco Biosystems, Houston, Texas), which records opercular movements as a function of time. The amount of oxygen taken up by the gills was determined by measuring the difference in the concentration of oxygen in water from chambers A and B. Oxygen concentrations were measured using a dissolved oxygen meter (YSI Model 58, Yellow Springs, Ohio). Contaminant uptake by the gills was determined by measuring the difference in the radioactivity in chambers A and B. Uptake efficiencies (percentages) were calculated by dividing the amount of contaminant or oxygen taken up by the gills by the amount in the exposure water (chamber A). Data expressed as the percentage of control were determined by dividing the measurement observed during exposures with humic acid present by that measured with no humic acid present (control exposures).

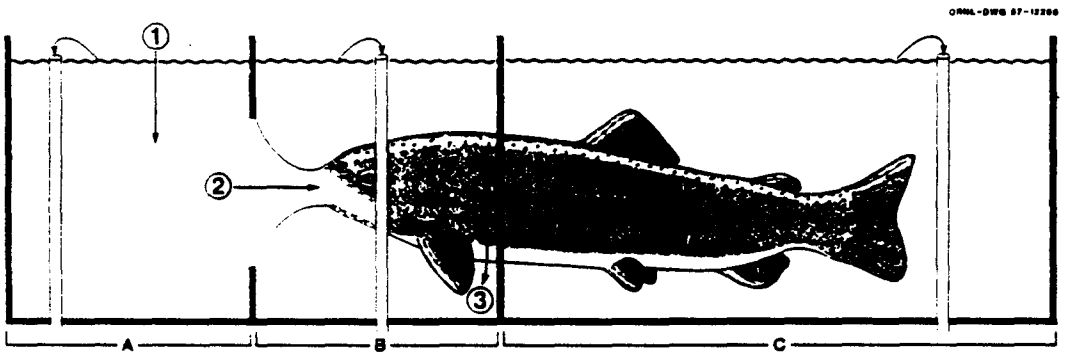

Figure II-1. The fish metabolic chamber.

(1) Exposure water with O_2 and the ^{14}C-labeled contaminant enters chamber A. (2) Water with the contaminant and O_2 is pumped over the gills by the ventilatory action of the fish. (3) Water minus the contaminant and O_2 extracted by the gills is expelled into chamber B.

Measurement of Contaminant Binding to Humic Acid

The percentage of BaP that was freely dissolved versus that bound to humic acid was determined using equilibrium dialysis (Carter and Suffet, 1982; McCarthy and Jimenez, 1985). Five milliliters of exposure water containing humic acid was placed in a dialysis bag (Spectra/Por 6, molecular weight cutoff of 1000 daltons) and equilibrated for 4 d in a ^{14}C-labeled BaP solution. The percentage of freely dissolved BaP was calculated from the analysis of radioactivity inside the dialysis bag (containing humic acid-bound BaP and freely dissolved BaP) and the solution outside the bag (freely dissolved BaP).

The percentage of TCB bound to humic acid was determined using a reverse phase separation method (Landrum et al., 1984). Five milliliters of radiolabeled exposure water with humic acid was applied to a C-18 Sep Pak cartridge (Waters Associates, Milford, MA). Tetrachlorobiphenyl bound to humic acid will not sorb to the C-18 resin and was determined from the analysis of radioactivity in the eluent. The percentage of freely dissolved TCB was calculated by subtracting the percentage bound from 100%.

Analysis of Radioactivity

The ^{14}C-radioactivity was measured using a Packard CDS 460 liquid scintillation counter with automatic quench correction. Five milliliters of water containing radiolabeled BaP or TCB were mixed with 10 ml of scintillant (ACS, Amersham Corporation). BaP and TCB concentrations ($\mu g/L$) were calculated from the specific activities of the stock solutions.

Statistical Analyses

Statistical analyses were performed using SAS Version 4.1 (Statistical Analysis Systems, Cary, NC). Specific tests are identified in the text.

Results and Discussion

Effect of Humic Acid on Respiratory Functions

Humic acid did not affect respiratory functions (ventilation volume, ventilation rate, oxygen extraction efficiency) in either the BaP or TCB exposures (Fig. II-2). Increases in humic acid concentration caused no significant changes in respiratory functions compared with measurements made during control exposures ($p < 0.05$, Duncan's Multiple Range Test). Mean values (±SEM) for respiratory functions were: oxygen extraction efficiency, 57.6 (±2.5)%; ventilation volume, 183.0 (±10.1) mL/min; and ventilation rate, 97.4 (±2.4) strokes/min.

Binding of BaP and TCB to Humic Acid

The compounds used in this study, BaP and TCB, have different affinities for binding to humic acid. The partition coefficent (K_p) for BaP measured from equilibrium binding experiments with humic acid was 3.6 (±0.5) x 10^6 (n = 9). This value is similar to previously reported values of 1.8 x 10^6 (McCarthy and Jimenez, 1985) and 2 x 10^6 (McCarthy and Black, 1987). The K_p for TCB measured in this study was 5.8 (±0.4) x 10^4 (n = 12). Another study reported a similar value (7.1 x 10^4) using a gas purging method (Hassett and Milicic, 1985).

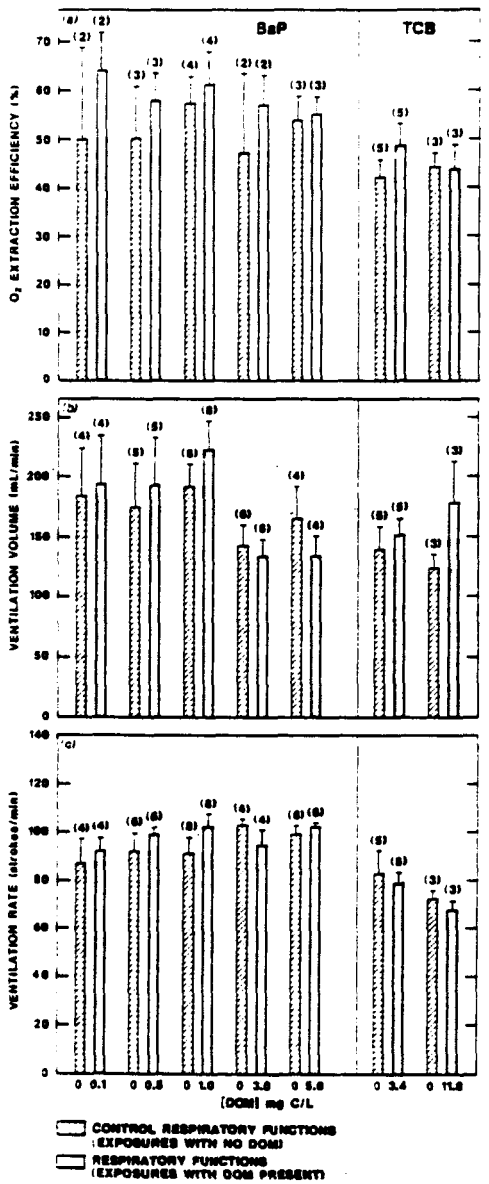

Figure II-2. Trout respiratory functions measured during exposures to BaP and TCB in the presence and absence of DOM.

Histograms represent the mean values (±SEM) for oxygen uptake efficiency (a); ventilation volume (b), and ventilation rate (c). There were no differences between control measurements with no DOM added and measurements made with DOM present ($p < 0.05$, Duncan's multiple range test.

Effect of Humic Acid on Uptake Efficiency of BaP and TCB

Binding to humic acid appeared to affect the the apparent uptake efficiency of BaP and TCB by trout gills (Table II-1). With increasing humic acid concentrations the uptake efficiencies of both BaP and TCB decreased. Because respiratory functions remained constant at all DOM concentrations, decreases in uptake must reflect the effect of sorptive interactions between DOM and the contaminant.

The magnitude of the reduction in uptake was related to the amount of BaP or TCB bound to humic acid (Table II-1), such that reductions in contaminant uptake correlated with reductions in the freely dissolved contaminant. These observations support the hypothesis that in these experiments only the freely dissolved BaP or TCB is available for uptake by trout gills. Figure II-3 shows this correlation with measurements of the percentage of freely dissolved HOC plotted against the percentage of control uptake efficiency (uptake measurements made in the presence of humic acid divided by control uptake measured with no humic acid present). The resulting regression equation was $y = 0.86x + 8.41$, where x is the percentage of control uptake and y is the percentage of freely dissolved contaminant; $r^2 = 0.91$, $n = 24$. The 1:1 relationship between these two measurements predicted by the hypothesis that only the freely dissolved contaminant is bioavailable falls within the 95% confidence limits of the regression of the data points. Thus, for all humic acid concentrations, the following relationship was observed between reductions in biological uptake and

Table II-1. Physicochemical and biological uptake measurements for BaP and TCB exposures at all DOM concentrations.

	PHYSICOCHEMICAL PARTITIONING			TROUT GILL UPTAKE	
HOC	log Kp	[DOM] mg C/L	% FREELY DISSOLVED	UPTAKE EFFICIENCY	CORRECTED UPTAKE EFFICIENCY
BaP	6.3	0	100	52 ± 1.9 (6)	52
		0.1	66 ± 3.1 (3)	38 ± 1.0 (4)	57
		0.5	37 ± 0.5 (3)	17 ± 1.4 (6)	45
		3.0	14 ± 0.8 (3)	8 ± 1.2 (4)	56
TCB	4.8	0	100	50 ± 9.8 (6)	50
		3.4	85 ± 3.1 (6)	44 ± 8.0 (6)	51
		11.8	57 ± 24 (3)	26 ± 4.5 (3)	47

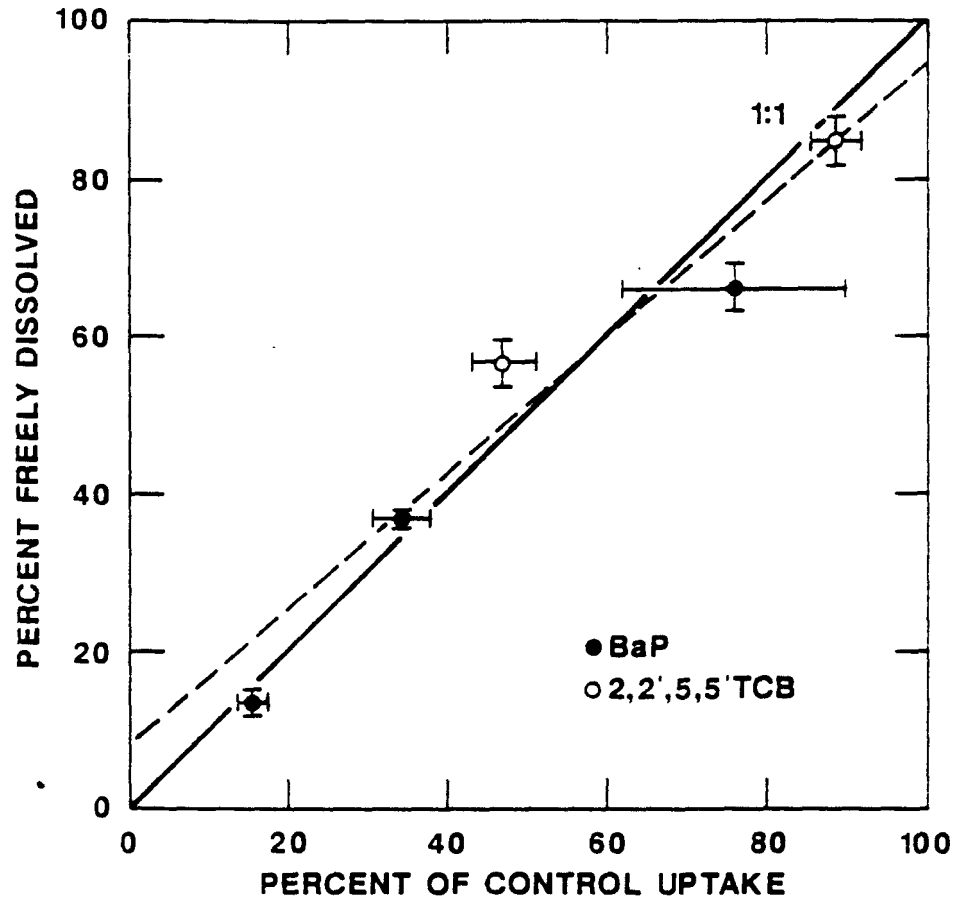

Figure II-3. The linear relationship between reductions in uptake efficiencies for BaP and TCB (percentage of control uptake) and reductions in the percentage of freely dissolved toxicant.

Percentage of control uptake is calculated as HOC uptake in the presence of DOM divided by HOC uptake with no DOM present for BaP and TCB. The 95% confidence interval of the regression of the data points (dashed line) is not significantly different from the 1:1 line (solid line) predicted by the hypothesis that only the freely dissolved BaP and TCB is available for uptake by trout gills.

the physicochemical form of the HOC due to binding to humic acid (HA) in these experiments:

$$\frac{\text{uptake efficiency (with HA)}}{\text{uptake efficiency (no HA)}} = \text{fraction freely dissolved HOC} . \qquad (1)$$

Uptake of DOM

Humic acid and HOCs bound to humic acid did not appear to diffuse across the gill membrane. Total organic carbon measurements of exposure water collected from chambers A (before contact with gills) and B (after flowing across the gills) were not statistically different at experimental humic acid concentrations, which ranged from 0.01 to 11.8 mg C/L ($p < 0.05$, Student's t- test).

Although DOM, including the commercially prepared humic acid used in these experiments, is considered to be a aggregate of molecules of varying molecular weights (ranging from less than 1000 to greater than 100,000 daltons (Thurman et al., 1982; Cole et al., 1984), binding studies using dialyzed DOM from natural and commercial sources have demonstrated that only the higher molecular weight fractions of DOM (molecular weight > 1000 daltons) will bind to HOCs, including BaP and several tetrachlorinated and hexachlorinated PCB congeners (McCarthy and Jimenez, 1985; personal communication, L. E. Roberson and J. F. McCarthy). Diffusion of DOM and contaminants bound to DOM across the gill membrane may be hindered by the large size of the DOM molecule. Furthermore, the presence of polar functional groups on DOM (Perdue, 1985; Thurman, 1985) may hinder the ability of DOM to penetrate and partition within the hydrophobic lipid bilayer of the gill membrane.

The observation that humic acid concentrations were the same before and after passing over the gills in the present study supports the hypothesis that humic acid and BaP and TCB bound to humic acid are not able to diffuse through gill membranes. The correlation between reductions in trout gill uptake efficiencies of BaP and TCB and reductions in the fraction of freely dissolved contaminant due to binding to humic acid lends further support to the hypothesis that HOCs bound to humic acid will not diffuse across gill membranes.

Environmental Implications

Based on this study and on implications from previous studies discussed in more detail in the introductory section (pp. 7-9) contaminants bound to DOM do not appear to be able to diffuse across respiratory membranes of aquatic organisms. Therefore, consideration of the hydrophobic organic contaminant's potential for binding to DOM is essential when predicting contaminant dose and toxic effects for organisms in the natural environment.

The partition coefficient (K_p) for sorption of a contaminant to DOM is calculated from measurements of concentrations of the bound and freely dissolved compound and the organic carbon concentration of the sorbent:

$$K_p = C_{dom}/(C_d \times [DOM]) \quad (2)$$

$$C_d = f_{free} \times C_T \quad (3)$$

$$C_{dom} = (1 - f_{free}) \times C_T \quad (4)$$

where C_{dom} = moles of HOC bound to DOM/mL solution, C_d = moles of freely dissolved HOC/mL solution, C_T = total HOC concentration (= C_d + C_{dom}), [DOM] = concentration of DOM in solution (g C/mL), and f_{free} = fraction of the total HOC concentration that is freely dissolved. By substitution and rearrangement the relationship between the concentration of freely dissolved HOC, the compound's affinity for binding to DOM (measured as the partition coefficient, K_p) and the concentration of DOM present in the environment can be demonstrated:

$$\text{fraction freely dissolved HOC} = 1/(1 + K_p \times [DOM]) \quad (5)$$

Because the biological uptake of HOCs is directly related to the concentration of freely dissolved HOC (Eq. 1), measurement of physicochemical parameters, such as K_p and DOM concentrations, should permit the prediction of reductions in contaminant uptake in the presence of DOM.

Because of its greater affinity for binding to DOM (a twentyfold higher K_p), a given concentration of humic acid caused greater reductions in the biological uptake of BaP than of TCB. At 3 mg C/L, uptake of BaP by trout gills decreased 85% relative to control uptake (no humic acid present), while TCB uptake was reduced by only 12% at a similar humic acid concentration (3.4 mg C/L) (Table II-1, p. 19). A similar effect was seen in comparing bioaccumulation of a series of three PAHs having log K_p values that ranged from 3 to greater than 6

by Daphnia magna in the presence of DOM (McCarthy et al., 1985). DOM concentrations of less than 1.5 mg C/L significantly reduced bioaccumulation of BaP and benzanthracene by the daphnids, while 60 mg C/L of DOM had little effect on bioaccumulation of naphthalene, the compound with the lowest affinity for binding to DOM.

In the present study, a commercially prepared source of humic acid was used to determine the effect of DOM on contaminant uptake by fish. A recent report criticized the use of commercially prepared DOM as a surrogate for naturally occurring DOM based on chemical differences between DOM from commercial and natural sources (Malcolm and MacCarthy, 1986). However, these chemical differences probably have little effect on the mechanism by which HOCs associate with DOM, which appears to involve a partitioning of the HOC within a nonpolar region of the DOM molecule (Chiou et al., 1986). Although DOM from different sources have widely different K_p's for binding HOCs (Means and Wijayaratne, 1982; Landrum et al., 1984; McCarthy and Jimenez, 1985; Chiou et al., 1986), the nature of the interaction between the HOC and DOM is hypothesized to be the same, regardless of the DOM source. This hypothesis has been verified with bioaccumulation experiments with P. hoyi (Landrum et al., 1985). A linear relationship was found between the reverse phase partition coefficients (K_{rp}) determined for a series of PAHs using DOM from different sources (including commercially prepared humic acid and DOM from sediment interstitial waters) and the biological partition coefficient (K_b) determined from bioaccumulation studies using the same DOM sources. No difference in the relationship between K_{rp} and

K_D was observed to result from the use of DOM from different sources. Therefore, the use of a commercially prepared humic acid to demonstrate the effect of DOM on contaminant uptake appears to be warranted, with the caveat that the magnitude of the dose reduction due to binding to DOM in the aquatic environment will depend on the affinity of the contaminant for binding to the DOM molecule, affected by the specific chemical composition and spatial configuration of the DOM molecule, in addition to chemical properties of the contaminant that affect binding.

Summary and Conclusions

The association of two hydrophobic contaminants (BaP and TCB) with humic acid rendered these compounds unavailable for uptake by trout gills. By using a direct measurement of gill uptake coupled with quantitation of the freely dissolved BaP or TCB in the exposure water, this study has demonstrated that only the fraction of the total BaP or TCB in the water that was freely dissolved was able to diffuse through gill membranes and be accumulated by the trout. Humic acid or solutes bound to humic acid, do not appear to be capable of diffusing through the gill. Since reductions in contaminant uptake were directly related to reductions in the freely dissolved compound, contaminant dose in the presence of DOM could be predicted from physicochemical measurements of the freely dissolved HOC, including K_D and determinations of the concentrations of bound and freely dissolved HOC.

For compounds that will bind to DOM, the fractions of the total HOC concentration that were bound and freely dissolved are determined by the affinity of these substances for binding to DOM and the concentration of DOM that is present. Greater reductions in uptake should be seen for compounds with high affinities for binding to DOM or in environments with high concentrations of DOM, although differences in the binding affinities of different sources of natural DOM must be considered. Neglecting to correct toxicant exposure concentrations to account for the truly bioavailable fraction of the HOC may result in overestimates of toxicant accumulation and of potential toxic effects in contaminated aquatic environments.

CHAPTER III

EFFECTS OF SUBLETHAL EXPOSURE TO CHLORINE ON THE UPTAKE OF POLYCHLORINATED BIPHENYLS BY RAINBOW TROUT, *Salmo gairdneri* (Richardson)

Introduction

Chlorine is a common industrial disinfectant and antifoulant. Dicharges of chlorine to the aquatic environment typically occur as pulsed releases of relatively high concentrations (0.5 mg/L) of chlorine, resulting from the use of chlorinated municipal water supplies for industrial cooling and process waters (Zeitoun, 1978) or as continuous releases of low concentrations (< 0.04 mg/L) as a component of industrial effluents or treated domestic sewage (Larson et al., 1978). Chlorine is acutely toxic to freshwater fish with the degree of toxicity influenced by the chemical speciation of chlorine, the fish species, and the lifestage of exposed fish. Cold-water species, including salmonids, are particularly sensitive to chlorine (Brungs, 1973). The 96-h LC_{50} values for adult rainbow trout (*Salmo gairdneri*), the test organism for this study, range from 0.014 to 0.07 mg/L total residual chlorine (TRC) (Mattice and Zittel, 1976; Ward et al., 1976). Because effluents often contain chlorine concentrations within this toxic range, rainbow trout and other sensitive fish species may be intermittantly or continuously exposed to damaging, but nonlethal concentrations of chlorine.

Avoidance behavior by fish often protects them from prolonged exposure to toxic chemicals in the aquatic environment. Schumacher

and Ney (1980) studied the avoidance behavior of rainbow trout to single pulses of chlorine released to a power plant discharge canal. Trout avoided concentrations greater than 0.05 mg/L TRC by swimming downstream to less contaminated water, but did not respond to 0.04 mg/L TRC, a potentially toxic concentration in prolonged or repeated exposures.

Brooks et al. (1982) found that rainbow trout were more sensitive to one 120-min exposure to chlorine (2-h LC_{50} = 0.66 mg/L) than to four 30-min exposures (2-h LC_{50} = 0.94 mg/L). These results indicate the importance of recovery between pulsed exposures to chlorine as an important variable in chlorine toxicity to fish.

Fish exposed to chlorine exhibit a typical suite of physiological and pathological responses involving the gills and respiration. Irritation of the gills and buccal cavity of chlorine-exposed fish results in the secretion of mucous by fish gills and a coughing reflex (Bass and Heath, 1977). Typical histological damage to fish gills includes hyperplasia, hypertrophy, and necrosis of epithelial cells, epithelial cell lifting with separation from the basement membrane, lamellar fusion, and hemangiectasis of lamellar vasculature (Bass et al., 1977; Middaugh et al., 1980; Wiley, 1982). Respiratory impairment is evident in fish exposed to acute and pulsed exposures of lethal doses of chlorine. Increased hematocrit, bradycardia, and increased ventilation rates of chlorine-exposed fish are all adaptive responses to insufficient oxygen consumption; all attempt to deliver more oxygen to the tissues of chlorine-stressed fish (Bass and Heath, 1977; Block et al., 1978; Middaugh et al., 1980; Wiley, 1982).

Compensatory respiratory changes often accompany chemically induced gill irritation. For example, fish exposed to zinc, a gill tissue irritant, show reduced oxygen uptake efficiencies, which results in internal tissue hypoxia (Skidmore, 1970; Hughes and Adeney, 1977; Tuurala and Soivio, 1982). This hypoxia is often accompanied by compensatory increases in respiration, primarily ventilation volume increases (Davis and Cameron, 1971; Kerstens et al., 1979; Lumholt and Johansen, 1979; McKim and Goeden, 1982). Although these compensatory increases in respiration are adaptive with respect to oxygen uptake, increases in the fish's ventilation volume could increase the effective exposure of fish to other contaminants in the water, as well as to oxygen.

Industrial and sewage effluents often contain toxic chemicals in addition to chlorine. These toxic chemicals can potentially have additive or synergistic effects with chlorine on exposed organisms. In a study comparing the LC_{50}s of rainbow trout exposed to phenol with and without pre-exposure to chlorine, Alexander and Clarke (1978) found that trout exposed to phenol alone had nearly twice the mean survival time of trout pre-exposed to chlorine, followed by exposure to phenol. These results indicate a potentiation of the toxicity of phenol due to the presence of chlorine. Therefore, the net toxic effects of fish exposure to a gill damaging agent, such as chlorine or zinc, concurrently with other toxic chemicals should depend on the relative magnitudes of changes in fish respiration and changes in contaminant uptake efficiency. Both are variables that affect the effective exposure to the gill irritant and the other toxicant.

The physiological responses of fish exposed to chlorine provide indirect evidence that chlorine also has a deleterious effect on oxygen uptake efficiency. The physiological and pathological effects of chlorine on fish gills that are proposed to alter the uptake efficiency of oxygen may have a similar effect on the uptake of other chemicals present in chlorine-treated effluents, in particular, those that are accumulated by fish via passive diffusion across fish gill membranes (Hunn and Allen, 1974; Norstrom et al., 1976; Opperhuizen et al., 1985). Potential co-contaminants include many hydrophobic chemicals persistent in the natural aquatic environment, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and polychlorinated dibenzodioxins .

In these experiments the effect of chlorine exposure on the uptake of three PCB congeners by trout was examined. The time course of the histpathological effects of a subacute, sublethal exposure to chlorine was characterized using rainbow trout exposed to chlorine in large flow-through tanks. In separate experiments using a fish respirometer-metabolism chamber, trout were exposed first to a PCB congener, and then to chlorine with the PBC congener in order to measure the effects of chlorine exposure on oxygen and PCB uptake and on trout respiratory functions.

Using this approach two hypotheses were tested. First, exposure to chlorine is hypothesized to have a similar effect on the flux of all passively diffusing molecules across the gill membrane, including oxygen and PCBs. Also, the degree that the uptake of oxygen and PCB

molecules is impaired should be related to the time of exposure to chlorine and the resulting time-dependent damage to the gills.

Materials and Methods

Fish

Yearling rainbow trout, Salmo gairdneri (Richardson) were obtained from the Buffalo Springs trout hatchery and acclimated at 15 °C in large flow-through tanks for at least 2 weeks. The mean trout weight (±STD) for the time course exposure to chlorine was 224 g (±49; n = 24). The mean trout weight (±STD) for the metabolic chamber exposures was 359 g (±104; n = 16). Trout were fed daily with trout chow (Purina)until 24 h before experimentation.

Chemicals

For the time course chlorine exposure, sodium hypochlorite (analytical grade, 4-6% chlorine, Fisher Scientific) was diluted to 3.5 times the desired final chlorine concentration with 9 L of deionized water. This dilute chlorine solution was pumped via a peristaltic pump into a 4-L mixing chamber and diluted with dechlorinated tap water to the desired exposure concentration (0.04 mg/L total residual chlorine) and flowed into the exposure tank at 1 L/min. The average chlorine concentration (±STD) in the exposure tank was 0.035 mg/L (±0.010; n = 12) total residual chlorine (TRC), measured at 8, 12, and 24 h.

Stock chlorine solutions (1.14 mg Cl/L) for the metabolic chamber exposures were prepared by diluting sodium hypochlorite in deionized water. The stock chlorine solution was pumped by a peristaltic pump

into a 4-liter mixing chamber where it was mixed with dechlorinated tap water and the PCB stock solution, yielding a final chlorine concentration of approximately 0.04 mg/L. This diluted solution containing PCBs and chlorine was then pumped into the fish metabolic chamber at a flow rate of 400 mL/min. The average chlorine concentration (±STD) in the metabolic chamber exposures was 0.035 mg/L TRC (±0.008; n = 16).

Radiolabeled PCBs ([ring-UL-^{14}C]2,2',4,4'-tetrachlorobiphenyl (22'44'-TCB), 9.89 mCi/mmol; [ring-UL-^{14}C]2,2',5,5'-tetrachlorobiphenyl (22'55'-TCB), 12.6 mCi/mmol; and [ring-UL-^{14}C]-2,2',3,3',5,5'-hexachlorobiphenyl (22'33'55'-HCB), 19.2 mCi/mmol) in toluene were obtained from Pathfinder Laboratories (Sigma Corp, St. Louis, MO). The radiochemical purity of the PCB congeners exceeded 99%, determined using high performance liquid chromatography. Dilute PCB solutions were prepared in glass distilled methanol (Burdick and Jackson, American Scientific Corporation) and diluted to the final exposure concentration (≤ 1.5 µg/L) with dechlorinated tap water. Concentrations of PCB congeners in the exposure water were below published aqueous solubility values (Mackay et al., 1980). Methanol in the exposure water never exceeded 0.01%.

Analysis of Total Residual Chlorine

Total residual chlorine was measured by amperometric titration (Method 408 C, Greenberg et al., 1985) using a Wallace and Tiernan Amperometric Titrator (Belleville, NJ). One mL of 5% potassium iodide and 1 mL of pH 4 acetate buffer were added to 250 mL of the exposure water containing chlorine. This solution was mixed and titrated with

0.0564 N phenylarsine oxide until no further amperage deflections were measured. The concentration of total residual chlorine (TRC) (mg/L) was calculated from the volume of phenylarsene oxide titrated.

Histopathological Analysis

Gill tissue samples were processed overnight in 10% neutral formalin, 95% ethyl alcohol, 100% ethyl alcohol, xylene, and paraffin using an Autotechnicon Mono Model 2A processor (Technicon Inc., Dublin, Ireland). The dehydrated tissue samples were then embedded in paraffin and 4 μm sections were cut with a microtome. The tissue sections were mounted on slides and dried at 80 °C for 20 minutes. Duplicate slides of each tissue were stained with Hematoxylin/Eosin, a general nuclear stain (Evans, 1974), and Alcian Blue/Periodic Acid-Schiff's base, a stain detecting mucopolysaccharides (Thompson, 1966).

Time Course Exposure to Chlorine

Twenty-four trout were placed in a flow-through tank and allowed to acclimate for 24 h. Trout were then exposed to 0.04 mg/L chlorine for up to 24-h total exposure time (Fig.III-1). Trout were removed after 0-, 8-, 12-, and 24-h exposure to chlorine for histological analysis of gill tissue. The tanks were flushed with dechlorinated water and two groups of trout remained in dechlorinated tap water for 24 and 48 h. At each sampling time four trout were removed from the experimental tank, killed, weighed, and the second gill arches removed and preserved in 10% neutral formalin for histological analysis. Water samples were taken from the exposure tank at each sampling period for analysis of chlorine concentrations (TRC).

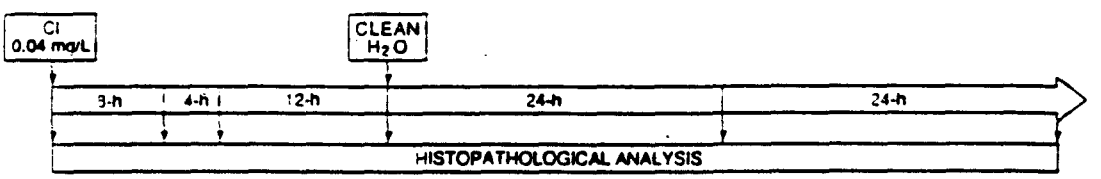

Figure III-1. Chlorine exposures in flow-through tanks.

Rainbow trout were exposed to 0.04 mg/L chlorine in 400 L flow-through tanks for 24-h, followed by 24 and 48-h recovery periods in dechlorinated water. The sampling times for removal of gill tissue from exposed trout for histopathological analysis are indicated by vertical arrows.

Exposure to Chlorine and PCBs (Fish Metabolic Chamber)

Surgical procedures. Trout were anesthetized with MS-222 (tricaine methanesulfonate, Crescent Research Chemicals, Paradise Valley, AZ) and placed on a fish surgical table (McKim and Syrett, 1982). Each trout was immobilized by spinal transection and a latex membrane was sewn to the trout's mouth. Trout were transferred to the fish metabolic chamber (McKim and Goeden, 1982) and the latex membrane was attached to the chamber to form the barrier between inspired and expired water. Each trout recovered from the surgical procedures overnight ($\geq$18 h) in flowing dechlorinated tap water.

Metabolic chamber. The fish metabolic chamber is described in detail elsewhere (McKim and Goeden, 1982). It is a modified respirometer-metabolism chamber (Fig. III-2), divided into three chambers (A, B, and C) by latex membranes. A latex membrane sutured to the trout's mouth separates inspired and expired water in chambers A and B, respectively. Chambers B and C are separated by a latex collar that is secured to the metabolic chamber and fitted snugly around the fish's midsection posterior to the gills. Standpipes in each chamber drain the excess water.

Ventilation volume (mL/min) was determined by measuring the volume of water overflowing the standpipe in chamber B as a function of time. Ventilation rate (ventilatory strokes/min) was measured using a physiograph (Narcotrace 40, Narco Biosystems, Houston, TX) which recorded opercular movements as a function of time. Oxygen concentrations (mg/L) were measured in water from chambers A and B

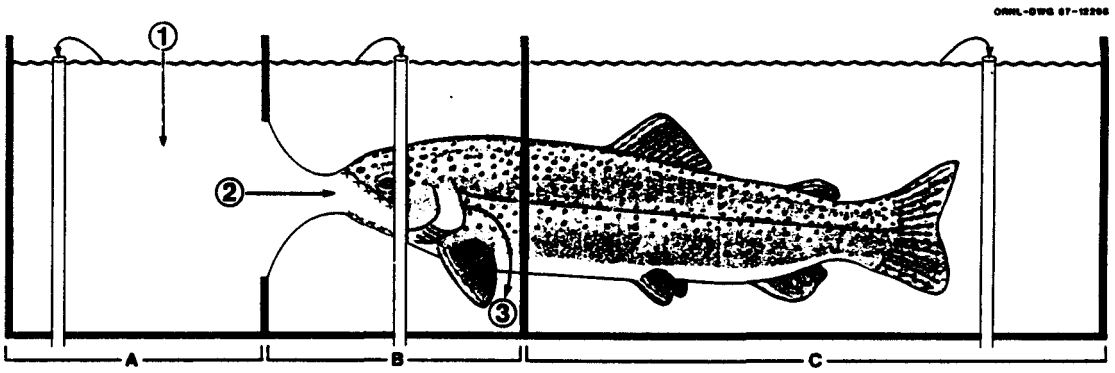

Figure III-2. Repeat of the fish metabolic chamber.

(1) Exposure water with O_2 and ^{14}C-labeled PCB enters chamber A.
(2) Water with PCB and O_2 is pumped over the gills by the ventilatory action of the fish.
(3) Water minus PCB and O_2 extracted by the gills is expelled into chamber B.

(before and after passing over the gill) using an oxygen electrode (Model 58, YSI Corporation, Yellow Springs, OH). PCB concentrations were measured in chambers A and B by liquid scintillation counting of 5 mL water samples. Oxygen and PCB uptake efficiencies (percentages) were calculated by dividing the difference in oxygen or PCB concentrations in chambers A and B (amount taken up by the gills) by the exposure concentration (concentration in chamber A) and multiplying by 100%.

Exposure to chlorine and PCBs. The metabolic chamber exposure protocol is diagrammed in Fig. III-3. Trout were pre-exposed to a PCB congener (22'44'-TCB, 22'55'-TCB or 22'33'55'-HCB) for 3 h. Baseline measurements of respiratory parameters (ventilation volume and ventilation rate) and oxygen and PCB uptake efficiencies were made during the pre-exposure period (no chlorine) at 2 and 3 h. After baseline measurements were made, chlorine (0.04 mg/L) was pumped into the metabolic chamber along with the selected PCB congener. Simultaneous exposure to chlorine and PCBs continued for 24 h, with fish respiration and oxygen and PCB uptake measured at 4, 8, 12, and 24 h. Total residual chlorine concentrations in the exposure water were measured after 10 and 20-h exposure to chlorine. After the 24-h chlorine/PCB exposure, one group of fish were allowed to recover in dechlorinated water for 24 hours. The selected PCB congener was withheld from the chamber except for the first 4 h and final 3 h of the 24 h recovery period to conserve costs and maximize human safety during the overnight exposure. Oxygen and PCB uptake and respiratory

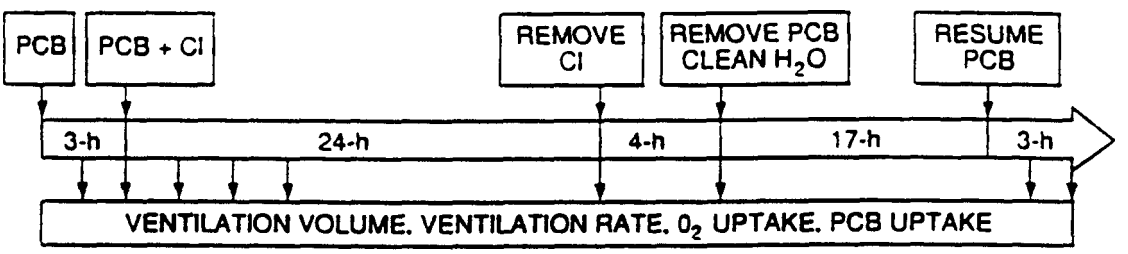

Figure III-3. Simultaneous exposure to chlorine and PCBs in the fish metabolic chamber.

Rainbow trout were exposed to a PCB congener in dechlorinated water for 3-h to obtain baseline respiration and uptake measurements. Trout were then exposed to the PCB congener and chlorine for 24 h, and allowed to recover in dechlorinated water for 24 h, with continued exposure to the PCB congener for the first 4 h and final 3 h of the recovery period. Respiratory functions and PCB and O_2 uptake were measured at the times indicated by the vertical arrows.

functions were measured at 4 and 24 h to determine the progress of recovery from the chlorine exposure.

Analysis of radioactivity. The radioactivity in the water samples was measured using a Packard CDS 460 liquid scintillation counter, equipped with automatic quench correction. Five mL of water containing a ^{14}C-labeled PCB congener were added to 10 mL of scintillant (ACS, Amersham Corporation). The PCB concentrations were calculated using the specific activity of each congener.

Statistical Analyses

Most statistical analyses were performed using PC-SAS Version 6 (Statistical Analysis Systems, Cary, NC) and Lotus 1-2-3 Version 2.01 (Lotus Development Corp., Cambridge, MA). Sokal and Rohlf's Biometry (1981) was the reference text for manual calculations. Specific tests are identified in the text.

Results and Discussion

Effects of Chlorine on Trout Gill Histopathology

Chlorine-induced damage to trout gills was evident as early as after 8-h exposure, compared to normal gill lamellae (Fig. III-4). After 8-h exposure to chlorine, trout gill lamellae had a threefold increase in the number of mucous cells, increasing to approximately a fivefold elevation in numbers at 12 and 24 h (Fig. III-4b, Fig. III-5). Mucous cell numbers remained elevated throughout the 48-h recovery period in dechlorinated water, although their numbers appeared to be decreasing (Fig. III-5).

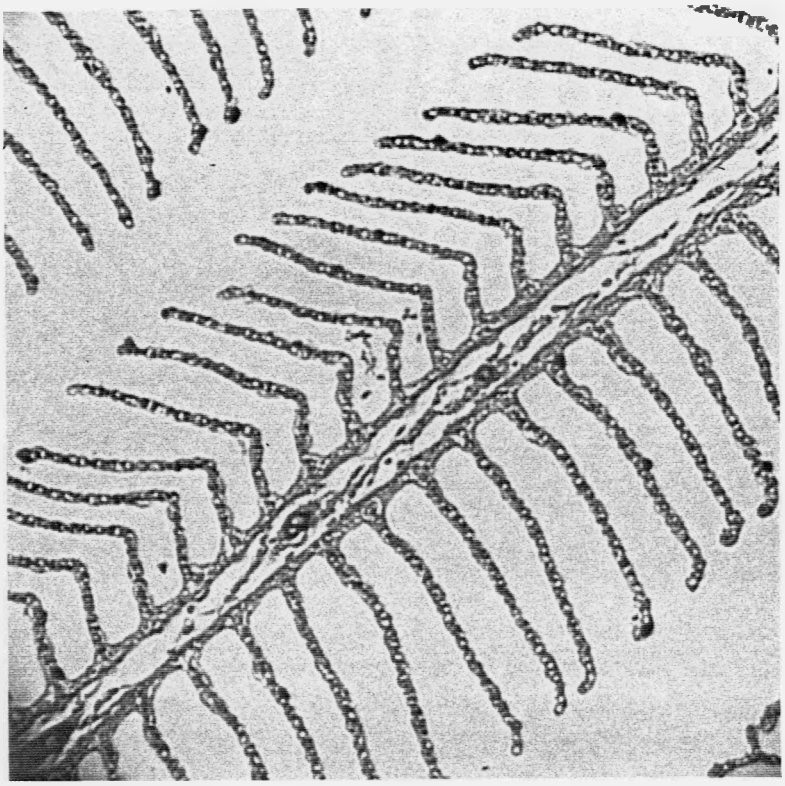

Figure III-4. Histopathological evidence of chlorine-induced damage to trout gill lamellae.

(A) Normal gill lamellae from control trout (no chlorine).

Hematoxylin/Eosin, 100X.

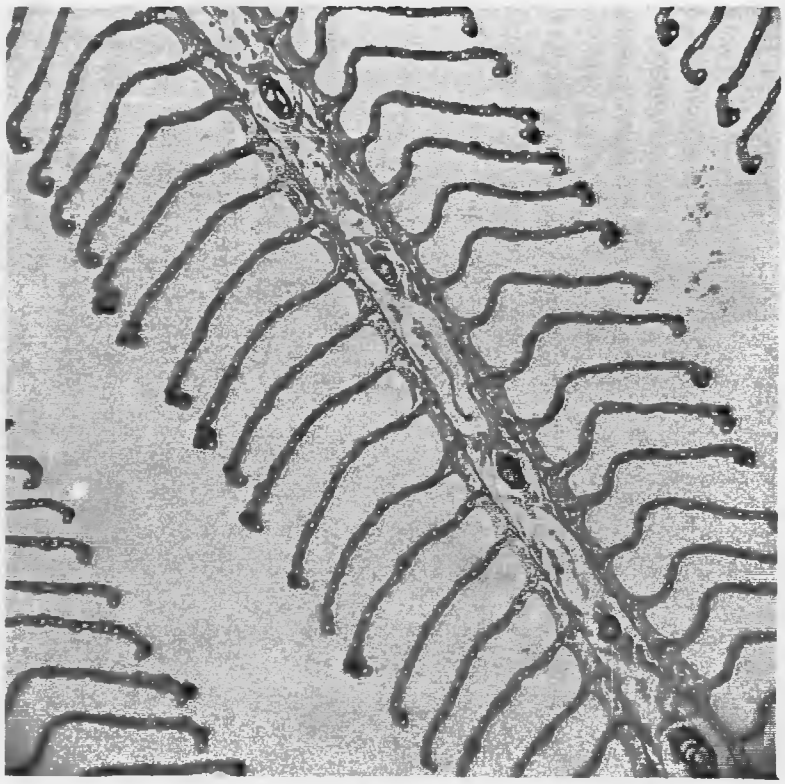

Figure III-4 (continued)

(B) Proliferation of mucous cells and clubbed lamellae observed after 12 h exposure to 0.04 mg/L chlorine.

Alcian blue/PAS, 100X.

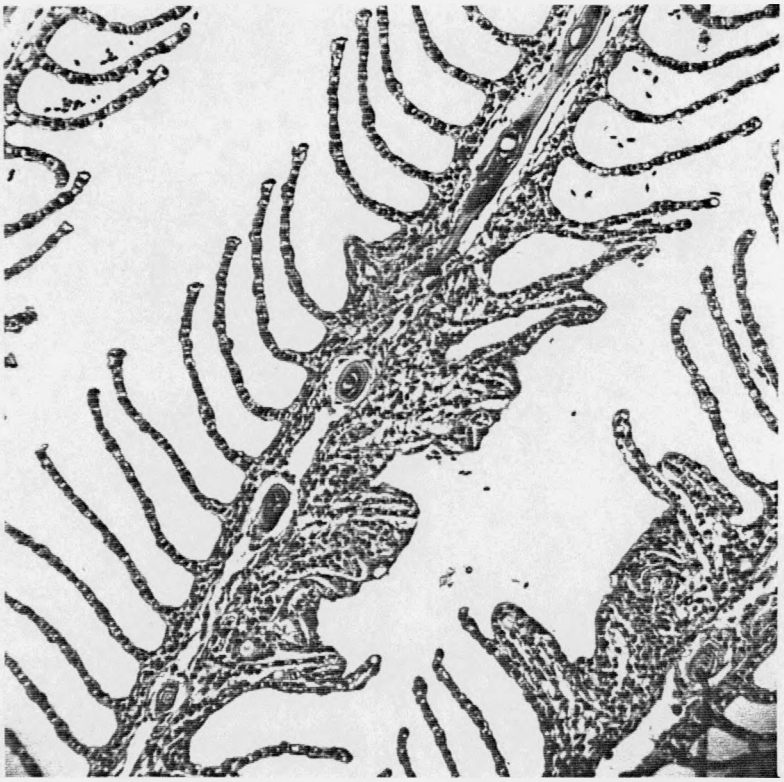

Figure III-4 (continued)

(C) Hyperplasia of lamellar epithelial cells observed after 12-h exposure to 0.04 mg/L chlorine.

Hematoxylin/Eosin, 100X.

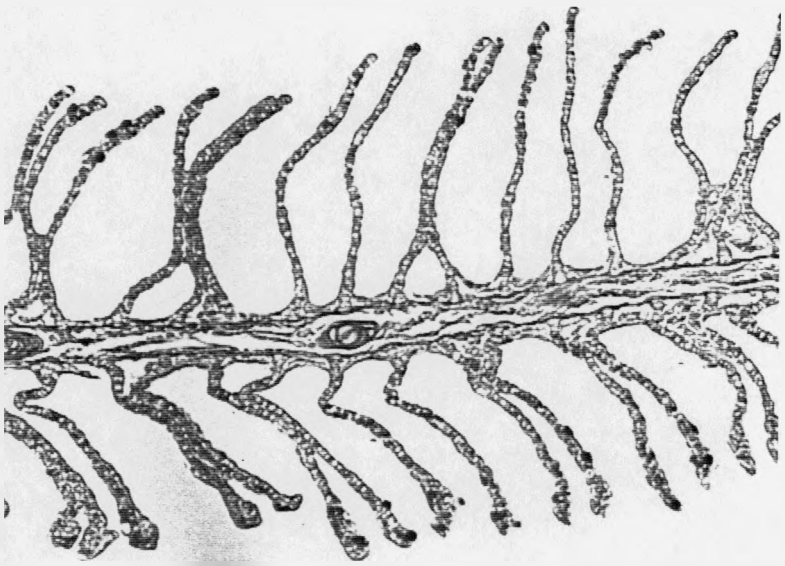

Figure III-4 (continued)

(D) Fused lamellae observed after 24-h exposure to 0.04 mg/L chlorine Hematoxylin/Eosin, 100X.

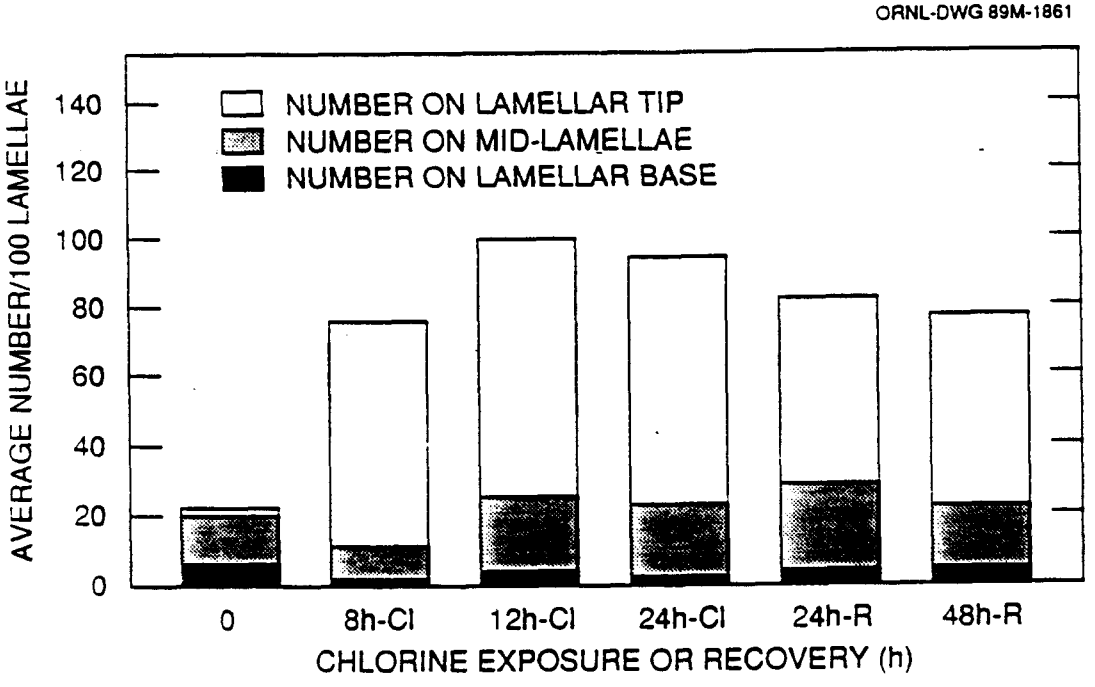

Figure III-5. Mucous cell counts from trout gill lamellae from control and chlorine-exposed trout.

Histograms represent the average number of mucous cells per 100 gill lamellae counted in control trout (0 h) and in trout during the 24-h exposure to 0.04 mg/L chlorine and the 48-h recovery period in dechlorinated water. The shaded area represents the average number of mucous cells found on the base of the lamellae. The stippled area represents the average number counted on the mid-lamellar region. The unshaded area represents the number counted on the lamellar tips. Mucous cell proliferation reached a maximum (fivefold) increase after 12-h exposure to chlorine, with the numbers still elevated, although showing a declining trend, after 48-h recovery in dechlorinated water.

Mucous cell proliferation occurred primarily at the tips of the gill lamellae, presumably the area with the greatest contact with chlorine (Fig. III-4b, p. 41). A similar pattern of mucous cell proliferation was observed on the lamellae of rainbow trout exposed to five or more pulses of a high dose (0.4 mg/L) of chlorine (Bass et al., 1977). Although migration of some cell types from the base to the tips of lamellae has been observed (personal communication, W. H. Gingerich, National Fishery Research Laboratory, LaCrosse, WI), the pattern of mucous cell proliferation did not suggest that cell migration occurred in chlorine-exposed trout. Since no sequential increase in the number of mucous cells was observed at the base or mid-sections of the lamellae, which would indicate mucous cell migration, it is more likely that lamellar epithelial cells were transformed to become mucous-secreting cells. This mechanism has been proposed by Roy (1988) to explain the appearance of new goblet mucous cells on the skin and gills of Rita rita exposed to dodecylbenzene sodium sulfonate.

Mucous secretion is a protective mechanism to shield gill and other epithelial tissues from exposure to irritating agents (Carpenter, 1927, 1930; Mallatt, 1985). By increasing the number of distal lamellar mucous cells, chlorine-exposed trout can generate more mucous to protect the lamellar epithelial cells from the oxidizing effects of chlorine. However, the functional respiratory surface area of the lamellae will decrease as increasing numbers of respiratory epithelial cells are replaced by mucous-secreting cells. In addition, increases in the thickness of the mucous layer adhering to the surface

of the lamellae would increase the respiratory diffusion distance. Both of these effects of mucous cell proliferation could potentially interfere with the diffusion of oxygen and other molecules across the gill membrane.

Abnormal pathological lesions, including clubbed lamellae (Fig. III-4b, p. 41) and hyperplasia of lamellar epithelial cells (Fig. III-4c, p. 42), were visible on trout gills after 12-h exposure to chlorine. Lamellar fusion was seen after 24-h exposure to chlorine (Fig. III-4d, p. 43). In general, the severity of damage and the number of lesions observed were related to the time of exposure to chlorine, with the most severe damage observed after 24-h exposure.

These lesions may be adaptive in that they may protect the gill from uptake of irritating chemicals by providing a physical barrier, preventing uptake of the irritant (Mallatt, 1985). However, the increased diffusion distances and decreased gill surface areas can also hinder the uptake of oxygen and other molecules present in the fish's environment that are accumulated by passive diffusion across gill membranes.

Recovery of gill tissue from exposure to chlorine began to be evident after 48 h in dechlorinated water. At this time few abnormal lesions were observed on the gill lamellae, although the lamellae still had increased numbers of mucous cells.

Effects of Chlorine Exposure on Respiration and PCB Uptake

The changes in gill lamellar morphology seen in trout exposed to 0.04 mg/L chlorine would be expected to alter the diffusional capacity of the gills as a result of increases in the diffusion distance and

decreases in the functional surface area of the gill membranes. Uptake efficiencies of both oxygen and PCBs by trout gills were reduced in chlorine-exposed trout (Fig. III-6). The reductions in uptake efficiency corresponded with the observed progress of chlorine-induced damage to gill membranes and were related to the exposure time, with greater reductions in uptake measured as the time of exposure to chlorine increased. Significant reductions in PCB uptake efficiencies were measured after 4-, 8-, 12-, and 24-h exposure to chlorine. Oxygen uptake efficiencies were significantly reduced compared to controls after 12- and 24-h exposure to chlorine ($p < 0.05$, Tukey-Kramer method).

In all exposures to chlorine and PCB congeners, decreases in oxygen uptake efficiency were paralleled by similar decreases in the PCB uptake efficiency throughout the chlorine exposure period. This relationship is illustrated in Fig. III-7 in which oxygen uptake efficiencies are shown to be directly related to PCB uptake efficiencies. The resulting regression equations are listed in Table III-1. The slopes and intercepts of the regression equations were not significantly different between PCB congeners or from the 1:1 line ($p < 0.05$, Students t-test). These results indicate that the chlorine-induced changes in fish gill pathology had an equivalent effect on the diffusion of both oxygen and PCBs across the gill membrane. Trout respiratory functions were elevated in chlorine-exposed trout (Fig. III-8). After 24-h exposure to chlorine significant elevations in trout ventilation volume were measured ($p < 0.05$, Tukey Kramer method). Ventilation volume measurements ($\pm$SEM) reached a maximum of

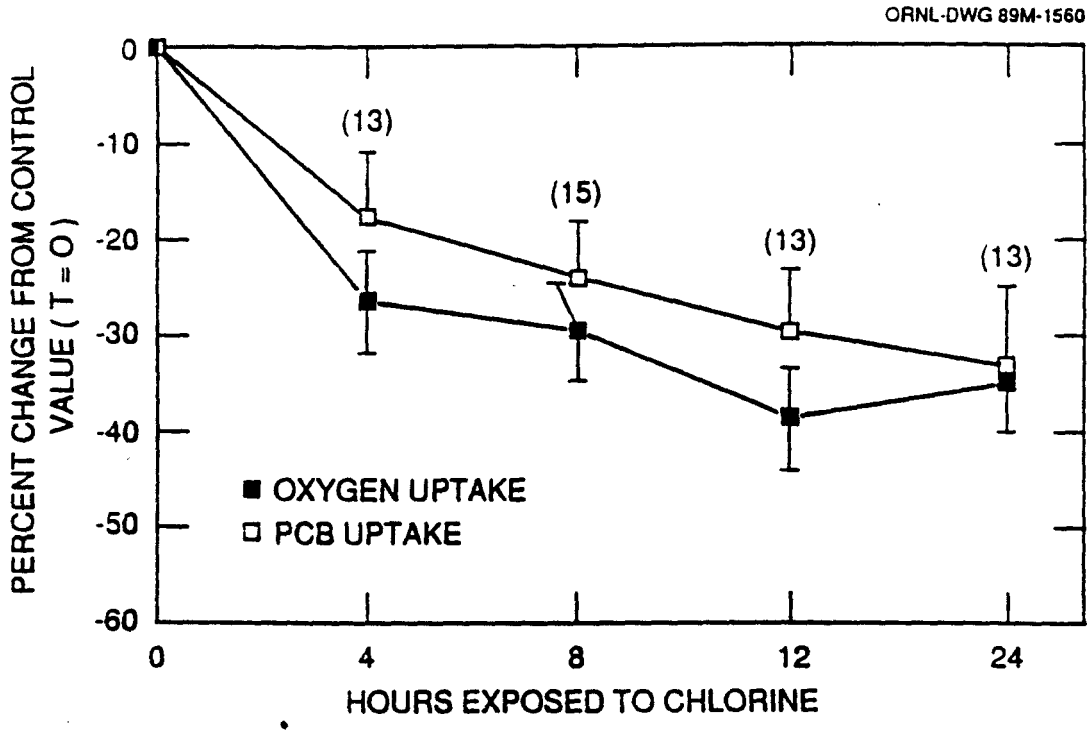

Figure III-6. Percentage change in oxygen and PCB uptake efficiencies during the 24-h exposure to chlorine and PCB congeners in the metabolic chamber.

Each point represents the mean percentage change (±SEM) from the control value (measured at T = 0, no chlorine). The number of observations is indicated in parentheses.

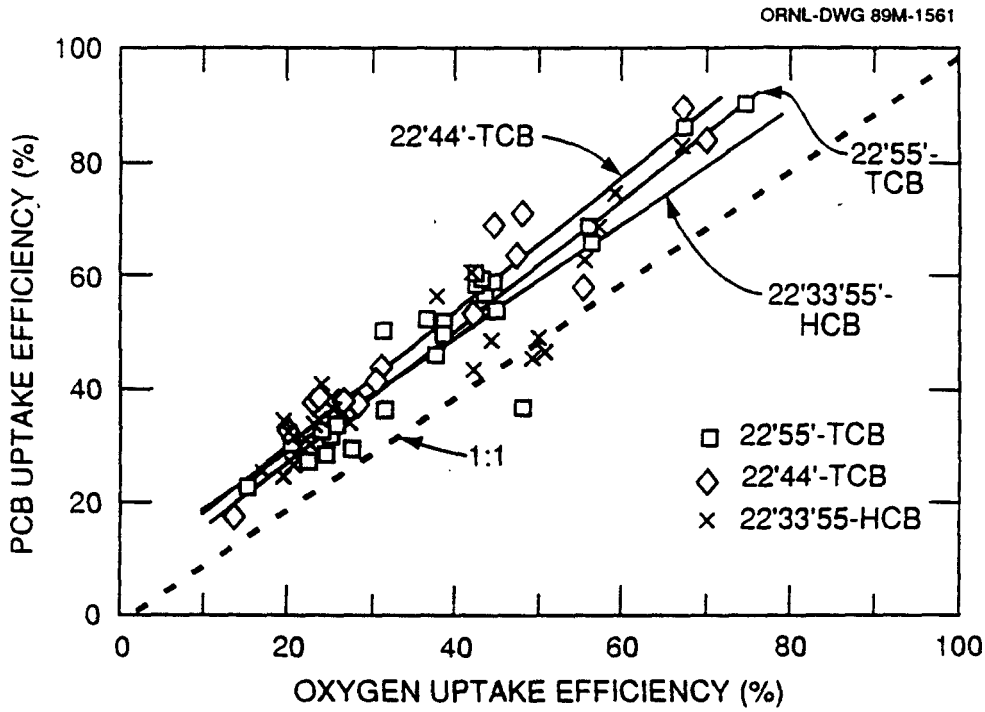

Figure III-7. Linear correlations between oxygen and PCB uptake efficiencies for each PCB congener during exposures to chlorine and PCB congeners.

The dashed line represents the 1:1 relationship predicted by the hypothesis that the uptake of both oxygen and PCBs are affected equally by exposure to chlorine. There were no significant differences ($p < 0.05$, Student's t-test) in the slopes or intercepts of the regressions for the three congeners and the 1:1 line or between the three congeners.

Table III-1. Regression equations demonstrating the relationship between oxygen uptake efficiency (x) and PCB uptake efficiency (y) for each congener.

PCB Congener	Regression Equation	n	r^2
22'44'-TCB	y = 1.18x + 7.1	16	0.92[a]
22'55'-TCB	y = 1.16x + 4.4	25	0.88[a]
22'33'55'-HCB	y = 1.00x + 9.0	30	0.83[a]

[a] $p < 0.001$

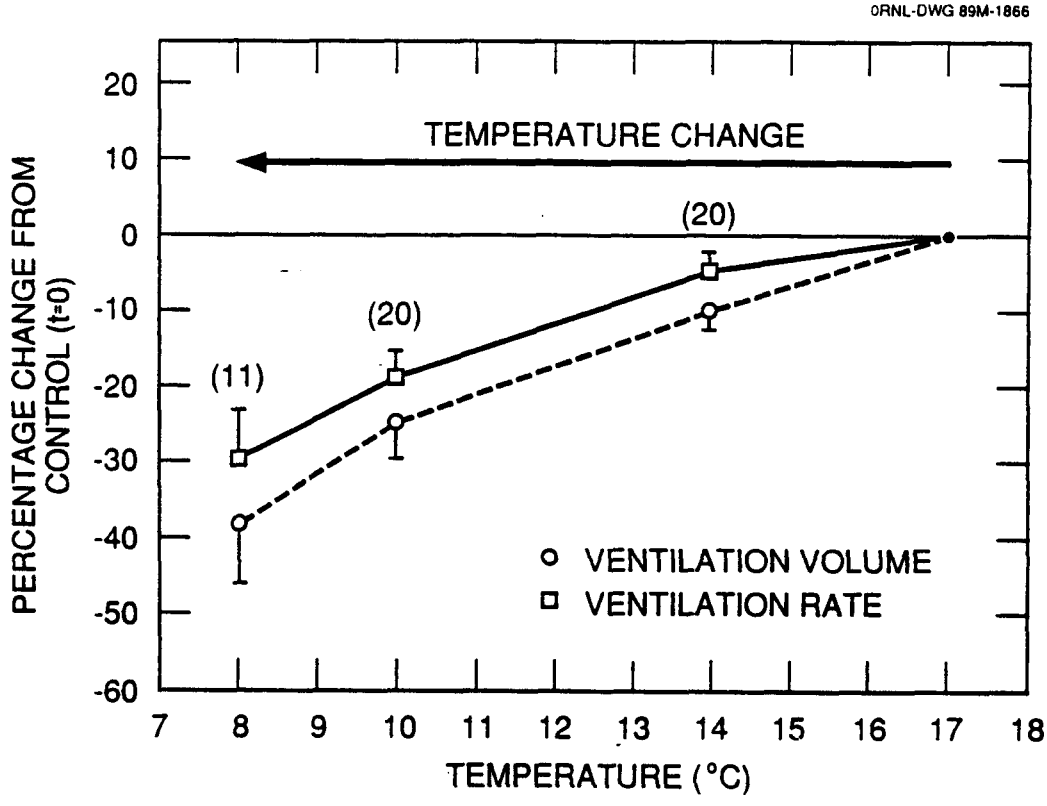

Figure III-8. The percentage change in trout ventilation volumes and ventilation rates during exposures to chlorine and PCB congeners.

Each point represents the mean percentage change (±SEM) from the control value (measured at T = 0, no chlorine). The number of observations is indicated in parentheses.

131% (±43) of the control value (no chlorine) at 24 h. Ventilation rate increases were much less. Significant increases in ventilation rate were measured at 12 and 24 h ($p < 0.05$, Tukey Kramer method), with a maximum increase of 16% (±8) measured at 24 h.

Oxygen uptake efficiency and ventilation volume measurements are both components determining oxygen consumption rates (mg O_2/kg/hr). The increases in respiratory functions measured in chlorine-exposed trout resulted in the maintenance of adequate oxygen consumption by trout throughout the 24-h exposure to chlorine (Fig. III-9). The average oxygen consumption values measured at 0 h (control values, no chlorine) were compared to oxygen consumption measured during the 24-h exposure to chlorine. At all sampling times oxygen consumption values were not statistically different from the control value (no chlorine) ($p < 0.05$, Tukey-Kramer method). Thus, trout appeared to compensate for the reduced oxygen uptake efficiencies caused by chorine-induced changes in gill pathology through increased exposure to the oxygen-saturated water through adjustments in ventilatory functions.

The calculated PCB uptake (μg PCB/kg/hr) remained constant over the 24-h exposure to chlorine (Fig. III-9). There were no significant differences between the control values (no chlorine, 0 h) for PCB uptake and those measured during the 24-h exposure to chlorine ($p < 0.05$, Tukey-Kramer method).

These results imply that compensatory adjustments in respiratory functions which allow for the maintenance of adequate oxygen supplies to the chlorine-exposed trout also maintain a constant dose of PCBs to the trout. These results also support the hypothesis that both

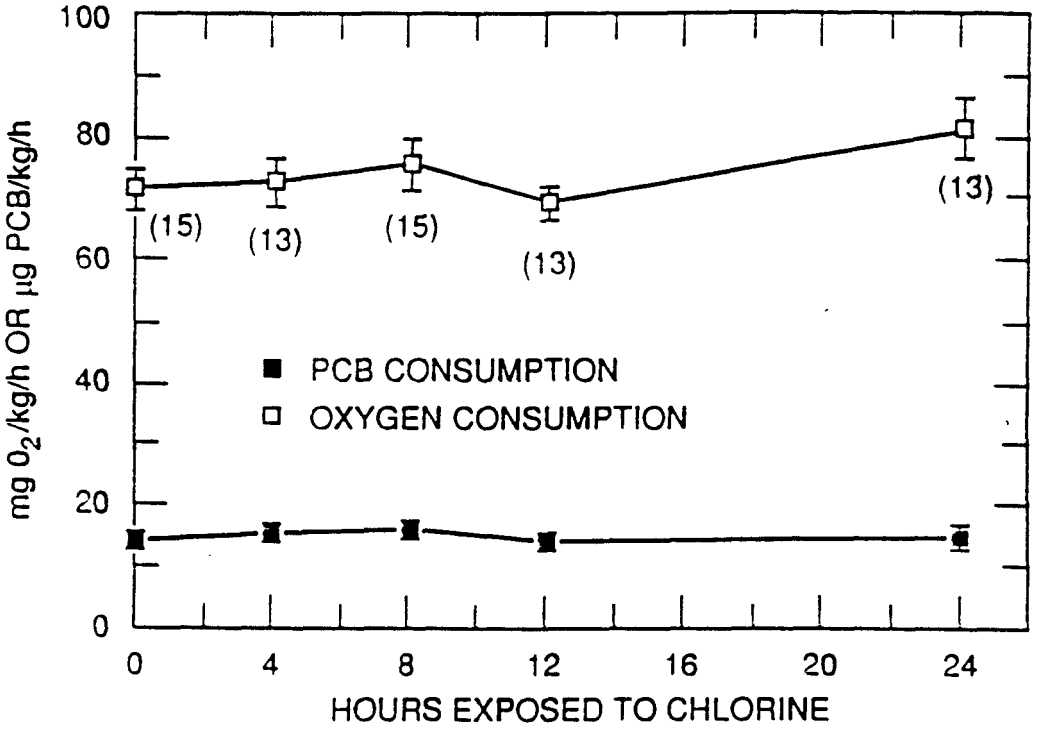

Figure III-9. Oxygen consumption and PCB uptake measured during exposure to chlorine and PCB congeners.

Each point represents the mean value (±SEM) for oxygen uptake (mg O_2/kg/hr) or PCB uptake (µg PCB/kg/hr). There were no significant differences between the 0 h (no chlorine) value and values at any exposure time for oxygen consumption or PCB uptake ($p < 0.05$, Tukey Kramer method).

molecules enter the gill by passive diffusion across the gill membrane (Hunn and Allen, 1974; Norstrom et al., 1976).

Recovery of Trout in Dechlorinated Water After Chlorine Exposure

After a 4-h recovery period in dechlorinated water trout ventilation volumes and rates remained elevated while oxygen and PCB uptake efficiencies were still reduced, compared to control values measured before exposure to chlorine. By the end of 24 h in clean water, recovery of both respiratory functions and uptake were evident. Ventilation volumes were within 4% of the pre-exposure values, oxygen uptake efficiencies were within 19% of pre-exposure values, and 22'55'-TCB uptake efficiencies were 5% greater than the average pre-exposure value.

Histopathological damage to gill tissue showed a similar, although slower, time course of recovery in clean water. Most lesions were absent by 48 h and mucous cell counts, although still elevated, appeared to be decreasing (Fig. III-5, p. 44). However, the observation that histopathological recovery was slower may be artifactual. The flow-through tank used in those experiments had a much greater volume (400 L) than chamber A of the metabolic chamber (2.5 L) and consequently, should have a greater lag-time in being completely flushed with clean water than the much smaller chamber A.

Physiological Implications

The histopathological results of this study indicate that acute exposure to a sublethal concentration of chlorine will result in structural and functional damage to trout gills. The extent of damage

was time-dependent and cumulative. Evidence of tissue irritation was observed within the first 8 h of exposure, followed by more extensive structural damage to the gills after 12- and 24-h exposure. The gill damage observed in these experiments appeared to interfere with the vital function of the gill as a respiratory surface for diffusion of oxygen into the fish's blood. Due to the chlorine-induced damage to the respiratory surface and the measured reductions in oxygen uptake efficiency, it can be inferred that chlorine-damaged gill membranes had a reduced diffusional capacity.

Chlorine-exposed trout had increased ventilation volumes and ventilation rates. Similar increases in ventilatory functions were seen in trout exposed to lethal doses of zinc (Skidmore, 1970) and fenvalerate (Bradbury et al., 1987), two chemicals demonstrated to cause gill damage similar to that caused by chlorine. In both experiments there was an inverse relationship between trout respiration and oxygen uptake efficiencies by exposed trout. By increasing ventilation volumes, trout were able to maintain constant oxygen consumption, even at low oxygen uptake efficiencies. Respiratory compensation continued until the trout were unable to tolerate the lethal dose, at which time ventilation volumes diminished and death occurred. Histological examination of trout exposed to each chemical revealed gill tissue abnormalities associated with gill irritation and damage. In both experiments, trout deaths were attributed to tissue hypoxia, resulting from the trout's inability to consume sufficient oxygen by the gills due to gill tissue damage by the toxic agent (Skidmore, 1970; Bradbury et al., 1987).

The relationship between fish ventilation volume and oxygen uptake efficiency has been investigated by others using non-invasive methods to increase respiration. Oxygen uptake efficiency was reduced in conjunction with experimental manipulations that increased ventilation volumes through increased activity (Saunders, 1962), hypoxia (Kerstens et al., 1979; McKim and Goeden, 1982), and artificial manipulation of gill ventilation flow rates (Davis and Cameron, 1971). In explaining these results, the authors hypothesized that the increases in ventilation volume decreased oxygen uptake efficiency due to the decreased contact time between the oxygen molecules dissolved in the ventilatory water and the gill membrane surface (Davis and Cameron, 1971; Kerstens et al., 1979; McKim and Goeden, 1982).

In the present experiments with rainbow trout exposed to chlorine, a similar inverse relationship was found between increases in ventilation volume and decreases in oxygen uptake efficiency. However, due to the nature of the damaging effect of chlorine on the gills of exposed trout, it seems counterproductive for the trout to respond to exposure by increasing their ventilation volumes and, consequently, exposing the gill membranes to more chlorine-laden water. The initial effect of chlorine on the fish respiration was a decrease in oxygen uptake efficiency presumably due to the altered diffusional capacity of the chlorine-damaged gills. The oxygen consumption data for the chlorine-exposed fish (Fig. III-9, p. 53) demonstrate that the fish maintained adequate oxygen consumption levels, accomplished by an increase in the fish's ventilation volume. In these experiments the concentration of chlorine was sufficiently

low enough to make the cost:benefit trade-off of increased ventilation volumes worthwhile. Perhaps ventilation volumes would decrease at higher exposure concentrations or during longer exposure durations, as was observed during the final time periods prior to death in the exposures with the gill-damaging agents, zinc (Skidmore, 1970) and fenvalerate (Bradbury et al., 1987).

The uptake efficiencies of the PCB congeners were also reduced, with reductions presumed to be due to the altered diffusional properties of the chlorine-damaged gills. The reductions measured in PCB uptake efficiencies were of the same magnitude as reductions in oxygen uptake for all three PCB congeners (Fig. III-7, p. 49). The 1:1 relationship between PCB and oxygen uptake efficiencies provides support for the hypothesis that chlorine-induced gill damage will have the same effect on the flux of both oxygen and PCB molecules across fish gill membranes.

Toxicological Implications

Many authors have related contaminant uptake dynamics to compound hydrophobicity and gill membrane permeability, characterizing the diffusion process as being limited by the molecule's diffusion through the hydrophobic and hydrophilic diffusion layers of the gill membrane (Flynn and Yalkowsky, 1972; Gobas et al., 1986). McKim and coworkers (1985) measured trout gill uptake efficiencies for chemicals having a wide range of octanol-water partition coefficients (K_{ow}). Although uptake efficiencies measured for compounds with low K_{ow}s (values $\leq 10^2$) were correlated with K_{ow}, the uptake efficiencies of compounds

with K_{ow} values ranging from 10^3 to 10^6 were nearly equivalent (approximately 55%). The authors postulated that for moderately hydrophobic compounds with K_{ow} values between 10^3 and 10^6, uptake of contaminants across trout gills is controlled by diffusion rates of the chemical through the aqueous diffusion layers at the inside and outside surfaces of the gill membrane, rather than by compound hydrophobicity. For these compounds similar values were measured for oxygen uptake efficiency (62.5%), further suggesting that uptake efficiencies were regulated by diffusional properties of the membrane, rather than the hydrophobicity of the diffusing molecule.

Oxygen and PCB uptake efficiencies were directly correlated over a wide range (uptake efficiencies ranged from 20 to 80%) (Fig. III-7, p. 49), presumably due to damage to gills resulting from exposure to chlorine. These data, coupled with the results of McKim and coworkers (1985), lend further support to the hypothesis that the uptake efficiencies of the three PCB congeners and oxygen are controlled by physical changes in the gill membrane, due to chlorine-induced gill damage. The calculated changes in oxygen consumption and contaminant uptake during exposure to chlorine result from changes in fish respiratory functions, in addition to changes in uptake efficiency of the diffusion molecule. These results suggest that factors that alter gill membrane diffusion properties or fish respiration may control the diffusion of oxygen, PCB congeners, and potentially other toxicants within this range of hydrophobicities to a greater extent than physicochemical interactions with membrane diffusion layers controlled by the molecule's hydrophobicity. This finding also

further supports the hypothesis that the uptake of oxygen, PCBs and perhaps other hydrophobic contaminants occurs by passive diffusion across the gill membrane.

The scientific literature is replete with data on oxygen consumption under a variety of physiological and environmental conditions, including environmental temperature changes; changing oxygen regimes; fish metabolism changes associated with species differences, age, activity, or reproductive status; and stress, including both physical and chemical stressors. This study has demonstrated that oxygen consumption is paralleled by PCB uptake, and that environmental and physiological change affects the uptake of both molecules to a similar extent. Based on these observations, values for oxygen consumption have potential utility in estimates of compound uptake for hydrophobic compounds with log K_{ow} values ranging from 3 to 6, whose uptake is proposed to be primarily controlled by fish respiration. This approach would be extremely useful, because the number of compounds having the potential for uptake by fish gills is extremely large, while the actual data base on their uptake and accumulation is relatively small. By using the much larger data base on oxygen consumption as the basis for extrapolation, models of contaminant uptake could be extended to include physiological and environmental changes that affect oxygen uptake, including growth, reproductive activity, changes in the environmental temperature, and fish activity changes. Using this approach, models of contaminant uptake can be adjusted to account for environmental or physiological

conditions that will alter fish respiration, thus improving predictions of contaminant uptake by fish in the natural environment.

Summary and Conclusions

1. Trout exposed to 0.04 mg/l chlorine for 24 h exhibited histopathological gill damage, including proliferation of mucous cells, clubbed lamellae, fused lamellae, and hyperplasia.

2. The progress of chlorine-induced damage to fish gills was correlated with increasing time of exposure to chlorine. Oxygen uptake efficiencies decreased as the severity of gill damage increased.

3. Chlorine-induced gill damage also hindered the uptake efficiency of PCBs. A 1:1 correlation was found between the uptake efficiencies of oxygen and all three PCB congeners over the entire range of measured uptake efficiencies (ranging from 20 to 80%).

4. The reductions in uptake efficiencies were accompanied by increases in fish ventilatory functions, resulting in a relatively constant oxygen consumption. Respiratory compensation also resulted in a constant dosage of the three PCB congeners.

5. The linear relationship between oxygen and PCB uptake efficiencies has potential utility for use in making predictions of toxicant

uptake by fish using the extensive literature base on oxygen uptake as the basis for extrapolation.

CHAPTER IV

EFFECTS OF TEMPERATURE-INDUCED CHANGES IN RESPIRATION AND GILL MEMBRANE PERMEABILITY ON THE UPTAKE OF BENZO[A]PYRENE, NAPHTHALENE, AND 2,2',5,5'-TETRACHLOROBIPHENYL BY RAINBOW TROUT, Salmo gairdneri (Richardson)

Introduction

Fish often encounter acute changes in environmental temperature as a result of short-term migrations during foraging or spawning activities. In addition, the ambient water temperature of some aquatic micro-environments including intertidal zones, riffle pools, shallow reaches, and areas receiving water from reservoirs or thermal pollution inputs can be quite variable during a relatively short time period. Fish may experience diurnal or intermittant temperature changes over a time span that is shorter than the 2-3 weeks necessary for them to acclimate to a new temperature (Peterson and Anderson, 1969; Campbell and Davies, 1975). Since the body temperature of aquatic poikilotherms conforms to their external thermal environment, these acute changes in water temperature can affect vital biochemical and physiological functions of fish living in these environments.

The effects of thermal increases on fish respiratory functions and oxygen consumption have been fairly well characterized in experiments utilizing a variety of fish species. Hughes and Roberts (1970) measured increased ventilatory frequencies and stroke volumes in rainbow trout, Salmo gairdneri, exposed to an acute 8 °C increase in temperature. Carp, Cyprinus carpio, experiencing a 4-6 °C increase in

temperature (Moffitt and Crawshaw, 1983) and Gobius cobitis, experiencing a 12.5 °C temperature increase (Berschick et al., 1987) both had increased ventilation rates. Oxygen consumption by rainbow trout (Heath and Hughes, 1973), Blennius pholis, (Campbell and Davies, 1975), carp (Moffitt and Crawshaw, 1983), and G. cobitis (Berschick et al., 1987) rose as experimental temperatures were increased.

The effects of progressive temperature decreases on fish respiration have received less attention in the scientific literature. Generally, temperature decreases have been assumed to lower fish respiratory demand, and result in corresponding decreases in fish ventilatory functions and oxygen consumption. Moffitt and Crawshaw (1983) confirmed these effects in carp experiencing a temperature decrease of 2-6 °C, where significant temperature-dependent decreases were measured for carp ventilation frequency, heart rate, and oxygen consumption. Others have measured lowered fish respiratory and cardiac functions and oxygen consumption at low acclimation temperatures, but these studies did not include measurements made during acute decreases in temperature (Ott et al., 1980; Soivio and Tuurala, 1981; Barron et al., 1987).

Changes in environmental temperature may also affect the exposure of fish to waterborne contaminants. The uptake of chemicals that are accumulated by passive diffusion across fish gills can potentially be altered through temperature-related changes in fish ventilatory functions or changes in the diffusional capacity of the gill membrane. Bioaccumulation of DDT by the mosquito fish (Gambusia affinis) increased threefold at 20 °C compared to exposures at 5 °C. Increases

in DDT accumulation were related to increases in oxygen consumption at the higher acclimation temperature (Murphy and Murphy, 1971). Bluegill sunfish (Lepomis machrochirus) had a twofold increase in the body burden of benzo[a]pyrene (BaP) with a 10 °C increase in exposure temperature. Greater body burdens measured at the higher temperature were hypothesized to result from increases in respiration rates which compensated for the increased metabolic demand, but also resulted in increased exposure of the gills to BaP (Jimenez et al., 1987). Increased accumulation of water-borne dieldrin by the filter feeding bivalve, Sphaerium corneum, was also related to increases in water temperature and corresponding increases in gill ventilation rates (cilia beat frequency) (Boryslawskyj et al., 1987). These studies indicate that respiration may be a controlling factor in contaminant accumulation by fish and other aquatic biota.

Others have manipulated fish respiration using other experimental approaches to determine the role of fish respiration in contaminant uptake by fish. Opperhuizen and Schrap (1987) found that uptake rates of guppies exposed to polychlorinated biphenyls (PCBs) under normoxic and hypoxic conditions were not affected by changes in oxygen concentrations and the corresponding changes in respiration rates. However, they did hypothesize that increases in ventilation rates induced by hypoxia would cause decreases in both oxygen and PCB uptake efficiencies. In an analysis of factors controlling the uptake of hydrophobic contaminants, Gobas et al. (1986) also hypothesized that the efficiency of uptake of hydrophobic compounds by fish gills may be regulated by the fish's ventilation rate. Black and McCarthy (1989)

demonstrated an inverse relationship between PCB uptake efficiencies and trout ventilation volumes in trout exposed to chlorine. However, they attributed the reductions in PCB uptake efficiency to the impaired diffusion properties of the gill membrane resulting from the damaging effect of exposure to chlorine, rather than to ventilatory increases. Increased ventilation was hypothesized to be a compensatory mechanism to deliver more oxygen to the gill surface in chlorine-exposed trout.

Changes in membrane permeability, resulting from temperature changes, may also influence the diffusion of contaminants across fish gill membranes. The diffusion of oxygen across fish gills may be facilitated at higher temperatures as a result of the corresponding lower viscosity of water, until oxygen concentrations reach levels too low to meet the fish's respiratory demand due to the decreased solubility of oxygen in water at higher temperatures. Viscosity reductions should lower the resistence of gill ventilatory movements and may also lower the resistance of the hydrophilic layers of the gill membrane (Hughes and Roberts, 1970). Membrane fluidity can also be altered by the effects of temperature on membrane lipids. Cooling a biological membrane results in a loss of membrane fluidity, until acclimatory changes in the membrane lipids can occur. Acclimatory changes, including increases in fatty acid chain length and desaturation of membrane fatty acids, will increase the membrane fluidity and may restore the membrane permeability and other properties, depending on the organism's capacity for acclimation (Hazel, 1979, 1984; Isaia, 1979).

Because acute temperature changes can cause alterations in membrane fluidity and permeability and changes in fish ventilatory functions, temperature is hypothesized to affect the diffusion of both oxygen and contaminant molecules across fish gill membranes, through either effect or a combination of both effects. In addition, changes in the uptake of oxygen resulting from the action of acute temperature change on trout gill membranes should be paralleled by similar changes in contaminant uptake, since both molecules are proposed to diffuse across the gill membrane by passive diffusion (Hunn and Allen, 1974; Norstrom et al, 1976; Neely, 1979; Bruggeman et al., 1981).

In these experiments the effects of a progressive decrease in temperature on fish respiration and on oxygen and contaminant uptake were quantified. Using a fish metabolic chamber, simultaneous measurements of respiration and uptake were made at timed intervals throughout the temperature change. The use of the metabolic chamber enabled the direct measurement of oxygen and contaminant uptake efficiencies and facilitated making comparisons between respiration and uptake measurements.

Materials and Methods

Fish

Yearling rainbow trout, Salmo gairdneri (Richardson) were obtained from the Buffalo Springs trout hatchery and acclimated at 17 °C in large flow-through tanks for at least 2 weeks. The mean trout weight (±STD) was 447 (±210) grams (n = 20). Trout were fed daily with trout chow (Purina Mills, St. Louis, MO) until 24 h before experimentation.

Chemicals

Radiolabeled benzo[a]pyrene (BaP), [7-^{14}C]benzo[a]pyrene, 11.7 mCi/mmol; 2,2',5,5'-tetrachlorobiphenyl (TCB), [ring-UL-^{14}C]2,2',5,5'-tetrachlorobiphenyl, 12.6 mCi/mmol; and naphthalene (NAP), [1-^{14}C]naphthalene, 4.7 mCi/mmol, were obtained from Pathfinder Laboratories (Sigma Chemical Corp., St. Louis, MO). Chemical purities determined by high performance liquid chromatography exceeded 99%. The stock solutions in toluene were diluted with glass distilled methanol (Burdick and Jackson, American Scientific Corp.) and mixed with dechlorinated tap water to obtain the final exposure concentration ($\leq$ 1.5 μg/L). The dechlorinated tap water used in all experiments had the following average water chemistry values: pH, 7.89; hardness, 131 mg $CaCO_3$/L; alkalinity, 104 mg $CaCO_3$/L, and conductivity, 263 μmhos/cm. The concentration of each compound in the exposure water was below the published aqueous solubility value (Mackay and Shiu, 1977; Mackay et al., 1980). Methanol in the exposure water never exceeded 0.01%.

Metabolic Chamber Exposures

Surgical procedures. Trout were anesthetized with MS-222 (tricaine methanesulfonate, Crescent Research Chemicals, Paradise Valley, AZ) and placed on a fish surgical table (McKim and Syrett, 1982). Each trout was immobilized by spinal transection and a latex membrane was sewn to the trout's mouth. Trout were then transferred to the fish metabolic chamber (Fig. IV-1) and the latex membrane was attached to the chamber to form the barrier between inspired and

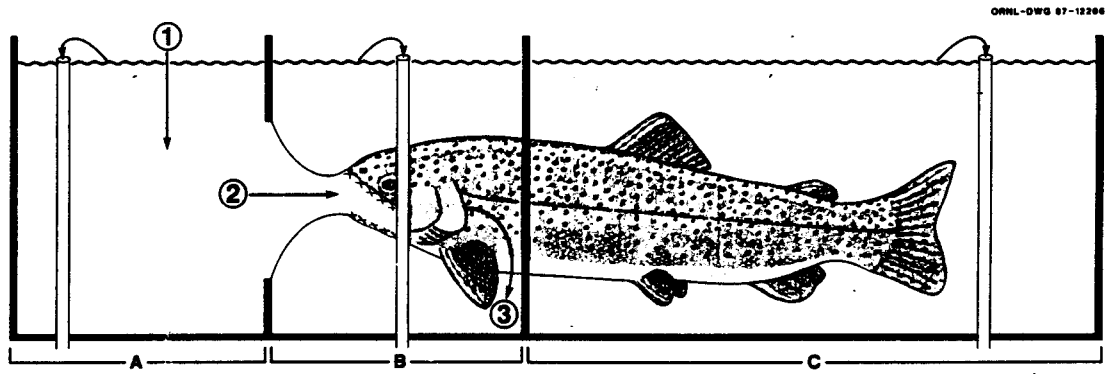

Figure IV-1. The fish metabolic chamber: Measurement of oxygen and contaminant uptake.

(1) Exposure water with O_2 and ^{14}C-labeled contaminant enters chamber A. (2) Water with the contaminant and O_2 is pumped over the gills by the ventilatory action of the fish. (3) Water minus the contaminant and O_2 extracted by the gills is expelled into chamber B.

expired water (McKim and Goeden, 1982). Each trout was allowed to recover from the surgical procedures overnight (≥ 18-h) in flowing dechlorinated tap water.

Metabolic chamber. The fish metabolic chamber is described in detail elsewhere (McKim and Goeden, 1982). It is a modified respirometer-metabolism chamber (Fig. IV-1, p. 68), divided into three chambers (A, B, and C) by latex membranes. Water containing a radiolabeled contaminant and oxygen is pumped into chamber A by a peristaltic pump. As the trout respires, the water is pumped across the gills and is expelled into chamber B, minus the oxygen and contaminant that the fish has extracted via the gills. Inspired and expired water in chambers A and B are separated by a latex membrane sutured to the trout's mouth. Chambers B and C are separated by a latex collar that fits snugly around the fish's body posterior to the opercula and is attached to the metabolic chamber. Standpipes in each chamber drain the excess water.

At each sampling time the following respiration and uptake measurements were made. Ventilation volume (mL/min) was determined by measuring the volume of water overflowing the standpipe in chamber B over two 1-2 minute time intervals. Ventilation rate (ventilatory strokes/min) was measured using a physiograph (Narcotrace 40, Narco Biosystems, Houston, TX), which recorded opercular movements as a function of time. Oxygen concentrations (mg/L) in chambers A and B (before and after passing over the gill) were measured using a YSI Model 58 oxygen electrode (Yellow Springs, OH). Contaminant concentrations (μg/L) in chambers A and B were calculated from the

radioactivity in five mL water samples and the known specific activity of the stock solutions. Oxygen and contaminant uptake efficiencies (percentages) were calculated by dividing the difference in oxygen or contaminant concentrations in chambers A and B (amount taken up by the gills) by the exposure concentration (concentration in chamber A). Oxygen consumption and contaminant uptake were calculated as the product of exposure concentration, uptake efficiency, and ventilation volume measured in each trout at each sampling interval.

Contaminant exposure: acute temperature change. Trout were exposed to the radiolabeled contaminant (BaP, TCB, or NAP) in the metabolic chamber at the acclimation temperature (mean temperature $\pm$STD = 16.7 $\pm$0.9 °C) for 3 h. Baseline measurements of respiration (ventilation volume and ventilation rate) and oxygen and contaminant uptake efficiencies were made after 2 and 3 h. After the baseline measurements were made, the temperature of the exposure water was gradually decreased approximately 8 °C (range = 6 to 10 °C) over the next three hours ($\leq$ 0.04 °C/min) to a minimum temperature of 8 °C (mean final temperature $\pm$STD = 9.2 $\pm$1.3 °C). During the 3-h period of acute temperature change, trout respiratory functions and compound and oxygen uptake efficiencies were measured at 15-min intervals (BaP exposures) or 30-min intervals (TCB and NAP exposures). The exposure temperature was measured at each sampling time, so that the uptake and respiration data could be correlated with exposure temperature at each sampling time.

One group of trout exposed to BaP during the temperature change exposures (n = 5) were maintained overnight in the metabolic chamber

in contaminant-free flowing water at the final water temperature (8 °C). Exposure to BaP was resumed after 15 h at 8 °C. Trout respiration and oxygen and BaP uptake were measured at 18 h, after a 3-h equilibration period.

Analysis of radioactivity. The ^{14}C radioactivity was measured using a Packard CDS 460 liquid scintillation counter, equipped with automatic quench correction. Five milliliters of exposure water containing ^{14}C-labeled BaP, TCB, or NAP were mixed with 10 mL of scintillant (ACS, Amersham Corporation) prior to analysis. Contaminant concentrations were calculated using each compound's specific activity.

Statistical Analyses

Most statistical analyses were performed using PC-SAS Version 6 (Statistical Analysis Systems, Cary, NC) and Lotus 1-2-3 Version 2.01 (Lotus Development Corp., Cambridge, MA). All manual calculations were made using Sokal and Rohlf's Biometry (1981) as the reference text. Specific tests are identified in the text.

Results and Discussion

Effects of Temperature on Trout Respiration

As the experimental temperature was lowered from 17 to 8 °C, trout respiratory functions (ventilation volume and ventilation rate) were decreased. The percentage change in ventilation volume and ventilation rate at the acclimation temperature of 17 °C and at 14, 10, and 8 °C are shown in Fig. IV-2. The lowest respiration values

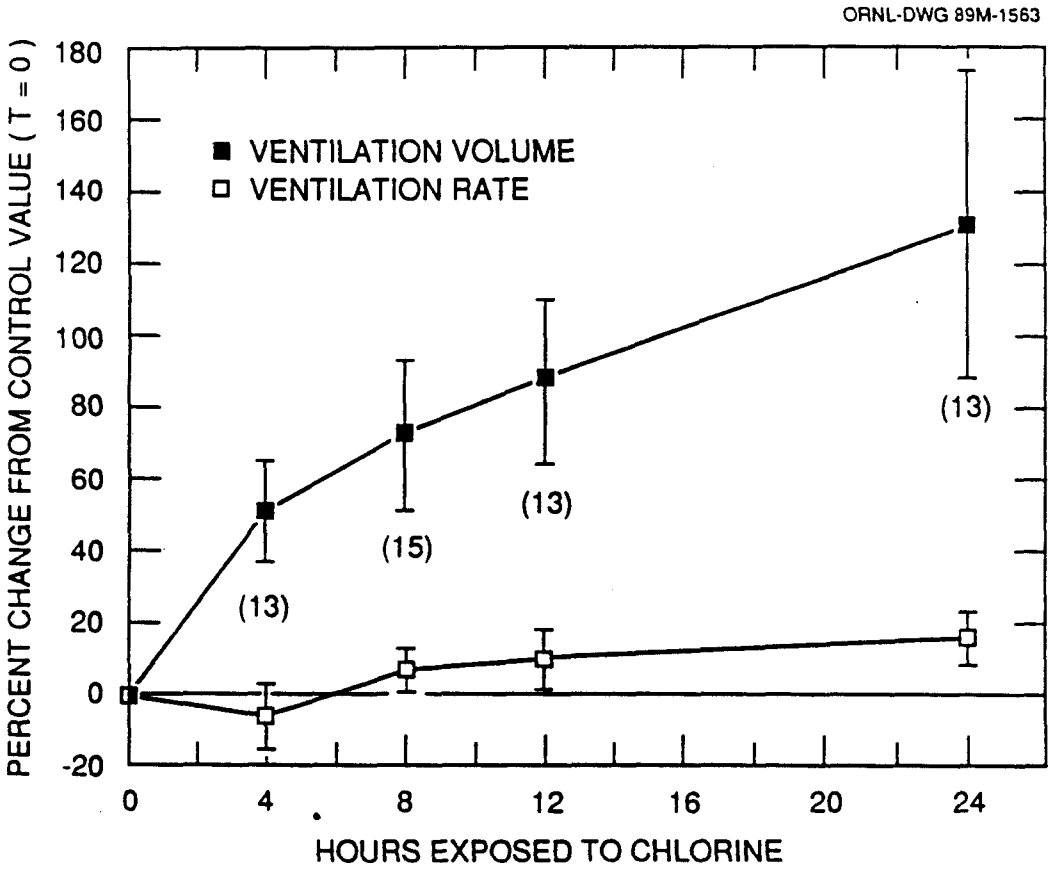

Figure IV-2. The percentage change in trout ventilation volumes and ventilation rates during an acute decrease in temperature.

Each point represents the mean percentage change (±SEM) from the control value measured at the acclimation temperature (17 °C). The number of observations is indicated in parentheses.

were measured at 8 °C, with a 38% total decrease in ventilation volume and a 29% decrease in ventilation rate, compared to measurements at 17 °C. Similar reductions in ventilation rate were observed in carp experiencing a 6 °C drop in temperature, although the magnitude of the decrease in carp ventilation rate was greater (-45%) (Moffitt and Crawshaw, 1983).

Changes in temperature cause predictable changes in the rates of physiological processes. Within limits, increased temperature results in a rate increase for most chemical reactions. Rate decreases are predicted when the temperature is lowered. The Q_{10} of a chemical or biological reaction is a calculation of the change in the rate of a physiological process with a 10 °C change in temperature. Typically, the rate of a chemical reaction doubles with a 10 °C increase in temperature, while most physiological reactions have a Q_{10} of two to three. The reaction rates of acclimated animals with perfect temperature compensation will have a Q_{10} of one. The equation used to calculate Q_{10} is: $Q_{10} = (R2/R1)^{10/(T2-T1)}$, where T1 is the initial temperature, T2 is the final temperature, R1 is the rate at T1, and R2 is the rate at T2 (Schmidt-Nielsen, 1983).

Q_{10} values were calculated for ventilation volumes and ventilation rates at all temperature intervals, including the entire temperature range between acclimation and final temperatures (Table IV-1). For ventilation rate measurements, no significant changes in Q_{10} were determined throughout the temperature change (Tukey-Kramer test, $p < 0.05$), with an average Q_{10} value of 1.53, calculated using the entire temperature range. This value is similar to the Q_{10} of 1.4 measured

Table IV-1. Mean Q_{10} values (±SEM) for respiration and uptake measurements. Values were calculated from experiments with BaP, TCB, and NAP (n = 20 trout, except as noted for the 10 to 8 °C interval).

Measurement	Temperature Interval			
	17-14 °C	14-10 °C	10-8 °C	Total
Ventilation Volume	1.96 (± 0.39)	1.83 (± 0.20)	4.47[a,b] (± 1.12)	2.02 (± 0.19)
Ventilation Rate	1.32 (± 0.14)	1.62 (± 0.14)	1.66[b] (± 0.41)	1.53 (± 0.17)
Oxygen Uptake Efficiency	1.56 (± 0.27)	1.69 (± 0.21)	1.76[b] (± 0.60)	1.47 (± 0.13)
Compound Uptake Efficiency	1.49 (± 0.27)	1.27 (± 0.11)	1.74[b] (± 0.35)	1.28 (± 0.09)
Oxygen Consumption	1.77 (± 0.31)	2.25 (± 0.20)	7.13[a,c] (± 2.95)	2.04 (± 0.13)
Compound Uptake	2.07 (± 0.46)	2.29 (± 0.27)	10.27[a,c] (± 3.62)	2.28 (± 0.17)

[a]Significant difference between adjacent temperature intervals ($p < 0.01$)

[b]$n = 9$

[c]$n = 10$

for rainbow trout experiencing an acute increase in temperature from 15 to 26 °C (Heath and Hughes, 1973), indicating that nonlethal temperature changes in either direction result in ventilation rate alterations occurring at similar rates of change.

Ventilation volume decreases had a biphasic response to temperature change, with significantly higher rates of change at the lowest temperature interval, from 10 to 8 °C (Q_{10} = 4.47), compared to the lower and more constant rates of change between 17 °C (Q_{10} = 1.96) and 10 °C (Q_{10} = 1.83) (Tukey-Kramer test, $p < 0.01$). The Q_{10} for ventilation volume determined over the entire temperature range was 2.02.

Effects of Temperature on Oxygen and Contaminant Uptake Efficiencies

Oxygen and contaminant uptake by trout, in terms of both efficiency of uptake as well as consumption were also reduced as the experimental temperature was lowered. Figure IV-3 shows the time course of changes in oxygen and contaminant uptake efficiencies corresponding with changes in experimental temperature. Mean uptake values (±SEM) were pooled from exposures with all three compounds, BaP, TCB, and NAP. The average decreases in oxygen uptake efficiencies were proportional to the average decreases in contaminant uptake efficiencies. Figure IV-4 demonstrates the linear relationship between these two variables when individual values for oxygen uptake efficiency for each contaminant exposure were plotted against contaminant uptake efficiencies measured at the same time. The regression equations for each compound are listed in Table IV-2. For exposures with TCB, the 95% confidence intervals of the slope and

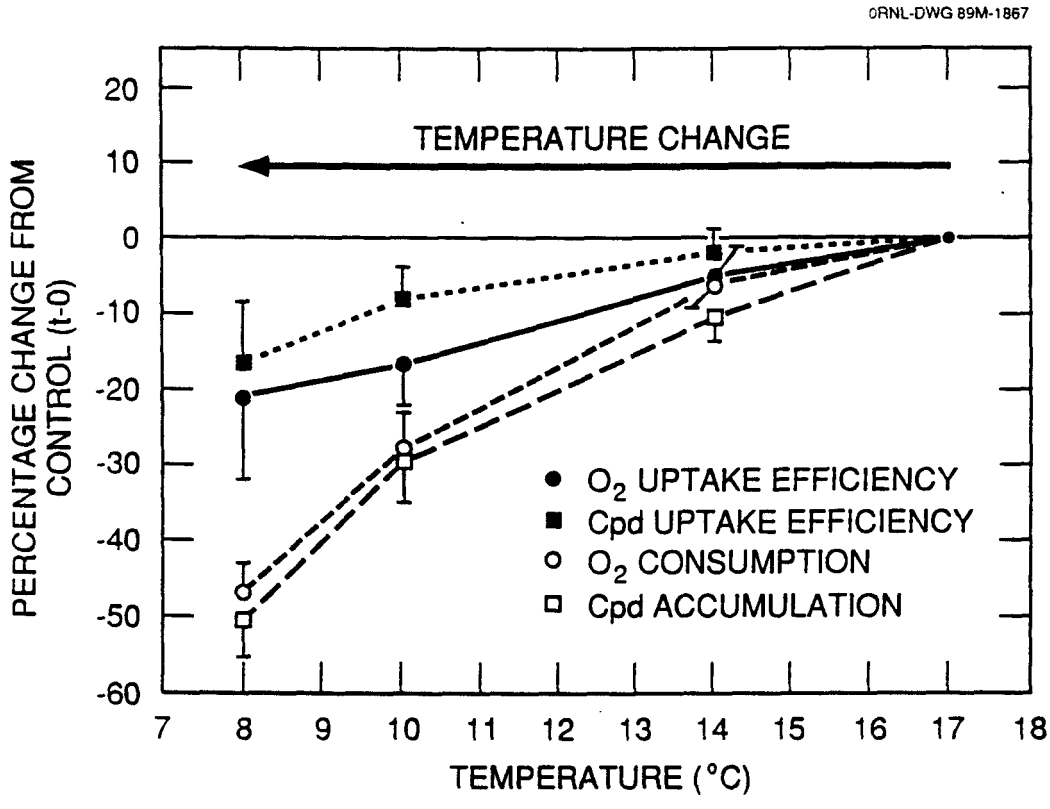

Figure IV-3. The percentage change in oxygen and contaminant uptake by trout during an acute decrease in temperature.

Each point represents the mean percentage difference (±SEM) from the control value measured at the acclimation temperature (17 °C). The number of observations is indicated in parentheses.

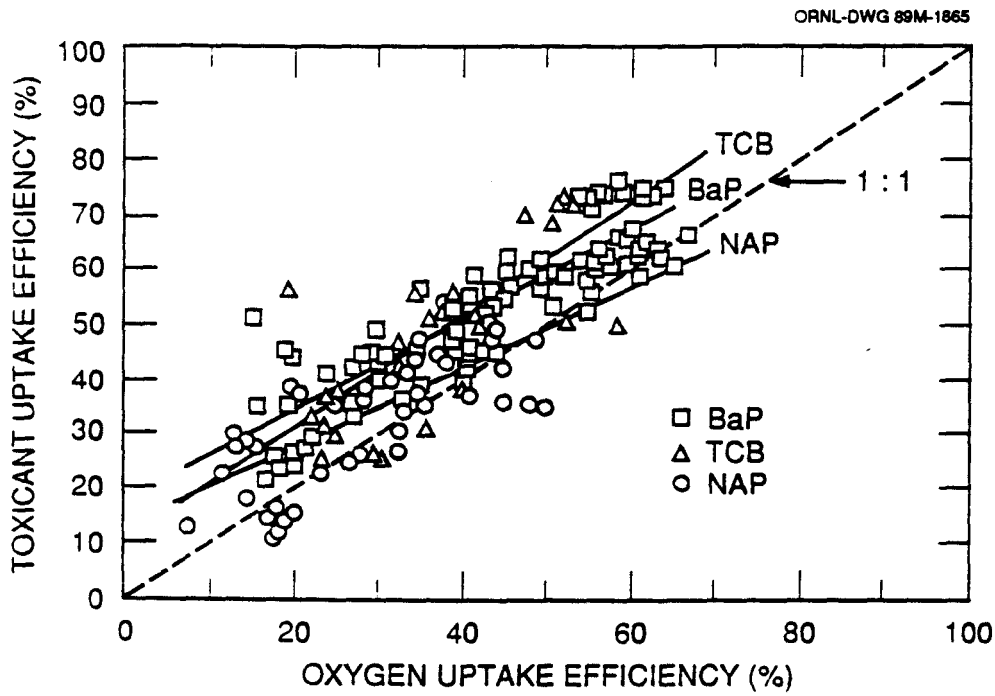

Figure IV-4. The linear relationships between oxygen and toxicant uptake efficiencies for exposures with BaP, TCB, and NAP during an acute decrease in temperature.

The dashed line represents the 1:1 relationship predicted by the hypothesis that the uptake of oxygen and compounds are affected equally by an acute change in temperature. A significant linear relationship was found between oxygen and contaminant uptake efficiencies for all three compounds. For exposures with TCB, this relationship was not significantly different from a 1:1 relationship. The individual regression equations are listed in Table IV-2 (p. 78).

Table IV-2. Regression equations for oxygen uptake efficiency (x) plotted versus compound uptake efficiency (y).

Compound	Regression Equation	n	r^2
BaP	$y = 0.82x + 11.78$	96	0.77[c]
22'55'-TCB	$y = 1.04x + 10.33$[a,b]	48	0.62[c]
NAP	$y = 0.74x + 12.57$[b]	52	0.56[c]

[a]Slope not significantly different from 1 ($p < 0.05$)

[b]Intercept not significantly different from 0 ($p < 0.05$)

[c]$p < 0.001$

intercept were not significantly different from those from a 1:1 regression (slope = 1; y-intercept = 0), indicating that temperature changes had an equivalent effect on the uptake efficiency of both oxygen and TCB. For exposures with BaP and NAP, changes in oxygen uptake efficiencies were correlated with proportionally lower changes in contaminant uptake efficiencies, with both regressions having slopes significantly less than one.

The rates of change in oxygen and contaminant uptake efficiencies were constant throughout the temperature change from 17 to 8 °C. There were no significant differences in the Q_{10} determinations (Table IV-1. p. 74) calculated for smaller temperature intervals within the entire range of temperatures for both measurements ($p < 0.05$, Tukey-Kramer test). Q_{10} values were 1.47 for oxygen uptake efficiency and 1.28 for contaminant uptake efficiency calculated using the entire temperature range (17 to 8 °C). These values compare favorably with a Q_{10} value of 1.35 measured for temperature-induced viscosity changes in cytosolic extracts prepared from muscle tissue isolated from white perch (Morone americanus). Changes in the diffusion rates of selected small molecules (molecular weights ranging from 40 to 166) through these cytosol extracts were attributed primarily to increased viscosity of the extracts as temperature was lowered from 25 to 5 °C (Sidell and Hazel, 1987). The Q_{10} observed for uptake efficiency in cooled trout may also be indicative of similar changes in the viscosity of the membrane's diffusion layer, the membrane layer predicted to have the rate-limiting effect on diffusion of many hydrophobic molecules (K_{ow} ranging from 10^3 to 10^6) such as those used

in this study, BaP, NAP and TCB (Flynn and Yalkowsky, 1972; Gobas et al., 1986).

Effects of Temperature on Oxygen Consumption and Contaminant Uptake

Lowered oxygen uptake efficiencies coupled with decreased ventilation by trout resulted in significant decreases in oxygen consumption and contaminant uptake at decreased experimental temperatures (Fig. IV-3, p. 76). Changes in oxygen consumption were linearly correlated with changes in contaminant uptake for all three compounds (Fig. IV-5). The data from all three compounds were pooled, since there were no significant differences between the slopes and intercepts of the individual regression equations. The regression equation for the pooled data from the three contaminant exposures was: $y = 0.79x - 8.90$, where y is the percentage change in contaminant uptake and x is the percentage change in oxygen consumption; $r^2 = 0.71$, $n = 50$. Table IV-3 lists the individual regression equations. Although there was not a 1:1 relationship between changes in oxygen consumption and contaminant uptake, the significant linear relationship between the two variables for all three compounds and the regression of the pooled data indicates that acute temperature change had a similar effect on the uptake of oxygen and the three compounds by fish gills.

Further evidence that decreased temperature affected the uptake of oxygen and contaminants in a similar manner is reflected in the magnitude and pattern of the Q_{10} measurements for each variable (Table IV-1, p. 74). Both oxygen consumption and contaminant uptake rates had a bi-phasic response to the decrease in temperature. During the

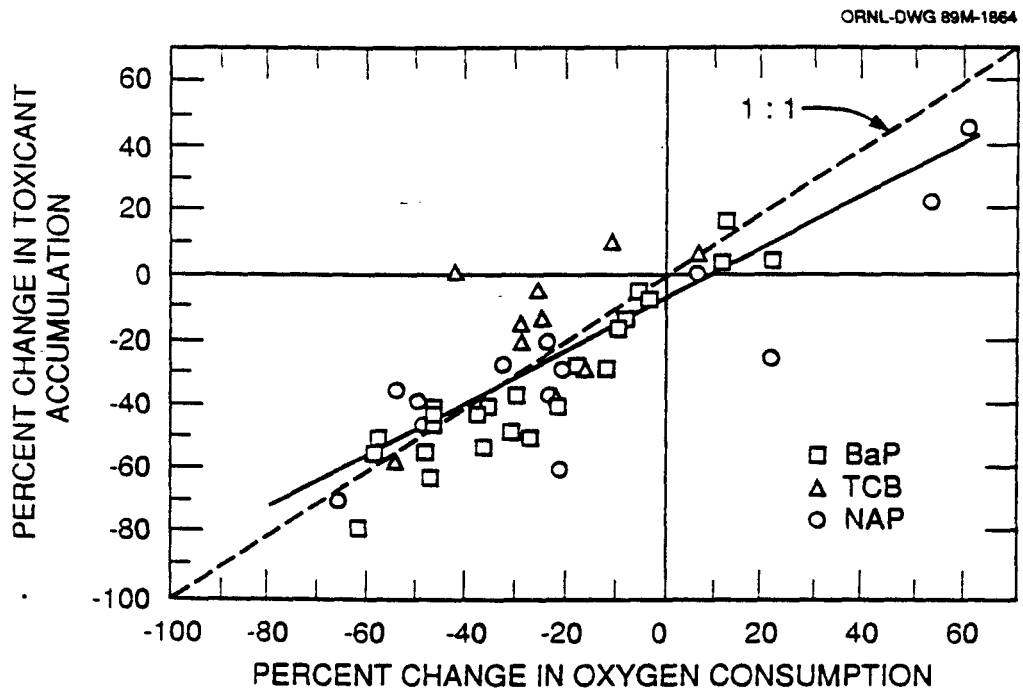

Figure IV-5. The linear relationship between changes in oxygen consumption and contaminant uptake by trout during an acute decrease in temperature.

The regression shown is for values pooled from the exposures with BaP, NAP, and TCB. Individual regression equations for the three compounds are listed in Table IV-3 (p. 82). The regression of the pooled data was significantly different from a 1:1 relationship between the two variables, indicated by the dashed line.

Table IV-3. Regression equations for the percentage changes in oxygen consumption (x) plotted versus the percentage change in contaminant uptake (y). Consumption and uptake values were measured at 14, 10, and 8 °C.

Compound	Regression Equation	n	r^2
BaP	$y = 0.94x - 8.62$[a,b]	24	0.87[c]
22'55'-TCB	$y = 0.67x - 0.83$[b]	12	0.34[d]
NAP	$y = 0.70x - 13.36$[b]	14	0.78[c]

[a]Slope not significantly different from 1 ($p < 0.05$)

[b]Intercept not significantly different from 0 ($p < 0.05$)

[c]$p < 0.001$

[d]$p < 0.05$

initial 7 °C drop in temperature, the Q_{10} for both variables was approximately two, signifying a halving of the rates of uptake for each molecule. However, for both variables Q_{10} changed dramatically during the final 2 °C drop in temperature. Between 10 and 8 °C, there was a sevenfold decrease in oxygen consumption rates and a tenfold decrease in contaminant uptake rates. Hughes and Roberts (1970) calculated a similar Q_{10} (4.2) for the oxygen consumption of brook trout as temperature was increased from 5 to 10 °C, whereas the Q_{10} value for the overall temperature change (5 to 20 °C) was 2.24, similar to the value calculated for the range of 17 to 8 °C in the present study (Table IV-1, p. 74). In the present study oxygen consumption and contaminant uptake are both calculated as the products of exposure concentration, uptake efficiency, and ventilation volume. Among these three variables, the only variable that also experienced significant changes in Q_{10} during that temperature interval was ventilation volume. Thus, the large decreases in oxygen consumption and contaminant uptake were probably due to the large decreases in ventilation volume.

Effects of Prolonged Exposure on Respiration and Uptake

No further changes in trout respiration or oxygen and contaminant uptake were measured after 18 h at 8 °C, the final experimental temperature (Fig. IV-6). There were no significant differences between measurements taken at 1 h and 18 h at 8 °C ($p < 0.05$, Paired comparisons t-test). This observation agrees with literature observations that for rainbow trout more than 18 h is required before acclimatory changes in respiration are observed following changes in

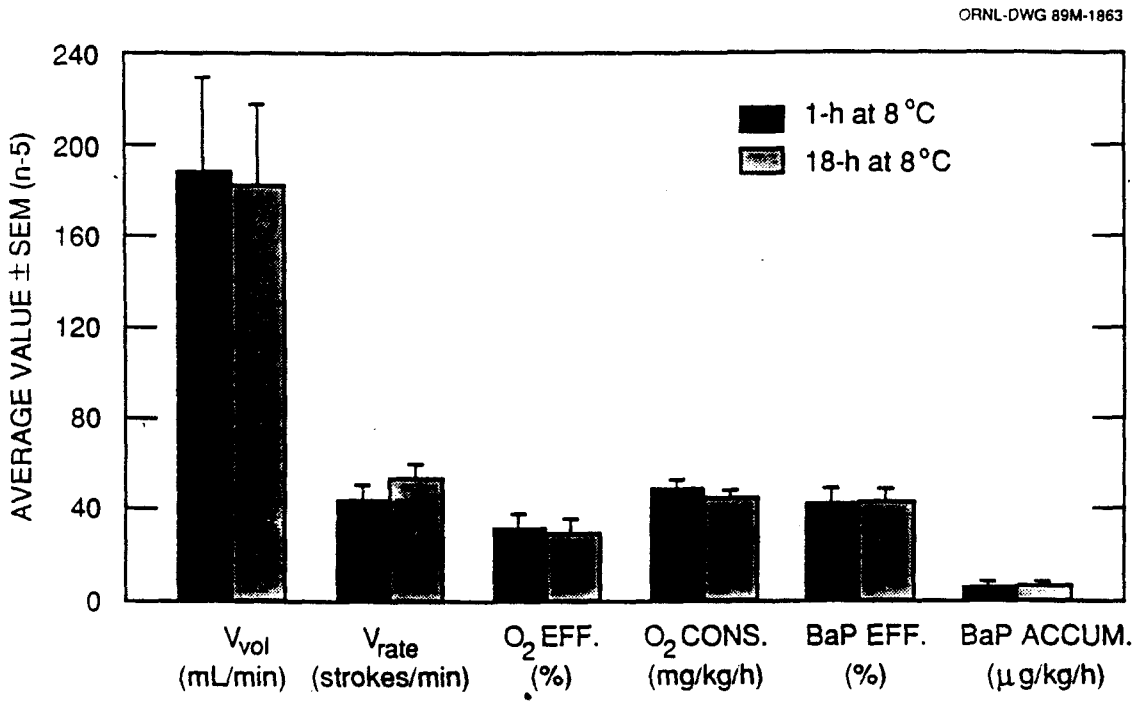

Figure IV-6. Comparison of trout respiration and uptake of oxygen and toxicants after 1- and 18-h exposure at 8 °C.

Histograms represent the mean value (±SEM) for each measurement (n = 5 trout). There were no significant differences between values measured at 1 and 18 h for each variable ($p < 0.05$, Paired comparisons t-test).

temperature (Peterson and Anderson, 1969; Campbell and Davies, 1975). Furthermore, stabilization of respiration and uptake measurements indicated that no overshooting occurred, and provides evidence that the decreases measured were in direct response to changes in experimental temperature, and were not influenced by time or the rate of temperature change.

Physiological Implications

Decreased oxygen consumption by trout as the experimental temperature was lowered reflected the trout's decreased metabolic demand at the lower temperature. Mean oxygen consumption values measured in these experiments at the lowest temperatures were consistent with with other measurements made using spinal-transected trout at 11 °C (McKim and Heath, 1983; McKim et al., 1986, Bradbury et al., 1987).

Rainbow trout and other salmonid species are "oxygen regulators," a term describing organisms that are able to maintain adequate blood oxygen levels in hypoxic or hyperoxic environments by changing their ventilation rates, ventilation volumes, and/or gill oxygen extraction efficiencies (Randall and Daxboeck, 1984; Hochachka, 1987). Changing ventilatory functions seems to be the predominant mechanism by which fish adjust oxygen uptake during hypoxia or hyperoxia. White sucker (Catostomus commersoni) maintained stable levels of oxygen consumption under hyperoxic conditions by decreasing ventilation volume by 50%, with no changes in oxygen uptake efficiency by the gills (Wilkes et al., 1981). Similarly, Wood and Jackson (1980) measured large

decreases in the ventilation volumes of rainbow trout exposed to hyperoxia. Gobius cobitis regulated oxygen consumption during hyperoxia by decreasing ventilation rates as the environmental PO_2 increased (Berschick et al., 1987). Smith and Jones (1982) found that trout exposed to both hypoxic and hyperoxic conditions maintained basal oxygen consumption rates and a constant blood PO_2 level primarily through compensatory changes in ventilatory functions. Hypoxia-induced increases in fish ventilation volume have been observed in a variety of fish species, and in all cases fish maintained stable oxygen consumption values through ventilatory compensation (Davis and Cameron, 1971; Kerstens et al., 1979; Lumholt and Johansen, 1979; McKim and Goeden, 1982). These results indicate that compensatory changes in ventilatory functions provide a mechanism for the physiological regulation of oxygen consumption and allow fish to maintain adequate internal blood PO_2 levels.

In these experiments decreases in oxygen uptake efficiency and ventilation volume both contributed to the observed reductions in oxygen consumption. However, the changes in ventilation volume were much greater than changes in uptake efficiency at all sampling times, and seemed to be the primary force driving consumption levels downward during the decrease in environmental temperature. This observation agrees with the previously described changes in ventilatory functions by fish exposed to hypoxia and hyperoxia, and supports the hypothesis that fish regulate oxygen consumption primarily by changes in ventilation volume, resulting in alterations in the amounts of oxygen-laden water flowing across the gill membrane.

Measurements of oxygen consumption are direct reflections of the combination of the effects of the volume of water flowing across the gills (ventilation volume), the concentration of oxygen in the exposure water and the efficiency by which these molecules are extracted by the gills (uptake efficiency). Temperature-induced changes in uptake efficiency may also affect the net consumption of oxygen and any other passively diffusing molecules present in the exposure water. Excluding the physiological interactions between oxygen and hemoglobin, oxygen uptake efficiencies should be dependent upon two variables: factors affecting the contact time and proximity of oxygen molecules in the ventilatory water to the gill membrane surface, and factors affecting the diffusional capacity of the gill membrane.

Some authors have hypothesized that fish ventilatory flows may limit uptake efficiencies in fish based on models of contaminant accumulation under changing environmental conditions, but without directly measuring fish ventilation (Gobas et al., 1986; Opperhuizen and Schrap, 1987). Others have measured an inverse relationship between trout ventilation volumes and oxygen uptake efficiencies under hypoxic conditions (Kerstens et al., 1979; McKim and Goeden, 1982). The inverse relationship between fish ventilation and uptake efficiency was proposed to result from a decreased contact between oxygen molecules and the gill membrane surface due to higher ventilatory flows (increased ventilation volumes and rates). This would explain the observed reductions in oxygen uptake efficiency coupled with a two to fourfold increase in ventilation volume observed

in fish during periods of hypoxia (Kerstens et al., 1979; McKim and Goeden, 1982). Conversely, decreases in ventilation should augment uptake efficiency by increasing the contact of the gill membrane with oxygen, but only until a steady-state transfer is attained. After a steady-state diffusion has been reached, oxygen uptake efficiencies would be affected only by internal physiological cues, such as regulation of hemoglobin's affinity and capacity for binding oxygen or by factors affecting the diffusional capacity of the gill membrane.

In these experiments oxygen uptake efficiencies were lowered with temperature decreases, but at the same time, ventilation volumes and rates were also decreased. A graph of ventilation volumes plotted against contaminant and oxygen uptake efficiencies revealed a perfect scattergram (Fig. IV-7). These results imply that at lower ventilatory flows steady-state diffusion has been reached and oxygen uptake efficiencies are not controlled by fish ventilation.

An alternate hypothesis is that reductions in uptake efficiencies may have been caused by temperature-induced changes in the gill membrane permeability. At reduced temperatures, biological membranes tend to be less fluid and less permeable to diffusing molecules (Hazel, 1979, 1984; Isiah, 1979). Without acclimation of the membrane lipids to colder temperatures, fluidity decreases may eventually result in a phase change in the membrane from a liquid to a gel state, resulting in a significant loss of membrane permeability (Hochachka and Somero, 1971). The temperature-dependent decreases in uptake efficiencies (Fig. IV-3, p. 82) are consistent with reduced gill membrane permeability as the experimental temperature was lowered.

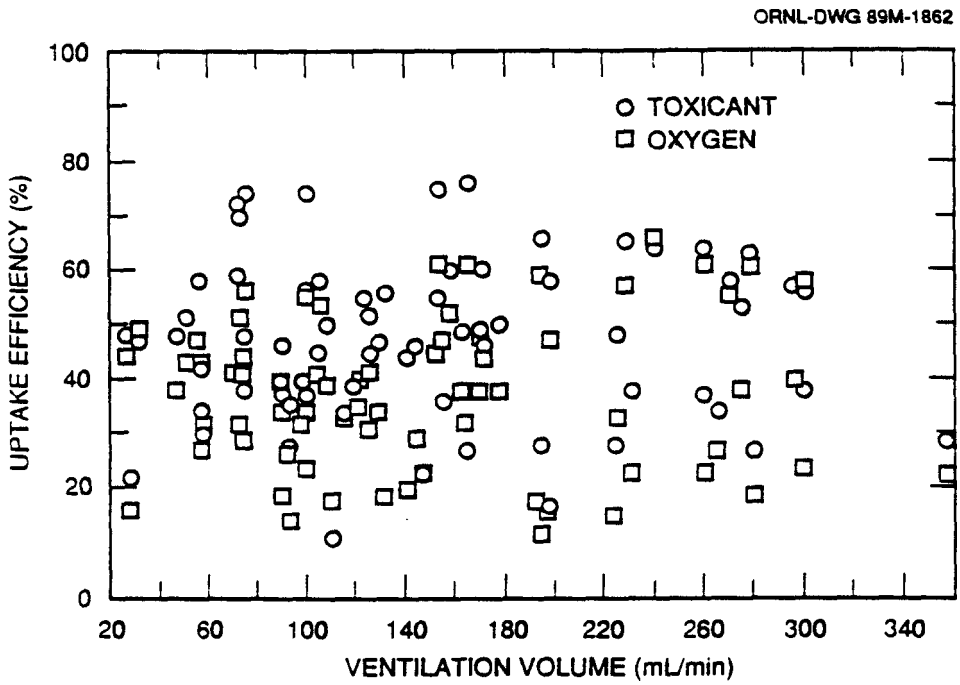

Figure IV-7. The relationship between trout ventilation volumes and uptake efficiencies for oxygen and BaP, NAP, and TCB.

The random scatter of the data points indicates that changes in trout ventilation volume do not cause consistent or predictable changes in uptake efficiencies during an acute decrease in temperature.

Constant Q_{10} values for oxygen uptake efficiency throughout the entire range of temperatures (Table IV-1, p. 74) imply that the decreases in uptake efficiency changes monotonically, without any abrupt decreases in the uptake efficiency of either oxygen or contaminant molecules, which might be indicative of phase changes.

Using the Fick's equation, the relationships between environmental temperature, physicochemical properties of membranes, and properties of diffusing molecules can be probed. Fick's first law has been used to quantify the rate of diffusion of oxygen and other molecules across the gill and other membranes (Butler and Metcalf, 1983; Gobas et al., 1986; Barber et al., 1988). A general form of the Fick's equation is:

$$M = (DA\Delta C)/X \qquad (1)$$

where M (nmol/min) is the flux of the diffusing molecule, D (cm^2/s) is the diffusion constant, A (cm^2) is the surface area of the gills, ΔC (nmol/L) is the concentration difference between blood and water for the diffusing molecule, and X (μm) is the membrane thickness. Diffusion constants (D) were calculated from the data on oxygen and contaminant uptake at the acclimation temperature (17 °C) and after the temperature change (8 °C), using a rearrangement of Eq. 1, substituting an average value from the literature (4.4 μm) for X, and using gill surface areas calculated for each trout from the following equation relating surface area to fish weight (W): $A = (3.14)W^{0.932}$ (Hughes, 1972; Hughes and Perry, 1976). The diffusion constants for oxygen and contaminants are presented in Table IV-4. Diffusion

Table IV-4. Diffusion constants (±SEM) calculated for oxygen and contaminants. Values were calculated using uptake measurements taken before and after the acute temperature change (at 17 and 8 °C, respectively) (Eq. 1). There were significant differences between the 17 and 8 °C values for each fish (Paired comparisons t-test, $p < 0.05$).

Exposure	Oxygen		Toxicant	
	17° C	8° C	17° C	8° C
BaP (n=8)	3.47×10^{-7} (1.92×10^{-8})	2.77×10^{-7} (1.67×10^{-8})	8.53×10^{-7} (4.89×10^{-8})	3.70×10^{-7} (2.77×10^{-8})
NAP (n=6)	2.87×10^{-7} (3.85 x 10-8)	2.58×10^{-7} (3.71 x 10-8)	8.35×10^{-7} (1.27 x 10-7)	2.83×10^{-7} (4.38 x 10-8)
TCB (n=6)	5.34×10^{-7} (3.21×10^{-8})	3.86×10^{-7} (1.83×10^{-8})	6.80×10^{-7} (4.39×10^{-8})	5.39×10^{-7} (4.71×10^{-8})

constants determined for oxygen and contaminants were both significantly lower after the experimental temperature was decreased from 17 to 8 °C for all exposures ($p < 0.05$, Paired comparisons t-test). These data imply that gill membrane diffusion processes are reduced at lower temperatures.

Variables from the Fick's equation (Eq. 1) can also be used to calculate a transfer factor (T), which describes the diffusional ability of the gills (modified from Randall et al., 1967):

$$T = M/\Delta C \tag{2}$$

Using measurements from our metabolic chamber exposures, T can be calculated from measured values for the oxygen or contaminant concentration ($[C]_A$), uptake efficiency (Eff), and ventilation volume (V_G) at each sampling time:

$$T = \{([C]_A)(Eff)(V_G)\}/[C]_A \tag{3}$$

By rearrangement and substitution of Eq. 1, the following relationship is also found for T:

$$T = DA/X \tag{4}$$

Combining Eq. 3 and 4 and solving for Eff yields:

$$Eff = (DA)/(XV_G) \tag{5}$$

Based on this equation increases in ventilation volume or gill membrane thickness would be predicted to result in decreased uptake efficiencies. This model also predicts that uptake efficiencies would be augmented with increased gill surface area, a variable that is species specific, and is related to fish weight (Hughes, 1972) and an increased diffusion constant, which is related to physicochemical properties of the diffusing molecule and the membrane. Stryer (1981) presents an equation to calculate D for molecules diffusing through a model membrane or solvent system:

$$D = (kT)/(6\pi nr) \qquad (6)$$

where k is the Boltzman constant, T is temperature, n is the diffusion layer viscosity, and r is the frictional resistance factor for the diffusion media. From Eq. 5 and 6 it follows that uptake efficiency would be influenced by changes in temperature and changes in the diffusional resistances within the membrane, including viscosity (also temperature controlled) and the frictional resistance. Frictional resistance is also likely to be influenced by physicochemical properties of the diffusing molecule, such as molar volume and chemical interactions between the molecule and the membrane. These predictions combined with the determination of decreased diffusion constants at lower temperatures lend further support to the hypothesis that reductions in uptake efficiencies at lower temperatures result from temperature-induced decreases in gill membrane permeability.

Based on experimental results and model predictions, it appears that independent factors may control changes in uptake efficiency and ventilation volume. In these experiments, gill membrane permeability was probably reduced at the lowered temperatures due to the effect of temperature on membrane fluidity, causing the measured reductions in oxygen and contaminant uptake efficiencies. Changes in ventilation volume may reflect an adaptation to the fish's reduced oxygen demand at lowered temperatures and are probably controlled by an internal oxygen receptor, similar to the receptor thought to control heart rate during hypoxia described by Smith and Jones (1978) and others.

Toxicological Implications

These data confirm that a significant linear relationship exists between changes in trout oxygen consumption rates and hydrophobic contaminant uptake rates. Although there was not a 1:1 relationship between the two variables, within a reasonable physiological range of oxygen consumption (decreases between 0 and 60% of the original consumption rates) contaminant uptake values were within 10% of the oxygen consumption values. Based on these considerations it seems reasonable to predict the relative magnitude of changes in compound uptake due to environmental and physiological changes based on established relationships on their effect on oxygen demand.

For example, Rao (1968) measured oxygen consumption in rainbow trout at 5 and 15 °C, finding a 49% decrease in consumption at the lower temperature relative to consumption at 15 °C. Accumulation of DDT by rainbow trout with similar weights (30-110 g) was decreased by

45% over the same temperature interval (5 and 15 °C) (Reinert et al., 1974). This experimentally determined value (45%) is almost identical to the percentage change in accumulation (49%) that would have been predicted based on Rao's data on oxygen consumption.

A large database exists relating oxygen consumption to a number of physiological and environmental variables. In many cases this relationship has been modelled, so that oxygen consumption under varying conditions can be calculated. Due to the linear relationship found between oxygen consumption and contaminant uptake in this study, these calculated or measured changes in oxygen consumption may be predictive of the magnitude and direction of changes in contaminant uptake by fish gills. Based on the changes in oxygen consumption, literature values for contaminant uptake, either measured experimentally or calculated using QSARs, can then be adjusted for the physiological or environmental change.

In the previous example of DDT accumulation by rainbow trout (Reinert et al., 1974), predictions of DDT accumulation at 5 °C could be made by multiplying the value obtained at 15 °C by the percentage decrease in oxygen consumption (45%) measured by Rao (1968) over the same temperature interval (Table VI-5). The predicted accumulation of 3.35 μg/g is similar to 3.76 μg/g, the body burden measured at 5 °C. Conversely, accumulation of DDT at 15 °C predicted fron values obtained at 5 °C were also similar to the measured values.

Changes in oxygen consumption have been related to other environmental and physiological changes. The relationships developed between temperature-induced changes in oxygen consumption and

Table IV-5. Measured and predicted values for DDT accumulation by rainbow trout at 5 and 15 °C. Calculations of predicted accumulation are based on the percentage changes in oxygen consumption (data from Rao, 1968 and Reinert et al., 1974).

DDT Accumulation (μg/g)	5 °C	15 °C
Measured values[a]	3.76	6.82
Predicted values[b]	3.35	6.26

[a]Data from Reinert et al., 1974

[b]Predicted values for DDT accumulation at 5 and 15 °C were calculated using the percentage differences in oxygen consumption at these temperatures calculated from data from Rao, 1968. The difference in oxygen consumption from 15 to 5 °C was 49.1%. The difference from 5 to 15 °C was 204%.

contaminant uptake may also be applicable in other situations involving similar changes in oxygen consumption.

Oxygen consumption (standard metabolic rate) has been mathematically related to fish weight in many fish species (eg. Fry, 1957; Beamish, 1964a; Glass, 1969; Brill, 1987; and others). The typical relationship between oxygen consumption and fish weight is a power function with the general equation: $y = a\ x^b$, where y is oxygen consumption (mg O_2/hr), x is fish weight (kg), and a and b are species-specific fitted parameters. For most fish species, the fish weight exponent (b) is less than one, signifying that weight-specific oxygen consumption decreases as fish weight increases. Therefore over the lifespan of a fish, weight-specific oxygen consumption would be expected to decrease. Based on the results of this study, these decreases in oxygen consumption would be expected to result in decreased weight-specific uptake of waterborne hydrophobic contaminants as fish size increased as a result of growth and maturity.

Beamish (1964b) measured two and threefold increases in the metabolic rates of male brook and brown trout during their spawning seasons. Metabolic rate increases in female brook trout were similar to those in males, while female brown trout had no increase in metabolic rates during spawning. Since spawning periods can last up to several months for some fish species, these seasonal changes in oxygen consumption could result in significant changes in contaminant uptake by males and females during spawning periods. For gravid females found to have increased oxygen consumption during spawning,

increased contaminant uptake during spawning predicted by our hypothesis could have deleterious effects on fecundity by affecting the success of oogenesis or egg maturation. The higher doses of the contaminant could be transferred to the developing embryos via the yolk, and impair growth and development of the young. In addition, a reduced ability to detoxify certain contaminants resulting from estradiol-induced reductions in the activity of the MFO detoxification systems could also greatly increase the contaminant body burden of spawning females (reviewed in Jimenez, 1989).

Changes in oxygen consumption have also been related to exercise and activity levels in fish. At a maximum swimming speed, exercised rainbow trout had nearly an eightfold increase in oxygen consumption, compared to trout resting metabolic rates (Kiceniuk and Jones, 1977). Ventilation volumes were elevated proportionally, to achieve the increase in oxygen consumption by increasing the exposure of the gill membrane to oxygen in the ventilatory water. Swimming white suckers (Catostomus commersoni) had a similar increase in oxygen consumption compared to nonswimming fish, although two more quiescent species, carp (Cyprinus carpio) and brown bullhead (Ictalurus nebulosus) had no change in oxygen consumption with increased activity (Saunders, 1962). Although the relationship between oxygen consumption and activity may not be consistent in all fish species, for species that do increase oxygen consumption with activity, contaminant uptake via the gills may be proportionally increased.

This study has demonstrated that changes in oxygen consumption occur in trout exposed to acute changes in temperature. These changes

in oxygen consumption have been quantitatively related to changes in the uptake of BaP, NAP, and TCB via water exposure. This relationship can be useful in making predictions of contaminant uptake using well-documented physiological changes in oxygen demand, and will enable a more accurate prediction of contaminant dose and accumulation by fish over chronic exposure regimes, including seasonal exposures and exposures over an entire lifecycle.

Summary and Conclusions

1. Acute temperature decreases resulted in significant decreases in oxygen consumption rates and oxygen uptake efficiencies in trout at reduced temperatures.

2. There was no relationship between trout ventilatory functions and uptake efficiencies for either oxygen or contaminants. This finding suggests that uptake efficiency and gill ventilation are not linked at low ventilation volumes and rates. In these experiments trout ventilatory functions seem to be regulated by changes in metabolic demand and decreased oxygen and contaminant uptake efficiencies must be caused by temperature-induced decreases in gill membrane permeability.

3. There was a significant linear relationship between trout oxygen and contaminant uptake efficiencies over the temperature range from 17 to 8 °C. This relationship implies that both oxygen and contaminant molecules enter the fish by passive diffusion.

Furthermore, evidence was presented indicating that diffusion of contaminant and oxygen molecules was controlled by temperature-induced changes in membrane permeability.

4. There was a linear relationship between changes in oxygen consumption and contaminant uptake rates. This relationship forms the basis for predicting contaminant uptake using the magnitude of changes in metabolic demand or oxygen consumption under a variety of physiological and environmental conditions as the basis for extrapolation.

CHAPTER V

GENERAL CONCLUSIONS

Contaminant uptake by the gills of fish is hypothesized to be influenced by both environmental and physiological factors that interfere with the uptake process either by altering the physicochemical form of the contaminant, altering the diffusional capacity of the respiratory membrane, or by altering the fish's respiratory demand. This study has examined an environmentally relevant example of each of these factors. Quantitative data has been presented relating changes in contaminant uptake to changes in respiration, changes in gill diffusion properties, and changes in the physicochemical form of the contaminant.

The binding of dissolved or particulate sorbents to toxicants in the aquatic environment appears to reduce the bioavailability of the toxicant by decreasing the net uptake of the toxicant by the gills by an amount proportional to the percentage of the toxicant that is bound to the sorbent. Reductions in BaP and TCB uptake were correlated with measured reductions in the concentration of the contaminant in true solution, i.e., the concentration not bound to humic acid. The degree of binding of the toxicant to humic acid was related to the compound's affinity for binding to humic acid (K_p), which is quantitatively related to the compound's hydrophobicity and the physical characteristics of the DOM. These quantitative relationships form a basis for extrapolation of the effects of binding to natural DOM sources, based on measured K_p values or on models relating binding

properties to physicochemical properties of the sorbent and sorbate. Through these modelled relationships, the potential exists for extrapolation of the effects of binding to DOM to include estimations of reductions in uptake for other hydrophobic toxicants in the presence of DOM in the natural environment.

Uptake of contaminants is also hypothesized to be influenced by physiological changes within the exposed organism. Potential physiological changes could be induced by internal biochemical cues or by external events, controlled by the fish's environment. This study addressed an example of each type of physiological changes elicited by internal and external cues, by quantitating the changes in contaminant uptake associated with environmental temperature change and in the presence of chlorine, a gill damaging agent. Each of these experimental approaches altered fish respiration and resulted in predictable changes in contaminant uptake.

The efficiency of oxygen and toxicant uptake by trout appeared to be controlled primarily by changes in gill membrane permeability and diffusion capacity. In exposures with concomitant exposure to chlorine (Chapter III) and with acute temperature change (Chapter IV) uptake efficiencies of both molecules were decreased. The mechanism of action of each of these variables on membrane permeability and gill diffusional capacity were different. Chlorine-induced damage to gill lamellae was proposed to reduce the functional gill surface area and diffusion distance across the gill membrane. This hypothesis is supported by the time-dependent damage to gill lamellae gill seen in chlorine-exposed trout. Reduced temperature was proposed to reduce

the fluidity and permeability of the gill membrane. Although no direct measurements of membrane fluidity were made, the observed reductions in contaminant and oxygen uptake efficiencies were hypothesized to result from temperature-induced changes in membrane fluidity.

In all exposures involving changes in fish respiration, regardless of the effect on uptake or the experimental design, reductions in toxicant and oxygen uptake efficiencies were directly related to the extent of exposure to the proposed inducer of physiological changes in membrane diffusivity, due either to the magnitude (ie. temperature change experiments) or the duration of exposure (concomitant exposure to chlorine). The linear correlations between oxygen and contaminant uptake efficiencies observed in both experimental approaches lend further support to this hypothesis, since both molecules are assumed to enter the gill by passive diffusion across the gill membrane. Thus, changes in membrane permeability and diffusional capacity would be expected to affect the diffusion of each molecule to a similar extent. Although this relationship was not always a perfect correlation resulting in a 1:1 relationship between oxygen and contaminant uptake efficiencies, the magnitudes of the variables were in very close agreement for all compounds in both experimental treatments. In both cases where a significant 1:1 relationship was not observed (BaP and NAP exposures), oxygen uptake efficiencies were consistantly higher than contaminant uptake efficiencies. This is not surprising, considering the differences in the relative sizes and molecular weights of oxygen and these contaminant molecules and the

presence of other physiological mechanisms which will augment oxygen diffusion, but will not affect contaminant uptake, including mechanisms that alter the affinity and capacity for fish hemoglobin to bind to oxygen.

In both experimental treatments, oxygen consumption was directly related to contaminant uptake, regardless of the magnitude and direction of ventilatory changes. Reductions in trout ventilation at lower temperatures resulted in lowered oxygen consumption which was mirrored by similar reductions in contaminant uptake. During sublethal exposures to chlorine, oxygen consumption and contaminant uptake both were stabilized, resulting from increases in fish ventilatory functions, which were hypothesized to compensate for the lowered uptake efficiencies. In both approaches, alterations in trout ventilation had the same effect on the uptake of both oxygen and contaminant molecules by fish gills. By adjusting ventilatory functions, trout maintained adequate oxygen consumption levels to meet their respiratory demand.

These results confirm that the environment can have a direct and significant modulating effect on contaminant uptake by fish, through both natural and anthropogenic processes. Furthermore, the quantitative relationships presented in these studies can be incorporated in models of contaminant uptake to account for environmentally-relevant changes in contaminant bioavailability and fish respiration.

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