# UPTAKE, BIOAVAILABILITY, AND BIOACCUMULATION OF LIPOPHILIC XENOBIOTICS IN JUVENILE RAINBOW TROUT: AN ASSESSMENT OF THE ROLE OF GILLS AND SUSPENDED PARTICLES AS A SOURCE OF CONTAMINANTS

by

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lation of Lipophilic Xenobiotics in
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of the Role of Gills and Suspended
Particles as a Source of Contaminants.

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# ABSTRACT

Xenobiotics generally enter fish via the gills from the water when they breathe or via the gastrointestinal (GI) tract from ingested food. The relative contribution of gill uptake versus GI tract uptake to the overall chemical burden in fish is still under much debate and is the general focus of my thesis. Of particular interest was the influence of the lipid solubility of chemicals (as expressed by the octanol-water partition coefficient, log $K_{ow}$) and of the presence of suspended particles in the water (with low or high organic carbon content) on chemical uptake via these two routes. I used rainbow trout (*Oncorhychus mykiss*) as the test animal and three test chemicals that had different lipid solubilities, i.e., 1,2,4-trichlorobenzene (1,2,4-TCB), log $K_{ow}$ 3.98, 1,2,3,4,5-pentachlorobenzene (PeCB), log $K_{ow}$ 5.03 and 2,2'4,4'6,6'-hexachlorobiphenyl (HCBP), log $K_{ow}$ 7.55.

My first objective was to test the hypothesis that the gills are a more important uptake route for lipophilic chemicals than GI tract. Experiments were performed in which fish were exposed to either contaminated water or food. Uptake rate constants and concentration factors were calculated from measurements of water chemical concentrations, food chemical concentrations and fish body burden. Gill uptake rate constants for all three test chemicals were about 5-orders of magnitude greater than those for GI tract uptake. Further, a model simulating simultaneous exposure to water and food of differing chemical concentration ratios indicated that the two uptake routes would contribute equally to the fish body burden only when the food:water concentration ratio

was between $10^5$ to $10^{5.3}$ for the three test chemicals. According to the expected food/water chemical ratios in nature, the gills are predicted to act as the primary uptake route for 1,2,4-TCB and PeCB with log $K_{ow}$ values of 3.98 and 5.03, respectively, while the GI tract will contribute mostly to the uptake of HCBP with a log $K_{ow}$ value of 7.55.

My second objective was to test the hypothesis that the chemicals associated with suspended sediments containing low organic carbon from the Fraser River are bioavailable for gill uptake. Fish, with and without pharyngeal plugs to prevent ingestion, were exposed to the three test chemicals in aquaria containing sediments from the Fraser River. Uptake from the bottom sediments did not occur because the body burden did not differ between fish exposed to suspended sediments only and to both suspended sediments and bottom sediments. A 6- to 18-day exposure to sediment-laden water resulted in a rapid gill uptake of test chemicals within the first two days. A mass balance of the test chemicals in the water, sediment and fish compartments revealed that the body burden of the fish could not be accounted for solely by the amount of chemicals dissolved in the water at the time when the fish were introduced to the aquaria. I concluded that lipophilic chemicals associated with the suspended sediments were bioavailable to the fish via the gills.

My third objective was to test the hypothesis that dissolved organic matter does not necessarily reduce the uptake of lipophilic chemicals in fish. Body burdens of test chemicals were measured after exposure to different concentrations of humic acid. The results showed that only higher concentrations of humic acid (4.81 and 14.32 mg/L) reduced the uptake of HCBP to fish but did not influence the uptake of 1,2,4-TCB and

PeCB. A low concentration of humic acid (1.54 mg/L) actually elevated the accumulation of 1,2,4-TCB and PeCB compared with other concentrations of humic acid.

Collectively, my results show that the role of gill uptake of lipophilic chemicals from water, from suspended sediment and from humic acid may have been underestimated in the past, and that the factors affecting uptake of lipophilic xenobiotics in natural waters are likely more complex than previously thought.

# DEDICATION

**To my Mother, my Nephew and Nieces, Tao, Ming, Lu and Chen.**

# ACKNOWLEDGMENTS

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# CHAPTER 1

# REVIEW OF THE LITERATURE AND WORKING HYPOTHESES

# 1. INTRODUCTION

Xenobiotics are man-made chemicals that are often characterized by their persistence in the environment. Examples include polychlorinated biphenyls (PCB), dioxins, and organochlorine insecticides. Many xenobiotics are lipophilic and hydrophobic, i.e., they have greater solubility in lipids than in water, and are often toxic to organisms. The log $K_{ow}$ values (the partition coefficient of a chemical between octanol and water) for many xenobiotics ranges from 3 to 7. When such chemicals are released into aquatic systems, they can become bioavailable to fish. Bioavailability defines the degree to which a contaminant in an environment is available for uptake (Hamelink et al. 1994). Consequently bioaccumulation of these xenobiotics may occur, especially containing fat tissues, i.e., adipose tissue, myelin tissue around nerves, and fat droplets in muscle and blood. Thus uptake, bioaccumulation and bioavailability of lipophilic xenobiotics in fish are of considerable interest to the general population and toxicologists alike.

Bioaccumulation and bioavailability of lipophilic xenobiotics in fish involves many factors, such as chemical concentrations in different compartment in aquatic systems, the physical and chemical quality of water systems, the exposure routes and the hydrophobicity ($K_{ow}$ value) of a chemical. This literature review focuses on the main uptake routes in fish, namely the gills and the gastrointestinal (GI) tract, and one major aquatic compartments, i.e. suspended particles, that may affect the bioaccumulation of lipophilic xenobiotics in fish.

# 2. LIPOPHILIC CHEMICAL UPTAKE: GILLS AND GASTROINTESTINAL TRACT

## 2.1 INTRODUCTION

Uptake of lipophilic chemicals across the gills may involve bioconcentration, defined as the process by which an organism increases the tissue concentration of a chemical to many times that of the environment through transfer across a surface from water (Gobas et al. 1997). Skin may also contribute as a exchange surface but is not considered as a major uptake route in this study. Uptake across the GI tract may involve biomagnification, defined as the process by which an organism increases the tissue concentration of a chemical higher than that in the food it consumes (Gobas et al. 1997). The relative importance these two uptake routes is determined by morphological and physiological features of the absorption membrane, absorption rate, and exposure concentrations in the environment.

## 2.2 MORPHOLOGY AND PHYSIOLOGY

### 2.2.1 Gills

Gills represent the major portion of the external body surface area of fish and, as the primary respiratory organ, are the main entry site for $O_2$ dissolved in the water. The gill structure is well adapted for rapid uptake of oxygen, a lipophilic gas. The primary lamellae, or gill filaments, support two rows of secondary lamellae, which range in frequency from 10/mm to 60/mm (Hughes 1982); higher numbers are found in the more active species. This fine basket-like sieve of secondary lamellae provide both a large

surface area, which is 5 to 15 cm$^2$/g in a free-living and active fish (Evans 1993), and a short diffusion distance for the movement of $O_2$, $CO_2$, ions, water, and other chemicals to reach the blood circulation. The water-to-blood diffusion distance to cross the secondary lamellae is rarely more than 10 µm and often 1-2 µm (Hughes 1984; Laurent 1984). In addition, the blood and water flow in opposite directions (a counter current exchange) and this increases the efficiency of exchange of substances between water and blood. Blood and water transit times in fish gill lamellae are of the order of 1 second. This means that the transfer of $O_2$ from the water to the blood occurs both rapidly and efficiently.

2.2.2 GI tract

Unlike the gills, the fish GI tract has a much thicker diffusion barrier between food and the tissue (lamina propria) where blood capillaries are located. The structure of this the mucosa of fish GI tract consists of microvilli with 1-3 µm in length and mucosa epithelium with typical high columnar cells over 100 µm in length, followed by basal lamina and lamina propria and muscularis mucosa (Aida et al. 1995). When all these layers are included, the GI tract has a 100 to 1000 µm blood-food diffusion distance which is much larger than the gill blood-water diffusion distance. The surface area of the fish GI tract is about 1/10th of the gill, i.e. 1.5 to 2.0 cm$^2$/g for small fish (Steffens 1989). Digestion and uptake of food is usually measured in minutes and hours rather than seconds.

## 2.3 Uptake mechanism from gills and GI tract

The processes of gill uptake and GI tract uptake operating in fish have different control characteristics. Therefore, knowledge of the mechanisms involved in these processes is helpful in understanding the difference between the processes of bioconcentration and biomagnification.

### 2.3.1 Gill uptake

A waterborne lipophilic xenobiotic moves from the water into a fish via gills can be described as: 1) movement of water with the dissolved chemical through the gill lamellar "sieve" by the branchial pump; 2) diffusion of the chemical through water to gill epithelium; 3) removal of the chemical from the gill by the blood (Erickson et al. 1990; Hayton et al. 1990) and 4) removal of the chemical from the blood into tissues where storage, metabolism or excretion may occur. Most studies agree that lipophilic xenobiotics move across biological membranes by simple passive diffusion, which can occur rapidly across the lipid bilayer of the biological membrane provided a gradient exists. The rate of transfer of a chemical (solute) can be defined by the Fick Diffusion equation:

$$dQ_s/dt = D_s \cdot A \cdot dC_s/d_x \qquad\qquad (1.1)$$

where $dQ_s/dt$ is the rate of diffusion for a solute (chemical) s, $D_s$ is the diffusion coefficient of s, A is the cross-sectional area through which solute is diffusing, $dC_s$ is the concentration gradient of s, and $d_x$ is the diffusing distance (Eckert et al. 1988). $D_s$ varies with the nature and molecular weight of the chemical and of the solvent, i.e., water and cytoplasm. The chemical gradient dCs is clearly very important, since it directly determines the rate of diffusion. $d_x$ is also very important to favor diffusion when it is very short.

Because the ease with chemicals penetrate cell membrane and influence $D_s$ is related to the log $K_{ow}$, it is not surprising that a linear relationship between chemical partition coefficient and bioconcentration has been found in numerous studies for

chemicals with log $K_{ow}$ values ranging from 2 to 6 (Bruggeman et al. 1984; Oliver et al. 1984; Sabljic 1987; Connell 1990; Hawker 1990; Randall et al. 1990; Smith et al. 1990; Gobas 1990a; Nenza 1991). Above a log $K_{ow}$ = 6, chemicals tend to bioconcentrate less because their water solubility significantly decreases.

The molecular size of a chemical may also play a role here and was studied by some researchers. Opperhuizen et al. (1985) predicted that chemical molecules with minimum internal cross-sections greater than 9.5 Å would not permeate the polar holes of the epithelial membranes. Brooke et al. (1986) found that bioconcentration declined when restricted to chlorohydrocarbons having a molecular weight over 350. Moser et al. (1990) concluded that molecular weight exceeding 450 may reduce gill uptake. However, in another study, hydrophobic disperse dyes with a molecular size even smaller than 450 resulted low bioconcentration (ETAD 1990). Thus, there is no consistent result on this issue.

## 2.3.2 GI tract uptake

Due to a diversity of feeding strategies among fish, GI uptake of food and chemicals involves a number of complicated mechanisms associated with the digestive process. The process of biomagnification usually involves a sequential increase in chemical concentrations with a trophic level, which adds a further degree of complication.

The proximal portion of the intestine is where most of the absorption of food and lipophilic chemicals occurs (Adams et al. 1985). Although the process of absorption of food substances involves many passive and active mechanisms, facilitated and active transport generally are not considered as important mechanisms in GI in most studies. Gobas (1993b) indicated that the absorption of hydrophobic organochlorines appears to

be by simple diffusion rather than lipid co-transport. A suggested mechanism of GI tract uptake, the fugacity theory, was described by Mackay et al. (1991) and Gobas et al. (1993b&c). During digestion, fugacity in the GI tract, which is identical to partial pressure in ideal gases and described in units of Pa, is elevated during digestion to a level higher than that in the organism. This increasing fugacity gradient in GI tract results in a net uptake of chemicals across the intestine. Chemical hydrophobicity has an important and well documented effect on GI tract uptake. However, unlike gill uptake, in biomagnification, the most significant relationship for QSAR (Quantitative structure-activity relationship) is for lipophilic chemicals having log $K_{ow}$ value about 5 to 7 (Thomann 1989; Connolly et al. 1988; Opperhuizen 1991; Hamelink 1994), and the biomagnification continues for some chemicals with log $K_{ow}$ value higher than 8 (Connolly et al. 1988; Opperhuizen 1991). GI tract uptake may also involve enterohepatic recycling of these persistent lipophilic chemicals.

Like gill uptake, GI tract uptake of lipophilic chemicals is also influenced and controlled by many factors, such as chemical properties, feeding behavior, exposure rate, fish species and fat content of the food. The food ingestion rate in fish is often about 2 % of the body weight per day in hatchery conditions and less in the wild, but varies with fish size, fish species, environment temperature and diet composition (Nakashima, et al. 1978; Steffens 1989; Gerking 1994). Like gill uptake, the molecular size of the chemical may affect their uptake via GI tract. Niimi (1988) and Heath (1995) reported that compounds with molecular weights above 600 are poorly absorbed by the digestive tract.

Nonetheless, the process of biomagnification of lipophilic xenobiotics is still incompletely understood.

## 2.4 DEBATE ON IMPORTANCE OF UPTAKE ROUTE

Few questions in aquatic toxicology have generated as much discussion as the route of uptake of xenobiotics by fish between gills and GI tract (Murty 1986). For over two decades, a debate regarding the relative importance of either pathway in fish for lipophilic xenobiotics has continued without a satisfactory resolution. Conclusions are often contradictory and can be grouped in three categories as follows.

### 2.4.1 Gills as the primary uptake route

Studies favoring gill uptake as the primary uptake route involve different experimental designs. One approach is to compare fish exposed via water alone with fish exposed via both water and food. Using this approach Chadwick et al. (1969) found no additive accumulation of dieldrin (log $K_{ow}$ 5.4) in fish exposed to a combination of chemically spiked food and water for 3 weeks, compared with water exposure alone. In other studies, even though dieldrin (Reinert 1972) and DDT (log $K_{ow}$ 6.0) (Jarvinen et al. 1977) in the food was in equilibrium with water, the accumulation after 8 weeks from water alone in fish (guppy and fathead minnows) was still much higher than that from food exposure alone. In fact, in one study, the prey items used to feed the fish (*Daphnia*) accumulated dieldrin to a greater degree than the guppies (Reinert 1972), presumably through bioconcentration. Also, when the spiked food ration (dried, chopped clam meat)

was doubled, in the other study to double the dietary chemical exposure rate, the DDT concentration in fish was unchanged (Jarvinen et al. 1977). The bioconcentration of DDT from water was about one million times, whereas the mean bioaccumulation of DDT from food was only 1.2 times.

One concern with bioaccumulation studies is that marine fish drink water and both marine and fresh water fish take in some water when eating. It is known that marine fish drink approximately 5 to 12% of their body weight daily (Murty 1986). Thus, there could be xenobiotic uptake across the GI tract, but from water rather than food. On the other hand, fresh water fish gain water by osmosis and need to excrete about 10% of their body weight per day, and, drink little (Murty 1986). An approach to examine the effect of drinking is to compare the same species of fish in both fresh water (non-drinking) and sea water (drinking). Atlantic salmon exposed to [14]C- 2, 2', 4,5,5'-pentachlorobiphenyl (log $K_{ow}$ 5.92) under otherwise identical conditions had a higher body burden in fresh water compared with sea water, although the apparent initial chemical concentration in sea water was higher than in fresh water (Tulp et al. 1979). Thus, it would appear that drinking is not a major route of uptake. Furthermore, to determine whether endrin-induced death of fish was caused by chemical absorption either via gills or from swallowed water, Ferguson et al. (1967) exposed black bullheads to endrin (50 $\mu$l/L, log $K_{ow}$ = 4.53). Fish died equally rapidly whether or not the digestive tract was tied off near the esophagus to prevent drinking.

Other support for gill uptake comes from negative studies of biomagnification where there was no clear evidence of an increase in the concentration of xenobiotic

chemicals as the trophic level of the food chain increased. If gut uptake was important, then trophic transfer should have led to biomagnification. For example, marine organisms collected from eastern coast of Britain showed that the concentration of dieldrin in Atlantic cod (*Gadus morhua*) (0.006 ppm) was less than their main food item, sand eels (0.016 ppm), and the concentration of dieldrin in the planktonic crustacea (0.16 ppm) was greater than in any of the fish examined (Robinson et al. 1967). Similarly, PCB (Aroclor 1260) per unit lipid in catfish (16 ppm) was lower than the lower trophic levels of the food chain, which included mussel (*Mettles corscus*) (23 ppm), sea mullet (*Mugil cephalus*), fiddler crab (*Uca, sp*) (46 ppm) and bony bream (*Nematolosa*) (27 ppm) (Shaw et al. 1982). In another study, the lower level trophic organisms and macroplankton were found to be free of PCB and DDT when fish were exposed to contaminated water. Therefore, the measured fish body burden was believed to come from the water (Fowler et al. 1978). In a recent report, Leblanc (1995) further suggested that increased bioconcentration occurs with increasing trophic level and that it can be misconstrued as biomagnification, because of the fact that lipophilic organochlorine compounds consistently concentrate from the aqueous environment to greater levels in fish than in invertebrates. Therefore, he concluded that trophic-level differences in bioconcentration are due largely to increased lipid content and decreased chemical elimination efficiency of organisms in higher trophic levels organisms.

2.4.2 GI tract as the primary uptake route

The studies that support the GI tract as the major uptake route stress that the low freely dissolved concentrations of highly hydrophobic chemicals (log $K_{ow} > 6$) preclude appreciable gill exposure (Thomann, et al. 1984; Muir et al. 1985). Thus, dietary accumulation rather than direct uptake from water must explain observed fish body burdens. Batterman, et al. (1989) suggested that bioaccumulation of 2,3,7,8-TCDD (tetrachlorodibenzo-*p*-dioxin, log $K_{ow}$ 6.6-7.0) in lake trout occurs primarily through food chain transfer, simply because the chemical concentration in water is 5 to 7 orders of magnitude lower than that in food. Servos et al. (1992b) also explained that the body burden of polychlorinated dibenzo-*p*-dioxins (PCDD) could result from the shift from water to from food for the higher trophic level organisms when the water concentration declined to an extremely low level, although it was acknowledged that the water was the most important source for chemical accumulation in fish before the water concentration decreased.

Some studies have shown clear evidence of food chain biomagnification. For example, DDT and other pesticide concentrations were found 2- to 1,000- times higher in fish than in plankton (Rudd 1964). In addition, the reason of the different increase of PCB found between fish species in Lake Leman (France) was suggested by Monod et al. (1982) as the result of different diet basis. Biomagnification may also explain why carnivorous trout accumulated a significantly higher body burden of 2,5,4'-trichlorobiphenyl (log $K_{ow}$ 5.7) than the herbivorous carp (Crossland et al. 1987).

Having analyzed the data of food uptake efficiency from relevant literature and constructed a food uptake model, Gobas et al. (1988) suggested that uptake from food is the major pathway for organic chemical accumulation in fish, because the inflow ($D_I$ value) of the chemical is higher than outflow ($D_O$ value), as a result of food mass loss and composition change, whereas gills have same inflow and outflow of chemical ($D_V$).

2.4.3 Gill uptake and GI uptake are of equal importance

From a theoretical standpoint, gill ventilation rate is 4- to 6- orders of magnitude higher than food uptake rate. Thus, with such large differences in exposure rates for the gills and GI tract, the food chemical concentration has to be 4- to 6- orders of magnitude higher than water chemical concentration, in order to reach a similar fraction of fish body burden from each uptake route if the uptake efficiencies are similar. Such large differences in chemical concentration between food and water do exist, of course, for lipophilic chemicals because of their hydrophobicity and the lipid content of the food. Nevertheless, very few studies suggest that both pathways are of equal importance in the uptake of lipophilic xenobiotics.

Using a simple first order bioaccumulation model to assess the relative importance of the uptake route, Opperhuizen (1991) predicted that the gills and GI tract are equally important, because the efficiency of uptake of xenobiotics from the water by the gill and from food via the GI tract are both approximately 50% regardless of the different $K_{ow}$ values of chemicals. Some other papers have suggested that the fraction of fish body

burden from water and food seems to be equal from their experiments (Norstrom et al. 1975; Jarvinen et al. 1978; Macek, et al. 1979).

2.4.4 Summary

Despite a number of studies, differences in experimental results has caused debate over the relative roles of gill and GI tract uptake of lipophilic xenobiotics in fish, a consensus has yet to emerge. Some of the disagreement may arise because of differences in experimental design, and the unrealistic exposure concentrations and durations (Murty 1986). Moreover, laboratory studies may not show the same chemical bioaccumulation distribution patterns compared to the natural environment.

With so much debate regarding the uptake route of lipophilic xenobiotics in fish, it is necessary to define the uptake mechanisms carefully and in a reliable, quantitative manner. Although we have information on the delivery rate and exposure concentration for both routes, rarely do we have information of uptake constants. Therefore, we need such information to make a valid comparison. This is explained in the next section.

2.5 A BETTER METHOD FOR COMPARING GILL AND GI UPTAKE ROUTES AND CHEMICAL KINETICS

In nature, the chemical concentration in food is usually orders of magnitude higher than that in water depending upon the lipid content and the $K_{ow}$ value of the chemical. Therefore, based simply on exposure concentration, GI tract uptake is favored. However, based simply on the anatomical design features and exposure rates, gill uptake is favored.

A better method to compare the relative importance of uptake route is to integrate these factors using uptake constants (k values) and exposure concentrations for each uptake route independently.

The general equation commonly used to calculate the fish body burden from the gill uptake and GI uptake is as follows (Gobas, 1993a):

$$dC_f/dt \cdot V_f = C_w \cdot k_1 \cdot V_f + C_d \cdot k_d \cdot V_f - (k_2 + k_e + k_m) \cdot C_f \cdot V_f \qquad (1.2)$$

where $C_f$ (µg/L), $C_w$ (µg/L) and $C_d$ (µg/kg) are chemical concentrations in fish, water and diet, respectively; $V_f$ is volume of fish (L or kg, assuming 1 L $\cong$ 1 kg); $k_1$ (L/kg.day) and $k_d$ (kg/kg.d) are the uptake rate constants via gills and GI tract, respectively; $k_2$, $k_e$ and $k_m$ are the elimination rate constants from gills and GI tract and metabolism rate constant, respectively, which have units of 1/d.

The ratio of the chemical in fish taken from gills or GI tract can then be expressed as follows:

$$U_{gills}/U_{GI} = (k_1 \cdot C_w) \cdot V_f / (k_d \cdot C_d) \cdot V_f \qquad (1.3)$$

which can be simplified as:

$$U_{gills}/U_{GI} = (k_1 \cdot C_w) / (k_d \cdot C_f) \qquad (1.4)$$

where $U_{gills}$ is chemical mass in fish via gill uptake route and $U_{GI}$ is chemical mass in fish via GI tract uptake route, respectively, which have units of µg/kg.

In the above equations, the value $k_1$ incorporates the ventilation rate, the blood flow, and the membrane transfer efficiency for a contaminant entering the fish across the gill. The value $k_d$ incorporates the ingestion rate and the assimilation efficiency for the

selected food entering the fish across the GI tract (Hamelink et al. 1994). These two uptake rate constants are widely used in kinetic models of bioaccumulation in fish. However, experimentally derived $k_1$ and $k_d$ values for the same chemical in one species are very limited. Instead, most values are extrapolated from same chemical in one species are $k_1$ values range from $10^2$ to $10^5$ L/kg.day (Banerjee 1984; Oliver et al. 1985; Thomann 1989; Smith et al. 1990). In contrast, measured $k_d$ values are typically less than 1.0 (Macek et al. 1970; Lieb et al. 1974; Bruggeman et al. 1981; Skaar et al. 1981; Opperhuizen 1991).

In this thesis, gill versus GI uptake of three lipophilic chlorinated chemicals with log $K_{ow}$ value from 3.98 to 7.55 will be compared by first measuring $k_1$ and $k_d$ values and then modeling the chemical distribution in fish body from water and food, based on the k value and various food/water exposure concentration ratio.

# 3. SUSPENDED PARTICLES

## 3.1 Introduction

In the natural environment, an aquatic system not only includes water and food, but also other important compartments, such as sediment and suspended particles. With an aquatic system, the top few centimeters of bottom sediments and the flocculent mass are considered the "active layer" (Mackay 1991), because it is easily stirred by currents and by the action of the biota on, or in, the bottom which causes more suspended particles to be created in the water. It is known that the sediments and suspended particles can trap large amount of lipophilic chemicals so that these compartments may constitute the potential hazardous exposure environment for fish. The adsorption of lipophilic chemicals to suspended particles can greatly influence the redistribution of xenobiotics in water systems and alter bioavailability of lipophilic chemicals in organisms (Rand 1995). The impact of these compartments on fish coming into contact with them has been considered only recently. Their effect on the bioaccumulation of lipophilic chemicals in fish is far from understood. The relative importance of the uptake route of lipophilic xenobiotics in fish exposed to sediments has not been thoroughly examined either. Therefore, to learn the impact of these compartments, especially suspended particles, on the bioavailability, uptake and bioaccumulation of lipophilic xenobiotics in juvenile rainbow trout, including the role of the gill uptake of lipophilic xenobiotics, was my other interest in this thesis.

To understand the impact of the suspended particles on lipophilic chemical uptake, a review of the current information regarding particle components and the their interactions with lipophilic chemicals is necessary.

## 3.2 COMPONENTS OF SUSPENDED PARTICLES

Suspended particles are very fine naturally occurring particles with a density close to that of water (Mackay 1991) which do not readily settle in the water system. They are composed of undissolved solid-phase material, colloids, and dissolved particles (Rand et al. 1995). In general, the small size and the large surface area of particles favour the association of lipophilic chemicals. High concentrations of suspended particles are reported in many water systems. For example, in the Fraser River, the biggest river system in BC, the suspended sediment concentration is between 10 to 1,400 mg/L depending on the season (Environmental Canada 1995).

Suspended particles with low organic matter may be composed mostly of minerals. Suspended particles with higher organic matter, such as dissolved organic mater (DOM) is another important component. DOM is composed of a group of macromolecules including a variety of materials such as alkane, cycloalkane and aromatic groups with molecular weights of 500 - 5,000 (Thurman et al. 1982; Mackay 1991; Rand 1995). The reported size of DOM varies from 0.1 μm (Gobas et al. 1994) to 0.45 μm (Rand 1995). The DOM of large rivers ranges from 1 to 5 mg/L (Rand et al. 1995). In higher productive water systems, DOM can be as high as 10 to 25 mg/L (Mackay 1991, Rand et al. 1995) (Many articles use dissolved organic carbon, which is about 50% of

dissolved organic matter, as measure of DOM (Mackay 1991)). Most of the organic matter in fresh water is usually in the form of humic acid (as much as 80%) (Hamelink 1994).

## 3.3 ADSORPTION AND DESORPTION OF LIPOPHILIC CHEMICALS

The affinity of lipophilic chemicals to suspended sediments is determined by the $K_{ow}$ value of chemicals, the $K_{oc}$ (partition coefficient of a chemical between organic carbon in suspended carbon and water), and the organic carbon content in this compartment. The adsorption and desorption characteristics of lipophilic chemicals to suspended particles in water may be a key factor to influence the bioavailability of lipophilic chemicals in fish, and are not yet fully understood. This knowledge gap hampers attempts to describe and predict the importance of natural suspended particles in the transport and fate of organic pollutants in aquatic systems (Kukkonen et al. 1991). However, according to the different affinity of the particles based on their organic carbon content, it is possible that a chemical associated to the suspended particles with a low organic carbon content would dissociate more readily into the water column; the same may happen to chemicals with a lower log $K_{ow}$ value. In contrast, because of its relatively nonpolar features, DOM may readily bind lipophilic chemicals and the rate of desorption may be much slower than for low organic carbon particles (McCarthy et al. 1985b).

The desorption of lipophilic chemicals from suspended particles to water may contribute to fish uptake of dissolved chemicals. Although complete desorption of lipophilic chemicals is not commonly observed (DiToro 1985), studies have shown that

the reversible nature of sorption to small particles is an inherent kinetic property of lipophilic chemicals, including the super lipophilic chemicals (McCarthy 1985a&b; Gschwend et al. 1985). The same conclusion was reached by MacCarthy (1985b), who observed using equilibrium dialysis and fluorescence techniques, that benzo[a]pyrene (BaP) (log $K_{ow}$ 6.1) binding to and desorbing from humic acid was reversible, although the desorption process was slower than the association process.

To fully understand the desorption of lipophilic chemicals in water, the truly dissolved phase should be reviewed, since it can be argued that only the fraction of the lipophilic chemical in the water which is (initially) "truly dissolved" can be taken up by aquatic organisms (McCarthy 1983; Landrum et al. 1987; Mackay 1991; Servos et al. 1992b). The degree of chemical solubility in water may affect the bioavailability of the chemicals by fish, especially via the gills.

3.4 TRULY DISSOLVED PHASE

Once associated with suspended particles, lipophilic chemicals are considered to be non-soluble particles (Muir et al. 1985; Landrum et al. 1987; Mackay 1991; Gobas et al. 1994). To determine if the chemical associated with suspended chemicals is available to fish via the gills, a distinction between dissolved and associated chemicals in water is necessary. However, the definition of "truly dissolved" chemical concentration is controversial and the criteria used thus far are not consistent.

One approach is to use particles size and filtration techniques. Horzempa et al. (1983) suggested that the particles smaller than 1 μm represent dissolved particles.

Thurman (1985) also used diameters to categorize the dissolved and non-dissolved substances, as true solutes ($< 0.002$ μm), colloids ($0.001$ to $0.45$ μm) and solids ($> 0.45$ μm). Horowitz et al. (1996) listed many factors that may affect filtration to define dissolved trace element concentrations in natural water, such as pore size, diameter, volume of sample processed and the amount of suspended sediment in the sample. These factors may also affect the extraction of dissolved organic chemicals.

The other approach used by some studies to obtain the truly dissolved chemicals in water is to apply centrifugation at speeds from 760g to 20,000g (Voice et al. 1983; Gshwend et al. 1985; Servos et al. 1989b). Centrifugation and filtration methods were compared in determining low concentrations of suspended sediments in natural water (Campbell, 1975), in which filtration measured suspended sediment concentration with greater precision than centrifugation. However, there is a lack of comparative information in obtaining the dissolved chemicals regarding the centrifugation and filtration methods.

Other approaches to determine dissolved chemicals concentration include: headspace (Yin et al 1989; Resender et al. 1990), dialysis bag and reverse-phase techniques (Landrum 1984; McCarthy et al. 1985b; Servos et al. 1992b). These techniques are not used as frequently as filtration and centrifugation methods for general studies of chemical uptake.

How we can best discriminate between dissolved and non-dissolved chemical (and particulate) forms is not resolved, since there is insufficient evidence linking the size of chemicals and particles that can be dissolved in the water and would therefore be

bioavailable to organisms (Mackay 1991). No doubt this uncertainty may cause errors in the evaluation of bioavailability and bioaccumulation of lipophilic chemicals.

The most universally accepted working definition for dissolved constituents is: substances that pass through a 0.45 μm membrane filter (Mackay 1991; Horowitz et al. 1996). This definition is implemented by following the promulgated standard methods incorporated in the regulatory requirements made by water sample test authorities in North America. (Office of Water Data Coordination 1984; APHA 1989; ASTM 1995). Nevertheless, this is an operational definition because the separation is dependent on the filtration conditions.

## 3.5 THE IMPACT OF THE SUSPENDED PARTICLES AND CHEMICAL UPTAKE

The impact of sediments and suspended particles on chemical uptake in fish has been considered only recently. Although it is known that sediments and suspended particles can compete with organisms for sorption of large amounts of lipophilic xenobiotics (Maki et al. 1984), a portion of the chemical remains potentially bioavailable to organisms, including fish, inhabiting the sediments and suspended sediments (Ingersoll 1995). For example, several incidents of polluted suspended sediments causing toxic effects on fish have been reported (Servizi et al. 1987; Servizi et al. 1992; Newcombe and Jorgen 1996). They all indicate that contaminated suspended particles might have a negative effect on the survival of fish, presumably through the bioaccumulation of these chemicals, either via the gills or the GI tract, or both, and its effect on the bioaccumulation of lipophilic chemicals in fish is far from understood. The relative importance of the

uptake route of lipophilic xenobiotics in fish exposed to these compartments has also not been thoroughly examined.

Chemical association with suspended sediments might affect bioavailability in one of two ways. One is that they are not bioavailable for gill uptake. In this case, suspended sediments act as an absorbent for chemicals, reducing their dissolved phase in water, and bioavailability. However, information presented below shows that this may not always be the case. The alternative is that sorbed chemicals remain bioavailable. Uptake would then occur in one of three ways: 1. Uptake of sorbed chemicals via the GI tract by swallowing water either for drinking or associated with food (Murty 1986); 2. Chemical desorption from sediment to water and movement into fish via the gills; and 3. Particles and sorbed chemical accumulation across the body surface, the skin or the gills by phagocytosis (Newcombe et al. 1996). Compared with skin, gills have a larger surface area, higher exposure rate and shorter diffusion distance, and therefore have potential as an entry way for chemicals associated with suspended particles.

## 3.5.1 Suspended particles with low organic matter

There are not many studies available in the literature regarding the impact of low-organic-carbon-suspended particles on the bioavailability and bioaccumulation of lipophilic chemicals in fish. In one study, Opperhuizen (1988) found a higher bioaccumulation in particle-exposed guppies (*Poecilia reticulata*) than in a control group for chemicals with log $K_{ow}$ values between 5 and 6 (such as penta- and hexachlorobenzene, and tri- and tetrachlorobiphenyls) when fish were exposed to contaminated particles

(Chromosorb < 50 μm and 500 mg in 10 L water) that had low organic carbon concentration. Therefore, he concluded that if the number of contaminated particles is sufficiently high, particles can act as a source of lipophilic chemicals for fish. For lower log $K_{ow}$ chemicals, the bioaccumulation was determined by their very high water solubility, so that the uptake of the chemicals from the particles was negligible. For higher log $K_{ow}$ chemicals, both lower water solubility and desorption were considered as determining factors.

## 3.5.2 Suspended particles with high organic matter

More attention has been focused on the high affinity of organic matter for lipophilic chemicals. Finding that the bioaccumulation is less in fish exposed in the cages on the experimental lake bottom sediments than fish exposed only to the water column, Servos et al. (1992b) explained this effect as increased sorption of lipophilic chemicals to organic matter at the sediment-water interface. Black and McCarthy (1988) investigated the bioavailability of sorbed chemicals for gill uptake by rainbow trout (*Salmo gairdneri*). The effects of sorption of benzo[a]pyrene (log $K_{ow}$ 5.98), and 2,2',5,5'-tetrachlorobiphenyl (log $K_{ow}$ 6.10) to suspended organic matter (humic acid) on gill uptake was tested by comparing the extraction efficiency of the trout gills, with and without humic acid, and by using equilibrium dialysis techniques to analyze dissolved and sorbed chemicals in the water irrigating the gills. The results showed a decrease in extraction efficiency of 50 to 80 %, with an increase of the humic acid concentration (0 to 3.0 mg C/L and 11.8 mg C/L). Therefore, they concluded that the chemicals bound to

DOM did not diffuse across the fish gills. The chemical residue in fish, however, was not measured and the exposure time was brief (3 hours).

Kolok (1996) demonstrated that under roiled water, in which substantial resuspension of BaP contaminated sediment (organic carbon 10%) occurred, the concentration of BaP-equivalents were significantly greater in gizzard shad. Since this was true for fish with the gut ligated, the results suggest that ventilation of turbid water may be a significant source of BaP with gills as the main uptake route. Many studies suggest that the presence of DOM in a water system can compete with river suspended sediments and bottom sediments for the binding of lipophilic chemicals, and argue that this binding complex is much less bioavailable (Hassett et al. 1982; Voice et al. 1983; McCarthy et al. 1985b).

The general rule adopted by most researchers is that chemicals bound to dissolved organic matter are generally not bioavailable (McCarthy 1985a; McCarthy et al. 1985c; Landrum et al. 1987; Kukkonen et al. 1989; Schrap et al. 1990; Day 1991; Servos et al. 1992b; Muir 1994; Rowan 1994; Twiss et al. 1995). However, most of these studies involved organisms such as *Daphnia*, amphipod species, benthic organisms, and some bottom-living fish such as carp and suckers. It is therefore of interest to investigate the impact of suspended organic matter on the uptake of lipophilic chemicals by fish, such as rainbow trout, that are indirectly associated with bottom sediments.

## 3.6 SUMMARY

The presence of suspended particles may affect the bioavailability, uptake and bioaccumulation of lipophilic xenobiotics in fish exposed to the suspended particles, which may have either a high or a low organic carbon content. The contribution of suspended particles to the chemical bioaccumulation may have been previously overlooked or underestimated based on the literature. Although the true solubility of lipophilic chemicals in water is controversial, and the mechanisms incorporating the chemical associated with suspended particles are unclear, it is of great interest to investigate the impact of contaminated suspended particles on uptake and bioavailability of lipophilic chemicals in free-living fish, such as rainbow trout, especially via the gill uptake route, since these fish are continuously exposed to these particles when water flows through the gills.

# 4. WORKING HYPOTHESES

The review of the literature indicates that many questions remain regarding uptake, bioavailability and bioaccumulation of lipophilic chemicals in fish, especially with respect to lipophilic chemicals in water laden with suspended particles water. My focus is on the relative contributions of two uptake routes (GI tract and gill) for lipophilic xenobiotics, and on the impact of suspended particles on this uptake. Largely because of a lack of appropriately designed studies, it is my belief that the role of gill uptake of high $K_{ow}$ xenobiotics has not been fully appreciated in water and in sediment-laden water. The underlying aims of this thesis are to investigate: (a) if the gills are an important uptake route for lipophilic xenobiotics with a log $K_{ow}$ between 4.0 and 7.6 when compared with the GI tract; (b) the impact of suspended sediments on lipophilic chemical bioavailability, uptake and bioaccumulation; and (c) the impact of varying DOM concentrations on test chemical bioavailability, uptake and bioconcentration in juvenile rainbow trout *(Oncorhychus mykiss)*.

My working hypotheses, experimental approaches and consequences are summarized in Fig 1.1. They are explained in the following section.

## 4.1 COMPARISON OF UPTAKE ROUTES

Based on a review of the literature, the role of gill uptake appears to be underestimated. My first working hypothesis is that the gill uptake route is more important than the GI uptake route for lipophilic chemical bioaccumulation when fish are exposed to contaminated water and food. To test this hypothesis, chemical body burden

in fish arising as a result of either water or food exposure was measured. From these data, the uptake rate constants for the gill ($k_1$) and the GI tract ($k_d$), and exposure concentration from either water and food experiment were calculated. The importance of gill versus uptake routes using the values k, exposure concentration and log $K_{ow}$ of test chemicals was then evaluated in a simulation model.

## 4.2 EXPOSURE TO SUSPENDED SEDIMENTS WITH LOW ORGANIC CONTENT

In view of impact of the suspended particles on fish, my second working hypothesis is that lipophilic chemicals associated with suspended particles containing low organic carbon (from the Fraser River) are available for uptake by juvenile rainbow trout.

To directly test my second hypothesis, the chemical mass balance was measured, for fish exposed to chemically spiked water containing suspended sediments from the Fraser River (Chapter 4). The same three test chemicals were used to provide information on role of $K_{ow}$ of chemicals. The concentrations of these chemicals were measured in fish tissue, water, suspended sediments. A technique to block the GI tract was used in this study to prevent the uptake of sorbed chemicals via GI tract. In addition, the fish were compared with and without exposure to bottom sediments to test the sub-hypothesis that chemicals associated with bottom sediment were not bioavailable to juvenile rainbow trout via the GI tract.

To obtain accurate mass balance, I needed a reliable measurement of dissolved chemical concentration. Therefore, to quantify the dissolved chemical concentration from

sediment-laden water was further investigated in this study, based on the methodology in literature. I compared chemical concentrations by using filtration of sediment-laden water with a 0.45 μm filter membrane and centrifugation at 2,000g for 30 minutes as alternative methodologies (see Chapter 4).

4.3 EXPOSURE TO DISSOLVED ORGANIC MATTER

According to the literature review, DOM, as an important compartment in water systems, affects the lipophilic chemical bioavailability and uptake in fish. These effects may result from changes in concentration of DOM. Therefore, my third working hypothesis is that a reduction in the uptake of lipophilic chemicals is not influenced by low concentrations of DOM. To test this hypothesis, the chemical body burden of the in fish was compared after exposure to water containing different concentrations of humic acid (see Chapter 5). Again, the same three test chemicals were used to assess the impact of their hydrophobicity.

The main premise of this thesis is that the gills are the most important uptake route for the bioaccumulation of the xenobiotics that have moderate to high hydrophobicity, i.e., log $K_{ow}$ 4 to 7.
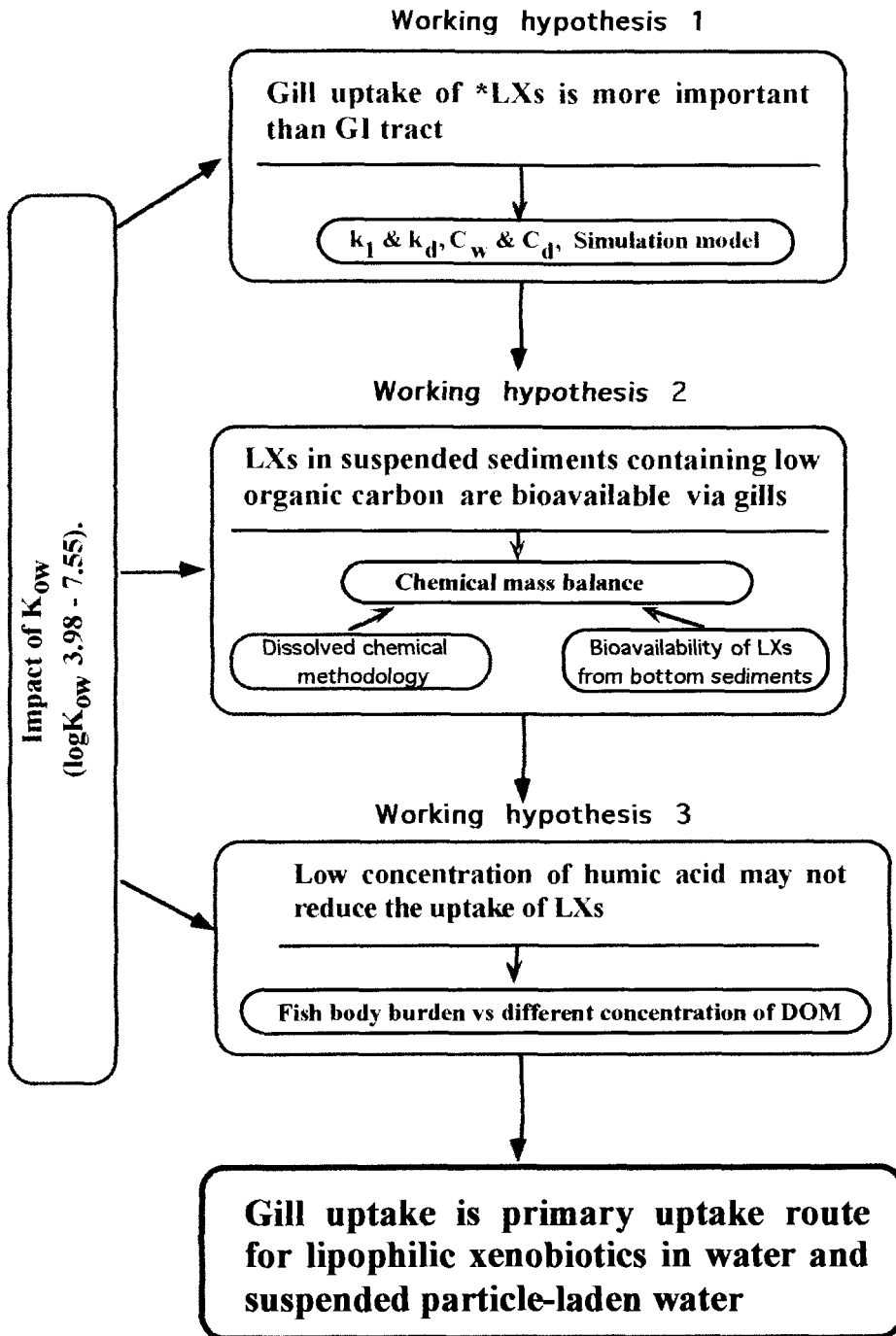
Figure 1.1 Summary of working hypotheses.

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# CHAPTER 2

# TEST MATERIALS AND CHEMICAL ANALYSIS

# 1. TEST MATERIALS

## 1.1 TEST ANIMALS

Rainbow trout (*Oncorhychus mykiss*) were chosen in this study because of their importance as a commercial and recreational fish species. Rainbow trout were also being used broadly in aquatic toxicology, because of the large information base on their physiology.

Juvenile fish, weighing from 2.5 g to 3.5 g, were obtained from Westcreek Fish Farm in Langley, B.C. This size fish ensured that the fish loading density in 50 L test aquaria was usually less than 1.0 g/L and never exceeded 2.0 g/L. Fish were held and acclimated in a large flow-through tank (500 L) with dechlorinated water at around $12^{o}C$ for at least 3 weeks prior to the experiments. The water was aerated continuously and the dissolved oxygen was more than 8 mg/L. Water pH was 6.1 to 6.3. The hardness of the water was 17.1 mg/L as $CaCO_3$ and alkalinity was 17.1 mg/L as $CaCO_3$. The fish were fed daily with either Silvercup trout chow or Clark's dry extruded fish feed until 2 - 4 days prior to the experiments. The food ingredients included fish meal, canola meal, fish oil, whole wheat, feather meal, can molasses, ethoxyquin and vitamins. Total crude protein was 47% and total fat was 14%, as analyzed in Chapter 3.

## 1.2 TEST, SURROGATE AND INTERNAL STANDARD CHEMICALS

Three test chemicals, three surrogates and one internal standard chemical were employed in experiments and chemical analysis. Their chemical-physical properties are described as below.

## 1.2.1 Test chemicals

The three lipophilic chemicals used in this study were: 1,2,4-trichlorobenzene (TCB; Aldrich Chemical Co., Inc.; 99%), 1,2,3,4,5-pentachlorobenzene (PeCB; Aldrich Chemical Co., Inc.; 98%) and 2,2',4,4',6,6'-hexachlorobiphenyl (HCBP; AccuStandard; 100%). The chemical properties of the three test chemicals are listed in Table 2.1. Test chemicals toxicities are summarized in Table 2.2. The concentrations of the test chemicals in water and food were more than 100-times lower than the 96-h $LC_{50}$ toxicity doses and the maximal exposure period was less than 18 days. Therefore, the exposure done in this study should not have adversely affected the fish physiology.

## 1.2.2 Surrogate and internal standard chemicals

The surrogate chemicals were used during chemical extraction procedures so that any loss of test chemicals from the sample extraction could be quantified. The chemicals used as surrogates were: 1,3,5-trichlorobenzene (1,3,5-TCB, Aldrich Chemical Co., Inc.; 99%) for 1,2,4-TCB; 1,2,3,4,5,6-hexachlorobenzene (HCB, Aldrich Chemical Co.; 99%) for PeCB; and 2,2',5,5'- tetrachlorobiphenyl (TCBP, AccuStandard; 100%) for HCBP. Each surrogate had similar physical-chemical characteristics (see Table 2.3) to its corresponding test chemical (Table 2.1). The internal standard chemical, 1,2,4,5-tetrachlorobenzene (TeCB, Aldrich Chemicals Co., Inc.; 99%), was added to the purified extract before gas chromatograph (GC) analysis to standardize GC operation. The chemicals (except HCBP) were kindly donated by Dr. Gobas.

## 2. CHEMICAL ANALYSIS

Extracts were analyzed by GC and calibrated by using surrogate and internal standard chemicals. Surrogates were added into the sample during the extraction procedure and internal standard was injected at least once into the GC to insure that the GC system was operating properly before the sample analysis began.

### 2.1 GAS CHROMATOGRAPH

GC analysis was carried out on a Varian model 3500, equipped with a 30 m DB-1 capillary column (J&W Scientific, Fulsome CA.) and $^{63}$Ni electron capture detector. The injector temperature was 250 °C and the detector temperature was 300 °C. The column temperature was programmed from 100 to 300 °C in 24.5 minutes. The carrier gas was ultrapure high grade helium delivered at 1.5 mL/min. and the split ratio was 64:1. The injection mode was splitless, with an injection volume of 1 μL. In this study, duplicate injections of each sample were applied for GC detection. The mean of these values represented one value for a tissue, sediment, or water sample. The GC analysis protocol is outlined in Figure 2.1. The retention times of test chemicals, surrogates and internal standard were 3.70 min. for 1,3,5-TCB, 4.17 min. for 1,2,4-TCB, 6.02 min. for 1,2,4,5-TeCB, 8.44 min. for PeCB, 10.765 min. for HCB, 13.2 min. for TCBP, and 14.90 for HCBP (Fig. 2.2).

## 2.2 CALCULATING CHEMICAL CONCENTRATION

To calculate the chemical concentration in each compartment, three steps were followed:

1. The response factors (RF) of test chemicals and surrogates were calculated with the following equations and are presented in Table 2.4.

$$RF(C) = (Cc/Ac)/(Ci/Ai) \qquad (2.1)$$

$$RF(S) = (Cs/As)/(Ci/Ai) \qquad (2.2)$$

where:

RF(C): response factor for test chemicals

RF(S): response factor for surrogates

Cc: concentration of chemicals

Cs:     concentration of surrogates

Ci:     concentration of internal standards

Ac:     GC peak area of chemicals

As:     GC peak area of surrogates

Ai:     GC peak area of internal standards


2. Test surrogate and chemical concentrations before calibrating for the loss due to extraction were calculated separately with following equations:

$$Cc \ (\mu l/mL) = (Ac \cdot Ci) / (RF \ (C) \cdot Ai) \qquad (2.3)$$

$$Cs \ (\mu l/mL) = (As \cdot Ci) / (RF \ (S) \cdot Ai) \qquad (2.4)$$

3. Calibration to take into account the sample volume and possible loss during extraction by using the following equation

$$Cc(final) = Cc \cdot Cs(injected) / Cs \cdot 2 / V \qquad (2.5)$$

where 2 is the volume of extract solution (mL), and V is sample weight (kg) or volume (L). The recovery rate of test chemicals were calculated using the recovery ratio of the corresponding surrogates and known injection.

In this chapter, the commonly used materials, the test chemicals, the chemical extraction and the chemical analysis are described. Specific materials and methods are described in relevant chapters.

Table 2.1 The physical-chemical properties of test chemicals.

| | M.W. | log $K_{ow}$ | Water Solubility (mg/L) | Vapor Pressure Pa |
|---|---|---|---|---|
| 1,2,4-TCB | 181.45 | 3.98[a] | 46.09[a] | 60.6 [b] |
| PeCB | 250.3 | 5.03[a] | 0.83[a] | 0.219[b] |
| HCBP | 360.9 | 7.55[a] | 0.00041[a] | 0.012[c] |

a: Miller et al. 1985
b: Mackay et al. 1981
c: Shiu et al. 1986

Table 2.2 The toxicity of the test chemicals.

| | TCB mg/L | PeCB mg/L | HCBP (PCB) mg/L |
|---|---|---|---|
| 96h LC$_{50}$ | 3.36-21.4[a] | 0.25-0.83[a] | 61[b] |
| test species | Bluegill | Bluegill | Cutthroat trout |
| | Fathead Minnow | Fathead Minnow | yellow perch |

a. USEPA 1980
b. US Dept. of Interior/Fish & Wildlife Service 1986
* data for salmonid were not available in the literature

Table 2.3 The physical-chemical properties of the surrogate and the internal standard chemicals.

| | M.W. | log $K_{ow}$ | Water Solubility (mg/L) | v.p. pa |
|---|---|---|---|---|
| 1,3,5-TCB | 181.45 | 4.49[a] | 4.1[a] | 77[b] |
| 1,2,4,5-TeCB | 215.89 | 4.02[a] | 2.35[a] | 0.64[c] |
| HCB | 284.8 | 5.47[a] | 0.047[a] | 0.0015[c] |
| TCBP | 292 | 6.10[c] | 0.027[d] | 0.0031[e] |

a: Miller and Wasik 1985  
b: Mackay et al. 1982  
c: Shiu and Mackay 1986  
d: Miller et al. 1984  
e: Mackay et al. 1985

Table 2.4 The response factors of the test and surrogate chemicals.

| Chemical | RF value |
|---|---|
| 1,2,3-TCB | 0.64 ± 0.01 |
| HCB | 3.40 ● 0.073 |
| TCBP | 0.71 ± 0.012 |
| 1,2,4-TCB | 0.55 ± 0.011 |
| PeCB | 3.41 ± 0.069 |
| HCBP | 1.28 ± 0.038 |
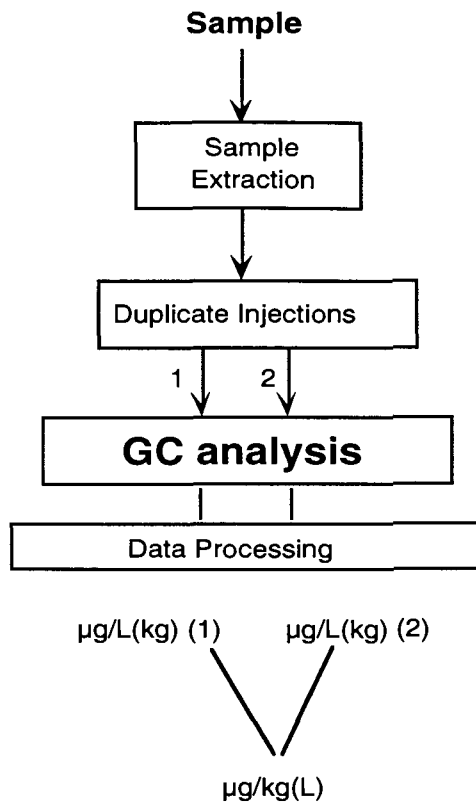
Figure 2.1 GC analysis protocol.

END

*        test chemicals

* 2,2',4,4',6,6'-Hexachlorobiphenyl        14.901 ————

**        surrogates

*** 2,2',5,5'-Tetrachlorobiphenyl        13.288

***        internal standard

** Hexachlorobenzene        10.765

* Pentachlorobenzene        9.444

*** 1,2,4,5 - Tetrachlorobenzene        6.016 ————

* 1,2,4 - Trichlorobenzene        4.124

** 1,3,5 - Trichlorobenzene        3.696 ————

INJECT
ECD B 8X10 1.0 CM/M 10%
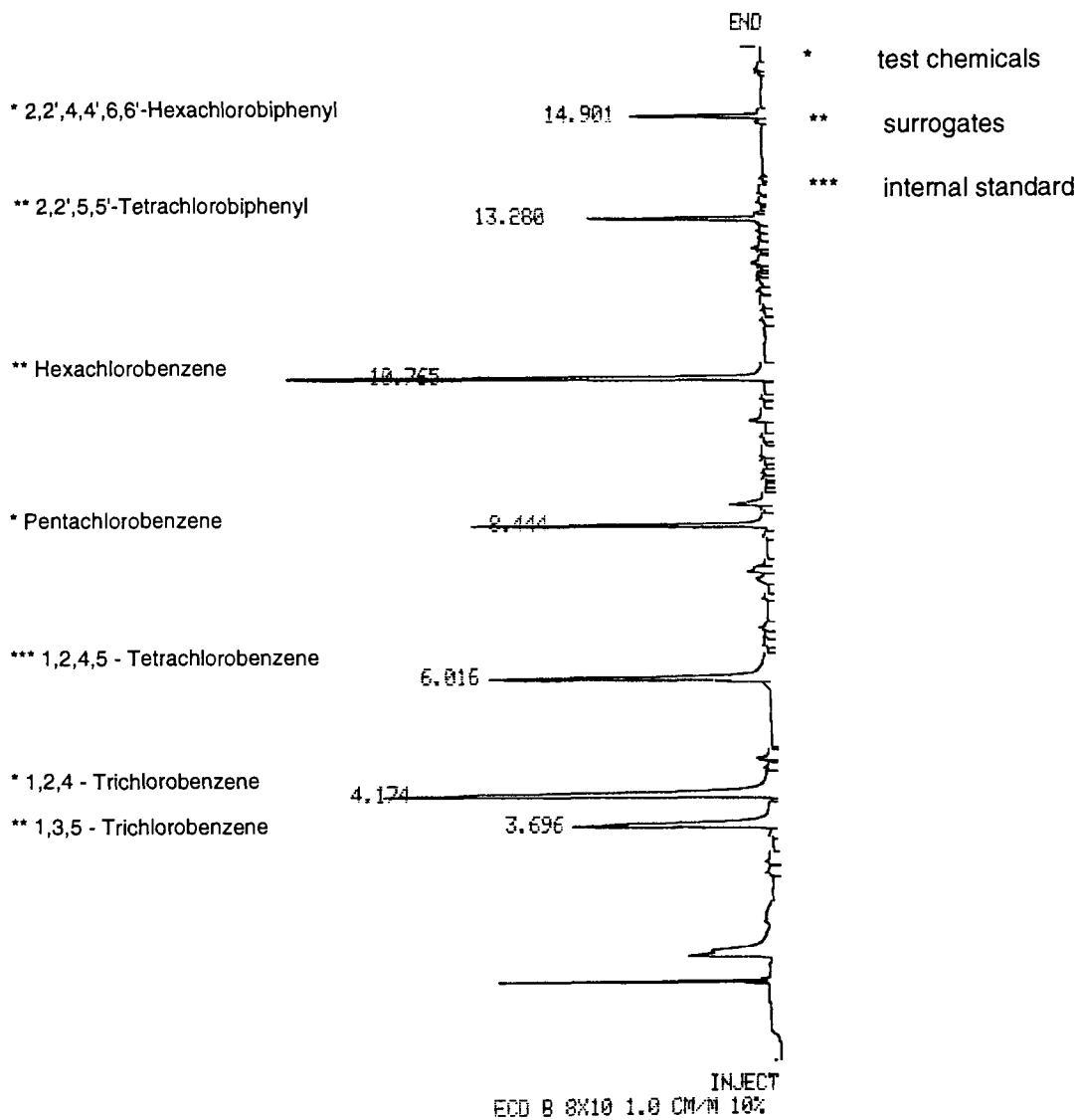
Figure 2.2 A representative GC trace showing the peaks for the mixture of test chemicals,

surrogate chemicals and internal standard.

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# CHAPTER 3

# THE COMPARISON OF UPTAKE OF LIPOPHILIC CHEMICALS

# BY THE GILLS AND GASTROINTESTINAL TRACT

# ABSTRACT

This study was designed to determine the relative importance of the gill uptake versus the GI tract uptake routes for lipophilic chemicals, 1,2,4-TCB, PeCB and HCBP, with log $K_{ow}$ values from 3.98 to 7.55. Juvenile rainbow trout (_Oncorhynchus mykiss_) were exposed separately either to chemically spiked water or food  for 4 days and 12 days, respectively. The chemical concentration in fish tissue and uptake rate constants ($k_1$ from water exposure, and $k_d$ from food exposure) were measured. The $k_1$ values for all three test chemicals were about 5-orders of magnitude greater than the $k_d$ values. Further, a model simulating simultaneous chemical exposure via water and food indicated that the GI tract accounted for nearly 100% of chemical uptake whenever the food/water concentration ratio for three test chemicals was $10^7$ or greater. In contrast, if the food/water concentration ratio was $10^3$ or lower, gill uptake accounted for 100% of the chemical uptake. The two uptake routes would contribute equally to the chemical body burden in fish only if the ratio of the food/water concentration was between $10^5$ to $10^{5.3}$ for three test chemicals. According to the expected chemical ratios of food : water in nature, the gills are predicted to act as the primary entry route for 1,2,4-TCB and PeCB (log $K_{ow}$ values of 3.98 and 5.03, respectively), whereas the GI tract would contribute mostly to the uptake of HCBP (log $K_{ow}$ of 7.55).

# 1. INTRODUCTION

The literature review in Chapter 1 (section 2.4) indicates that in previous studies, considerable controversy remains over the relative importance of gills versus GI tract. However, it is a fact that morphology and physiology features favor a rapid gill uptake of lipophilic chemicals in fish rather than the GI tract (Chapter 1, section 2.2), and much evidence (Chapter 2.4) shows that gill uptake of lipophilic chemicals may be underestimated. Therefore, a comparison of the relative importance of gill and GI tract for lipophilic chemicals is the purpose of this study.

As mentioned in Chapter 1 (section 2.5,) chemical mass in a fish body, either from gill or GI tract uptake route, is determined by uptake rate constant and the exposure chemical concentration. The ratio of chemical distribution in fish from each of uptake route then can be expressed as:

$$U_{gills}/U_{GI} = (k_1 \cdot C_w) \cdot V_f / (k_d \cdot C_d) \cdot V_f \tag{3.1}$$

which can be simplified as

$$U_{gills}/U_{GI} = (k_1 \cdot C_w) / (k_d \cdot C_f) \tag{3.2}$$

Measurement of $k_1$ and $k_d$ are key rate constants since they represent the potential uptake of lipophilic chemicals via either route, and once measured, they can be used to predict the uptake of chemicals in the body burden when the contaminant exposure level is known. Obtaining these uptake rate constants requires laboratory experiments with separate exposure to chemical-spiked water and food. Since uptake rate constants are independent of the exposure concentration, these data can be obtained, within certain limits of solubility and detection of chemicals, regardless of the chemical

exposure concentration in water and food. Thus, the chemical concentration selected for the three compounds 1,2,4-TCB, PeCB and HCBP in either water or food were not intended to simulate any specific real world situation.

Normally, in deriving $k_1$ and $k_d$, a constant exposure concentration is required until equilibrium between the fish and the medium is reached. The time to reach equilibrium, however, could be months or even years for chemicals with higher log $K_{ow}$ values. As an alternative approach, Gobas et al. (1992b) created the BIOFIT model which has proven successful in reducing the margin of error in the derivation of the BCF and k values in bioconcentration tests with short exposure duration and a non-steady-state water exposure concentration. In fact, in the field, chemical exposure concentrations often change (for both food and water) and an equilibrium may never be reached. The present study used the BIOFIT model to obtain uptake rate constants without reaching equilibrium. Exposure duration in this study was 4 days for water exposure and 12 days for food exposure. In addition to measuring $k_1$ and $k_d$, the bioconcentration factor (BCF) from water exposure, and biomagnification factor (BMF) for food exposure were calculated. To evaluate the relative importance of the two uptake routes, a model simulating simultaneous chemical exposure via water and food was developed, incorporating the measured $k_1$ and $k_d$ values and chemical ratios of food/water ranging from $10^3$ to $10^7$.

# 2. MATERIALS AND METHODS

## 2.1 TEST MATERIALS

### 2.1.1 Test animals and chemicals

Juvenile rainbow trout (*Oncorhychus mykiss*) weighing from 2.5 g to 3.5 g were used. Details of the fish acclimation and water quality are found in Chapter 2. The values for either for $k_1$ and BCF, or $k_d$ and BMF were estimated in separate experiments by exposing rainbow trout to either contaminated water without being fed or contaminated food with clean water. Details of the test chemicals 1,2,4-TCB, PeCB and HCBP are found in Chapter 2.

## 2.2 EXPERIMENTAL METHODS

### 2.2.1 Water exposure

The stock chemicals (1,2,4-TCB, PeCB and HCBP with concentrations of 925, 205 and 208 µg/L, respectively) were made up by first dissolving them in methanol and then diluting with water. The chemical stock solution was remade daily and covered with parafilm. Stock solution was added to the aquarium and mixed thoroughly to bring the concentrations to 1.85, 0.41 and 0.42 µg/L for 1,2,4-TCB, PeCB and HCBP, respectively. Thereafter a computer-controlled delivering apparatus with a pump-solenoid mixed the stock solution with a supply of aerated and dechlorinated tap water and delivered it to two 65 L aquaria with plastic covers (Figure 3.1) in a manner similar to that of Wood et al (1996). One aquarium held test fish and the other held control fish. Chemical stock was purged to the test aquarium every hour at 5 mL/min for 1.2 minutes and dechlorinated tap

water was delivered to both aquaria every hour at the rate of 1 L/min for 3 minutes. The concentration of methanol in water was less than 2 mL/L. The water turnover time in each tank was 16.2 hours. The outflow water from the test aquaria was passed through a filter with fiberglass and activated charcoal (1/8 inch pellets) prior to release to the sanitary sewer. The temperature was $13 \pm 1\ ^0C$ and the oxygen saturation was > 8 mg/L.

Forty-eight juvenile rainbow trout were placed in each of the tanks after the dosing system started running. Chemical concentrations were analyzed from water samples collected at 1, 4, 8, 12, 48 and 96 hours after introducing fish into the aquaria. Eight fish were sampled from each aquarium at each exposure time. Fish removed from the test aquaria were killed by a sharp blow to the head and immediately frozen at -80 °C. Storage was no longer than one month prior to chemical extraction.

## 2.2.2 Food Exposure

Clean commercial fish food (Moore-Clark Co. Vancouver, BC., Canada) was tested by the method as described below using GC analysis to confirm that no chemical contamination with test chemicals. Preparation of contaminated food was conducted one week before the experiment using a household kitchen centre, which included a blender, a mixing container and a hamburger-making die. Food was minced with the blender and transferred into the mixing container, where water was added and mixed until wet to the touch. Test chemicals were dissolved in methanol as described above, and then mixed with food for one hour. The test food was made into a favorable feeding size using a hamburger die with 3/32" sized holes. The pelleted food was dried in a fume hood. The concentration

of the chemicals in the food was confirmed by chemical analysis. The food concentrations of test chemicals, as determined by GC, were $49.2\pm1.6$, $17.9\pm0.5$ and $24.3\pm0.1$ mg/kg for 1,2,4-TCB, PeCB and HCBP, respectively.

Juvenile rainbow trout were held in 45 L glass aquaria containing aerated and dechlorinated water flowing in at a rate of 1.5 L/m. Fish were fed with chemical-spiked food at a daily rate of approximately 2% of body mass. To minimize chemical release from feces in the water, feces settling on the bottom were removed by a siphon tube twice a day. In addition, a filtration device (Aquaclear Mini, Rolf C. Hagen Inc. Montreal) filled with active charcoal and foam was installed in each of the aquaria to continuously remove the suspended particles and chemicals possibly released from the feces and food in the water. Seven aquaria were used for food exposure, with ten fish in each of six aquaria and four fish in the seventh. Sampling of 10 fish was conducted daily (day 1 through day 6), after the feeding trial, from one of the aquaria. On the 12th day, the last four fish were sampled. Fish removed from the test aquaria were killed by a sharp blow to the head and immediately frozen at -80 °C. Storage was no longer than one month prior to chemical extraction. Since feeding lasted 12 days, weight gain may have affected body concentration; thus, fish weights were recorded and statistically compared with a student's test ($p < 0.05$). Regression statistics (ANOVA) were conducted for the food exposure experiments.

## 2.2.3 Measurement of $k_1$ and $k_d$ values

In a two-compartment model, the distribution of chemicals between fish and water follows first order kinetics. Therefore, the rate uptake constants $k_1$ and BCF are based on a numerical integration of the following model, as established by Gobas et al. (1992c & 1993a):

$$dCf/dt = k_1.Cw - k_2Cf \tag{3.3}$$

According to the equation, BCF is independent of the duration of experiments when $k_1$ and $k_2$ are known. The BCF value under equilibrium follows the ratio of the uptake and elimination rate constants (BCF = $k_1/k_2$).

Biomagnification models generally assume the same mechanism as bioconcentration (Leblanc 1995). Therefore, the BIOFIT model was also used to calculate $k_d$ and BMF values, assuming that the chemical uptake via the GI tract represents constant uptake and the elimination of lipophilic chemicals, over time, follows first order kinetics. Therefore, the equation for food exposure is:

$$dCf/dt = k_d.C_d - k_e.C_f \tag{3.4}$$

The measured water concentration, food concentration and fish tissue concentration were used to solve the kinetic equation 3.1 for fish burden, which is derived from $k_1 \cdot Cw$, for water exposure, and $k_d \cdot C_d$ for food exposure. By using separate exposure experiments, one of the terms was assumed to be zero for calculation purposes.

2.2.4 A model simulating simultaneous exposure to chemicals in food and water

Predicting the chemical body burden in fish when there is simultaneous uptake via the gills and GI tract requires knowledge of $k_1$, $k_d$, and the prevailing chemical concentrations in water and food. Therefore, I used the measured values of $k_1$ and $k_d$ to estimate chemical body burden by assuming a ratio of chemical concentration in food to water ranging from $10^3$:1 to $10^7$:1. The proportion of body burden derived from either uptake route was calculated from the product of the k value and exposure concentration ($k_1 \cdot C_w$ for gills uptake and $k_d \cdot C_d$ for GI tract uptake). Since this simulation was performed for the three chemicals with different $K_{ow}$ values, it was possible to assess the role of $K_{ow}$ on uptake route partitioning. For such a comparison, an interpolation was made for the ratio of exposure concentrations that produced an equal contribution to the body burden from either uptake route. Comparison was also made for the predicted environmental concentration ratio for these chemicals based on the equation (Gobas 1993a):

$$C_d/C_w = K_{ow} \cdot L_d \qquad (3.5)$$

where $L_d$ is lipid content of the fish food, which is assumed as 2% for low level of trophic organism.

# 3. EXTRACTION OF CHEMICALS FROM WATER, FOOD AND FISH BODY

## 3.1 WATER SAMPLES

The water samples (50 to 500 mL) were collected at the same time as fish were sampled. The chemical extraction of water samples followed the protocol outlined in Figure 3.2. A solid reverse-phase extraction method was chosen, utilizing a octadecyl ($C_{18}$) (a non-polar sorbent 18 carbon straight-chain hydrocarbon) Bond Elute cartridge (Varian Co.) or a octadecyl ($C_{18}$) Empore$^{TM}$ disk (J.T.Baker), depend on sample volume. The $C_{18}$ column and disk were conditioned with methanol before use and was eluted by hexane. Each water sample was passed through either a cartridge column or an Empore$^{TM}$ disk from a glass funnel with flow controller, depending on the volume of the water sample. The chemicals were then eluted with hexane, as described in detail by Blevis et al. (1993). Compared with preliminary experiments using a liquid/liquid extraction method, the solid reverse phase extraction technique with a $C_{18}$ column reduced preparation time by more than 60%, reduced solvent usage by 90%, improved reproducibility and enabled high recoveries. The extracts were cleaned up as described below, and concentrated by $N_2$ in room temperature to 2 mL, followed by GC analysis.

## 3.2 TEST FOOD AND FISH TISSUES

The chemical extraction for test food (0.2 g) and fish tissue (0.5 g) was similar and is shown in Figure 3.3. The fish was thawed immediately prior to tissue analysis and the body surface was washed gently with distilled water and blotted dry. The fish was weighed and then the muscle was minced. Each fish sample was homogenized in a 15 mL

hand-held homogenizer (Pyrex Co., England) using a 2 mL acid buffer solution and 0.5 mL of the surrogate chemicals. The homogenate was then transferred to a 15 mL centrifuge tube with a screw cap, which was filled with 5 mL hexane and 3 to 5 mL buffer solution, and was shaken for 4 hours (American Optical Co., Richmond, CA.). It was then centrifuged at 3,000 g for 10 minutes. The supernatant was collected and another 5 mL of hexane was added in the centrifuge tube, followed by same procedure of shake and centrifugation. The supernatant were pooled and cleaned up, as described below, before GC analysis.

## 3.3 CLEAN UP

Before GC analysis, the water, fish, sediment and food extracts were cleaned up by transferring them into a 15 cm long clean-up column, containing (from bottom to top): a bead (#3000, Fisher Scientific), silica gel 40 (Kieselgel 40 Merck, 0.078 g), silica gel 60-200 (Mallinckrodt SilicRA, 0.2 g), a mixture of silica gel 60-200 and sulfuric acid (Fisher Scientific) with a ratio of 60:40 (0.2 g) and anhydrous sodium sulfate (Caledon 0.3 g). The column was washed with hexane before clean up. After the extracts had passed through the column, 5 mL of hexane was used to elute the column. The eluate was concentrated to 1.5 mL with $N_2$ at room temperature. Internal standard chemical (0.5 mL) was added into the extracts before GC analysis. The procedure for GC analysis and data calculation was presented in Chapter 2. Based on surrogate extraction, the recovery was 94 to 96% for TCB and 98% to 101% for PeCB and HCBP. The precision was >98%.

## 3.4 LIPID EXTRACTION OF FISH AND FISH FOOD

Lipid extraction was performed on a minimum of 3 g of either fish food or fish tissue, using a column (ID = 1 cm and length = 60 cm) filled with (from the bottom to top): a bead (3000#), silica gel 40 0.5 g, a 6 g mixture of Florisil 60-100 (FisherScientific) and silica 40 with a ratio of 4:1, Florisil 60-100 0.15 g, anhydrous sodium sulphate 1.5 g. and mixture of 3 g sample and 10 g of anhydrous sodium sulphate mixture anhydrous sodium sulphate of 10 g. Since the lipid content in fish was lower than that in fish food, lipid extraction from fish tissue used silica gel 60-200 instead of Florisil. The column was eluted with 300 mL of petroleum spirit (35-60C BDH) and dichloromethane (Caledon) with a ratio of 1:1. The solvent was then evaporated in a rotary evaporator (Yamato Scientific Co. Ltd., Japan) and then further dried in an oven at $60^0C$ for 1 hr (Gobas et al. 1993b). The lipid content was determined by weight. The lipid content was $5.22 \pm 0.40\%$ in the whole fish body, $3.52 \pm 0.20\%$ in fish muscle and $14 \pm 0.91\%$ in the food.

# 4. RESULTS

## 4.1 WATER EXPOSURE

Although the chemicals were supplied continuously during the entire water exposure experiment by a dosing system, the water concentration changed with the time (Fig. 3.4). During the first hour, water concentrations (3.9, 0.50, and 0.72 µg/L for 1,2,4-TCB, PeCB and HCBP, respectively) were higher than their expected concentrations (1.85, 0.41 and 0.42 µg/L, respectively). Chemical concentrations then decreased over the next three hours to levels about 37.5 to 60.5% of the initial concentrations. Water concentrations were reasonably steady from hour-12 through hour-48 at about 15 to 30% of the expected concentration for 1,2,4-TCB, 20 to 40% for PeCB and HCBP.

The chemical concentration in fish from water exposure are shown in Figure 3.5. The body burden of 1,2,4-TCB increased rapidly during the first 24 hours to about 250 µg/kg, but the accumulation rate then slowed. The 1,2,4-TCB body burden was not significantly different between 24-hour to 96-hour samples. PeCB showed a rapid uptake phase during the first 24 hours, similar to that for 1,2,4-TCB. The body burden of PeCB continued to increase significantly by almost 2.5-times until 96-h. The exposure concentration for HCBP was the same as PeCB (Figure 3.4), but bioaccumulation of HCBP started much slower than PeCB. At 24 hours, the body burden of HCBP was more than 3-times lower than body burden of PeCB. By 96 hours, the body burden of HCBP had increased significantly further 2-times, but remained about 6-times lower than that for PeCB. Test chemicals were not detected in the muscle tissues of any control fish collected in the same manner as the exposed fish.

## 4.2 FOOD EXPOSURE

The body burdens resulting from food exposure are summarized in Figure 3.6. 1,2,4-TCB accumulated quickly during the first 5 days of feeding, but the body burden did not change significantly from day 5 to day 12. Both PeCB and HCBP accumulated at a slower rate than 1,2,4-TCB during the first 2 days, but then accumulated steadily during the remainder of the exposure period (Fig. 3.6). PeCB and HCBP accumulation over the final 10 days appeared to have a linear relationship between chemical concentration in fish versus exposure time. Regression statistics showed that the correlation coefficient between body burden and time was 99.6% for PeCB (y = 64.55x - 34.72) and 98.1% for HCBP (y = 58.99x - 69.52). For PeCB, the body burden increased 2.5-times between day-5 exposure and day-12 exposure. Likewise, for HCBP, the body burden increased 4-times between day-5 exposure and day-12 exposure.

## 4.3 UPTAKE RATE CONSTANTS

The uptake rate constants for the food and water exposure experiments were calculated from BIOFIT model (Gobas et al. 1992b) and are presented in Tables 3.1 As expected from the body burden data, the $k_1$ values were much higher than $k_d$ values, i.e., all $k_1$ values for three test chemicals were about 5-orders of magnitude greater than the $k_d$ values (assuming L/kg is equivalent to kg/kg). Surprisingly, $k_1$ values for 1,2,4-TCB and HCBP were very similar, 258 L/kg.d and 257 L/kg.d, respectively (Table 3.1), as were the $k_d$ values, 0.0028 kg/kg.d and 0.0026 kg.kg/d respectively. Among the three chemicals, PeCB showed the highest ratios for $k_1/k_d$ (Table 3.1). The bioconcentration factors (BCF)

for 1,2,4-TCB and HCB were also similar, but the biomagnification factors (BMF) for three chemicals increased according to their log $K_{ow}$ values, i.e., 1,2,4-TCB < PeCB < HCBP.

During the feeding experiment, fish growth showed no significant increase from day 1 to day 5, but had increased significantly ($p < 0.05$) after day-6. At day 12, fish weight had increased 36%, and so increased body mass could have diluted $k_d$ by 36%.

4.4 MODELING SIMULTANEOUS EXPOSURE TO CHEMICAL IN FOOD AND WATER

The predicted contributions of gill and GI tract uptake to the chemical body burden in fish are shown in Table 3.3 and Figure 3.7. It was discovered that for the measured $k_1$ and $k_d$ values of all three chemicals, the GI tract accounted for nearly 100% of uptake if chemical concentration in food:water was around $10^7$ or greater. In contrast, when the food:water concentration was around $10^3$ the gill uptake accounts for 100% of uptake. The two uptake routes contributed almost equally to body burden when the ratio of chemical concentration in food to water was around $10^5$.

# 5. DISCUSSION

## 5.1 WATER CONCENTRATION

In the water exposure experiment, the chemical concentrations were not maintained constant by the dosing system until 8 hours into the experiment and most concentrations detected were lower than the expected chemical concentration (Figure 3.4). It was noticed that the chemical concentrations were higher than expected in the first hour. The reason might be that either the chemicals were not fully dissolved in the water and they had not reached equilibrium, or the chemical stock solution was initially not well mixed in the water aquarium. The lower than expected chemical concentrations for the rest of the experiment were probably due to rapid chemical absorption by the fish. By hour 4 the body burden of the fish was already more than 100-times the water concentrations of the test chemicals. The lower chemical concentration may also include volatilization, which, however, was probably minor because the aquarium was covered by a lid. The other possibility is that adsorption to the glass of the aquarium walls relative to the rate of chemical replacement by the dosing apparatus may have been greater during the first couple of hours. Nevertheless, adsorption and volatilization of the chemicals were not considered as significant factors since the stock was replaced daily and the fresh stock solution had no effect on the water concentration. Moreover, these changes in water concentration did not affect the calculation of k and BCF since the BIOFIT model accounts for such changes and also compensates for the exposure duration being too short to reach a steady-state (Gobas et al. 1992b).

$k_l$ values for lipophilic chemicals are reportedly several orders of magnitude higher than $k_d$ values. Studies have shown that $k_l$ values ranged from $10^2$ to $10^6$ and $k_d$ values were mostly less than 1.0 (Macek et al. 1970; Lieb et al. 1974; Bruggeman et al. 1981; Skaar et al. 1981). In Opperhuizen's study (Opperhuizen et al, 1990 & 1991), which tested $k_l$ and $k_d$ on several classes of chlorinated hydrocarbons in small fish (< 5 g), $k_l$ values were between 100 to 10,000 L/kg.d whereas $k_d$ values were calculated as 0.004 to 0.016 kg/kg.d derived from the product of uptake efficiency and uptake rate. Thus, $k_l$ and $k_d$ can differ by as much as 4 to 6 orders of magnitude. The present study is therefore consistent with previous studies in that the $k_l$ for 3 test chemicals ranged from 257 to 1,360 L/kg.d, whereas the $k_d$ values ranged from 0.0026 to 0.0070 kg/kg.d, representing up to 5 orders of magnitude difference. Such differences may be partly related to the speed with which lipophilic chemicals potentially enter fish via gill uptake vs GI tract uptake.

In reviewing the fish bioaccumulation curves from two exposure experiments (Fig. 3.5 and Fig. 3.6), it is obvious that rapid gill uptake caused a significant body burden after only a few hours of exposure to contaminated water, while GI tract uptake was tardy during the first two days. This lag phase for GI tract uptake indicates that the initial absorption of lipophilic chemicals by a fish entering a contaminated system for the first time would occur via gill uptake, even if there was simultaneous exposure via the GI with the food chemical concentration much higher ($10^5$ to $10^6$ times) than that in the water. Such a situation might occur in nature with migratory fish moving into and feeding in a contaminated aquatic ecosystem.

The low $k_1$ of HCBP may have resulted from its very low concentration in water, i.e., HCBP might have not fully dissolved in the water because of its very high hydrophobicity such that the water concentration of HCBP was actually lower than that measured. Although the test concentration was below the solubility limit of HCBP in water (0.4 $\mu$g/L), there is no proof for the HCPB being dissolved. Other researchers have used generator columns (Burgmore et al. 1981; Opperheizen et al. 1985; Gobas et al. 1989a) to produce dissolved PCBs and other lipophilic chemicals, and limit the possible formation of crystals in water. Even so some chlorinated chemicals with log $K_{ow}$ value above 7 (Opperhuizen 1990) were found to have lower values of BCF than chemicals with a lower log $K_{ow}$ value. So other mechanisms might be involved in the lower bioconcentration of HCBP and these are discussed in section 5.4.1.

## 5.3 SIMULATING SIMULTANEOUS CHEMICAL EXPOSURE VIA WATER AND FOOD

It is the product of rate constant and exposure concentration that will determine the relative roles of gills and GI tract (see equation 3.1 and 3.2). Even though gill uptake of lipophilic chemicals has a much greater rate constant than GI tract uptake, food concentrations of lipophilic chemicals will be much higher than water concentrations. There are two ways to experimentally assess relative roles of the uptake routes. One is to experimentally vary the gills and the GI tract exposure concentrations and measure the changes in chemical concentration in fish. This would be very labor intensive and costly. An alternative is to model the exposure concentrations, as was done here using the measured $k_1$ and $k_d$ values.

The simulation model for Figure 3.7 assumes that fish exposure occurs in an expected chemical ratio that would be reached with a chemical equilibrium with the exposure medium. For simplicity, food/water elimination rates were not included in this model. A general finding was that food chemical concentrations needed to be at least 5-orders of magnitude higher than water chemical concentrations for GI tract and gills to contribute equally to body burden. Having established this fact, it is important to consider what food/water ratios might exist in the field. In the absence of field data for these chemicals, I estimated the probable ratios based on the lipid content of the food and the log $K_{ow}$ values of chemicals. The approximate concentration in food can be calculated by a simple equation $C_d/C_w = K_{ow}.(L_d)$ (Gobas 1993a), in which, $L_d$ is the lipid content of the fish food This was assumed to be 2%. Thus, the chemical concentrations of three test chemicals in food would be 191 ($10^{2.3}$), 2,350 ($10^{3.3}$) and 709,627 ($10^{5.9}$) µg/L for 1,2,4-TCB, PeCB and HCBP, respectively (Fig. 3.7), if the water chemical concentrations for three test chemicals was set as 1 µg/L. These concentrations were plotted on Figure 3.7 and the relative contribution of gill versus GI tract uptake to body burden were interpolated. From Figure 3.7, it is obvious that over 98% of a fish's body burden for 1,2,4-TCB and PeCB will come from the water via gill uptake; the GI tract accounts for over 85% of uptake of HCBP. This simulation model clearly illustrates the importance of log $K_{ow}$ on the relative roles of gill vs GI tract of lipophilic compounds in fish. With a chemical such as TCB or PeCB, having a log $K_{ow}$ of 3.98 and 5.03 respectively, the gills are the most important uptake route.

The lipid of food in this simulation model was 2% while the lipid content in the food exposure was 14%. The different lipid content of food might be a factor that affect the uptake of lipophilic chemicals and relative literature was reviewed. In a dietary study, Gobas et al. 1993b found that the uptake efficiency of lipophilic chemicals with a log $K_{ow}$ of 4.51 - 6.10 was not significantly different between high (13.5%) and low fat food (< 0.2%), but the uptake efficiency of chemicals with a very high log $K_{ow}$ (6.29 - 8) from low fat food was 30% - 50% higher than from high fat food. Therefore, according to the lipid content (14%) in this study, the food contribution to HCBP biomagnification from the simulation model may be underestimated at least 50%, but the conclusion that the majority of HCBP in body burden can be taken via GI tract does not change.

## 5.4 THE IMPACT OF HYDROPHOBICITY AND OTHER FACTORS ON UPTAKE OF TEST CHEMICALS

### 5.4.1 Water exposure

Many studies have suggested that bioconcentration is only directly related to log $K_{ow}$ over the range 2 to 6, and is inversely related to log $K_{ow}$ at values higher than 6 or 7 (Bruggeman et al. 1984; Oliver et al. 1984; Connell 1990; Gobas 1990a; Opperhuizen 1991). In the present experiment, the $k_1$ value and BCF for PeCB (log $K_{ow}$ 5.03) were higher than those for 1,2,4-TCB (log $K_{ow}$ 3.98) (Table 3.1), but lower than those for HCBP (log $K_{ow}$ 7.55). These findings are consistent with previous studies.

It is suggested that HCBP is not as readily absorbed by the gills as the other two chemicals. The reasons may be two-fold. Firstly, although HCBP has a log $K_{ow}$ value of 7.55, this does not necessarily indicate that the lipid solubility properties for HCBP is in

excess of those with lower log $K_{ow}$ values. Chemicals with log $K_{ow}$ values about 6 or higher, in fact, may exhibit declining lipid solubility (Dobbs et al. 1983), which might reduce the affinity of the chemical with the lipid membrane of gill epithelium. The ability to deliver chemicals to the gill is also important (Randall et al. 1990), which means that the water solubility of chemicals can determine the chemical delivery capacity to gills. At a log $K_{ow}$ value over 7, the decline in lipid solubility combined with the extremely low water solubility would decrease chemical uptake process because gill transfer and chemical delivery to the gills are both impaired. The second explanation may be that the molecular size of HCBP (360) is too large to absorb across the gill. Anliker et al. (1987) reported that dispersion dyes with a molecular weight of more than 360 exhibited limited bioaccumulation. Since this study did not measure the permeability of gills to the size of the chemicals, this possibility needs further study.

The slow increase of the HCBP in the fish body exposed to contaminated water does not necessarily mean that the bioconcentration of HCB would never result in a high body burden. A slow elimination rate from the organism could result in a high body burden with a much longer exposure.

## 5.4.2 Food exposure

The results from food exposure experiment support previous studies, in which the BMF was directly related to the log $K_{ow}$ value, even when the log $K_{ow}$ value of a chemical is above 7 (Connell 1990; Haffner et al. 1994; Heath 1995). The possible explanation for this is the fugacity theory (Gobas 1993b), which holds that the fugacity is elevated during

digestion to a level higher than that in the organism. This fugacity gradient results in a net uptake of chemicals across the intestine and may not be influenced by lower water solubility when the chemical has very high log $K_{ow}$ values. Another reason may be that, in food exposure, a reduction of elimination rates for certain chemicals with higher log $K_{ow}$ values results in higher biomagnification (Opperhuizen et al. 1988; Opperhuizen et al. 1990).

Heath (1995) summarized the bioaccumulation of lipophilic chemicals via gills and GI tract by stating that: Chemicals up to log $K_{ow}$ 3 are mainly taken up by gill; those with log $K_{ow}$ 3 - 6 are taken up by both gill and gut; and those above log $K_{ow}$ 6 are probably taken up entirely by gut uptake. The results of my study support this conclusion.

## 5.5 THE ELIMINATION OF 1,2,4-TCB

Unlike the other two chemicals, the accumulation of 1,2,4-TCB apparently stopped after 24 hours of water exposure (Fig. 3.5), and after one day of food exposure (Fig. 3.6). A more reasonable explanation is that uptake and loss (metabolism and excretion) of chemicals reached a balance. Loss of TCB must have proceeded at a rapid rate soon after the body burden reached a certain level, in this case, about 200 and 250 µg/kg from food and water exposure, respectively. Metabolism was suggested as important factor that cause faster elimination, and was reported by other studies. For example, 1,2,4-TCB was found only to have a 1- to 3-day half-life in bluegill sunfish and American flagfish (Barrows et al. 1980; Smith et al. 1990), and was found to be rapidly metabolized by other animals, such as rats and monkeys (Lingg et al. 1982). The mixed

function oxidase (MFO) biotransformation system may be enhanced for 1,2,4-TCB metabolism once body burden reached a certain level.

The instability of 1,2,4-TCB as a low log $K_{ow}$ test compound clearly caused problems in the bioconcentration and biomagnification experiments. A substitute chemical, 1,5-dichloro-2,4-dinitrobenzene (log $K_{ow}$ value 2.5), which is "not dissociated or metabolized" in the environment (Newman 1993, Letter from Montana State University), for 1,2,4-TCB was tested during the early portion of this study. However, its high lethal toxicity to fish and lower sensitivity to GC precluded its use in this study.

# 6. CONCLUSION

In this study, the relative importance of the gill and GI tract uptake routes was investigated. In the exposure experiments, the chemical accumulation in fish showed that gill uptake was the faster and more efficient route for absorption of lipophilic chemicals during the early stages of exposure (two days). The higher value for $k_1$ than $k_d$ indicated that the gill uptake route was potentially important for the bioconcentration of lipophilic chemicals in fish. A simulation model of simultaneous exposure to chemicals in food and water showed that, based on data obtained, gill and GI tract uptake would contribute equally to chemical body burden in fish when the chemical concentration in food was about 5-orders of magnitude higher than that in water.

In the natural environment, fish might expose to contaminated food and water with the ratio that is based on the knowledge of chemical hydrophobilicylicity and lipid content of food, in which, the concentration ratio of food/water for 1,2,4-TCB and PeCB are predicted lower than HCBP. Combining the $k_1$ and $k_d$ values for these chemicals, gill uptake would play an important role in the uptake of 1,2,4-TCB and PeCB, with moderate to high log $K_{ow}$. In contrast, GI tract would be relatively important for HCBP, which has a very high $K_{ow}$ value.

Table 3.1 Uptake clearance constants ($k_1$, $k_d$) derived from water and food exposure to three test chemicals, 1,2,4-TCB, PeCB and HCBP using juvenile rainbow trout.

| Exposure | | 1,2,4-TCB | PeCB | HCBP |
|---|---|---|---|---|
| Water | $k_1$ (L/kg.d) | 258 | 1360 | 257 |
| | BCF | 1,942 | 5,573 | 1,413 |
| Food | $k_d$ (kg/kg/d) | 0.0028 | 0.0070 | 0.0026 |
| | BMF | 0.0056 | 0.0542 | 0.9184 |
| $k_1/k_d$ | | 93,478 | 194,285 | 98,846 |
| BCF/BMF | | 346,785 | 102,882 | 1,538 |

Table 3.2  Weights of juvenile rainbow trout during the 12 day food exposure experiment.

| day(control) | $g \pm (SE)$ |
|---|---|
| 0 | $2.18 \pm 0.15$ |
| 1 | $2.16 \pm 0.167$ |
| 2 | $1.84 \pm 0.13$ |
| 3 | $2.53 \pm 0.144$ |
| 4 | $2.36 \pm 0.15$ |
| 5 | $2.30 \pm 0.17$ |
| 6 | $2.79 \pm 0.20*$ |
| 12 | $2.98 \pm 0.21*$ |

* $P < 0.05$ (student t-test)

Table 3.3 Simulation model predictions of the distribution of the three test chemicals, 1,2,4-TCB, PeCB and HCBP, in fish tissues assuming that the fish are exposed simultaneously to food and water with chemical concentration ratios ranging from $10^3$ to $10^7$.

| food/water | chemical mass from water | chemical mass from food | chemical mass total | from water % | from food % |
|---|---|---|---|---|---|
| **1,2,4-TCB** | μg | μg | μg | | |
| 10,000,000:1 | 258 | 27,620 | 27,878 | 0.93 | 99.07 |
| 2,500,000:1 | 258 | 6,905 | 7,163 | 3.60 | 96.39 |
| 625,000:1 | 258 | 1,726 | 1,984 | 13.02 | 86.98 |
| 156,250:1 | 258 | 432 | 690 | 37.45 | 62.54 |
| 39,063:1 | 258 | 108 | 366 | 70.55 | 29.45 |
| 9,766 | 258 | 27 | 285 | 90.55 | 9.45 |
| 2,441 | 258 | 7 | 265 | 97.45 | 2.54 |
| 1,000 | 258 | 3 | 261 | 98.94 | 1.06 |
| **PeCB** | | | | | |
| 10,000,000:1 | 1,361 | 70,420 | 71,780 | 1.90 | 98.10 |
| 2,500,000:1 | 1,361 | 17,605 | 18,965 | 1.17 | 92.83 |
| 625,000:1 | 1,360 | 4,401 | 5,762 | 23.62 | 76.39 |
| 156,250:1 | 1,360 | 1,100 | 2,460 | 55.29 | 44.71 |
| 39,063:1 | 1,360 | 275 | 1,635 | 83.18 | 16.81 |
| 9,766 | 1,360 | 68 | 1,429 | 95.19 | 4.81 |
| 2,441 | 1,360 | 17 | 1,377 | 98.75 | 1.25 |
| 1,000 | 1,360 | 7 | 1,368 | 99.48 | 0.52 |
| **HCBP** | | | | | |
| 10,000,000:1 | 257 | 25,900 | 26,158 | 0.98 | 99.02 |
| 2,500,000:1 | 257 | 6,475 | 6,732 | 3.82 | 96.17 |
| 625,000:1 | 257 | 1,619 | 1,876 | 13.73 | 86.27 |
| 156,250:1 | 257 | 405 | 662 | 38.89 | 61.11 |
| 39,063:1 | 257 | 101 | 359 | 71.80 | 28.20 |
| 9,766 | 257 | 25 | 283 | 91.06 | 8.94 |
| 2,441 | 257 | 6 | 263 | 97.60 | 2.40 |
| 1,000 | 257 | 3 | 260 | 99.00 | 1.00 |
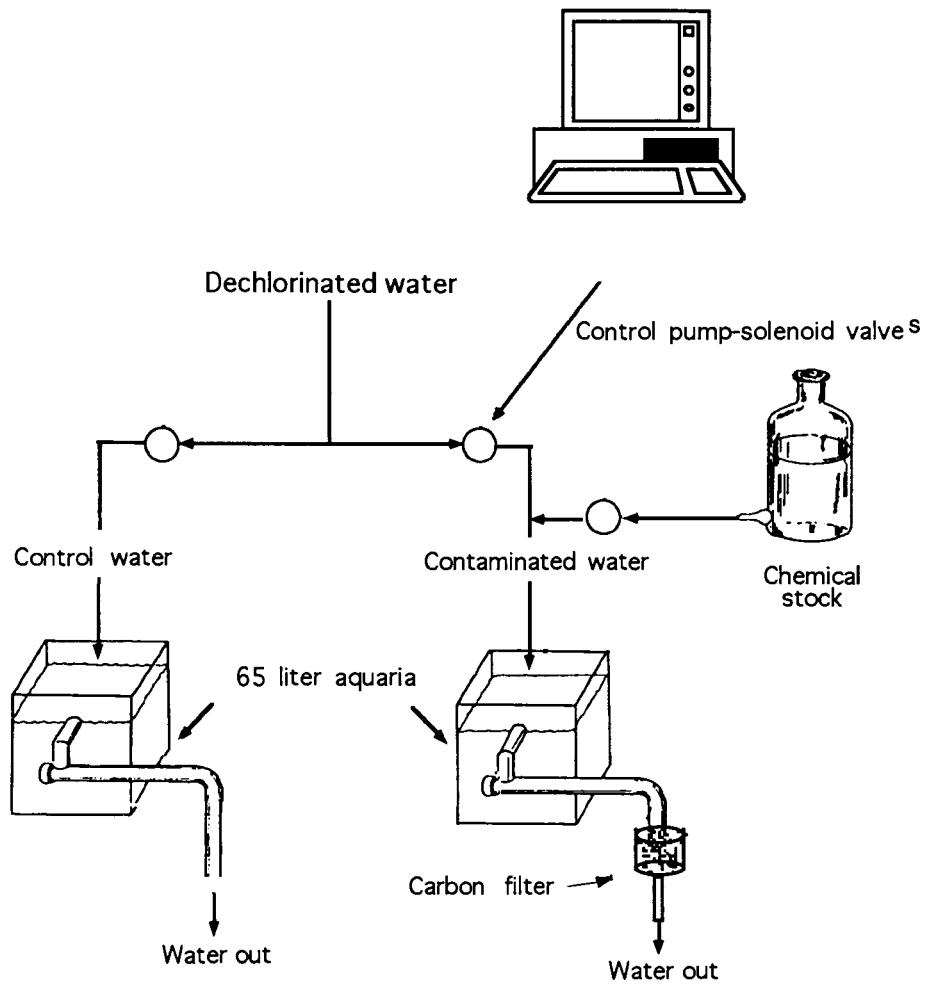
Figure 3.1 Apparatus used for chemical exposure via water for the juvenile rainbow trout.
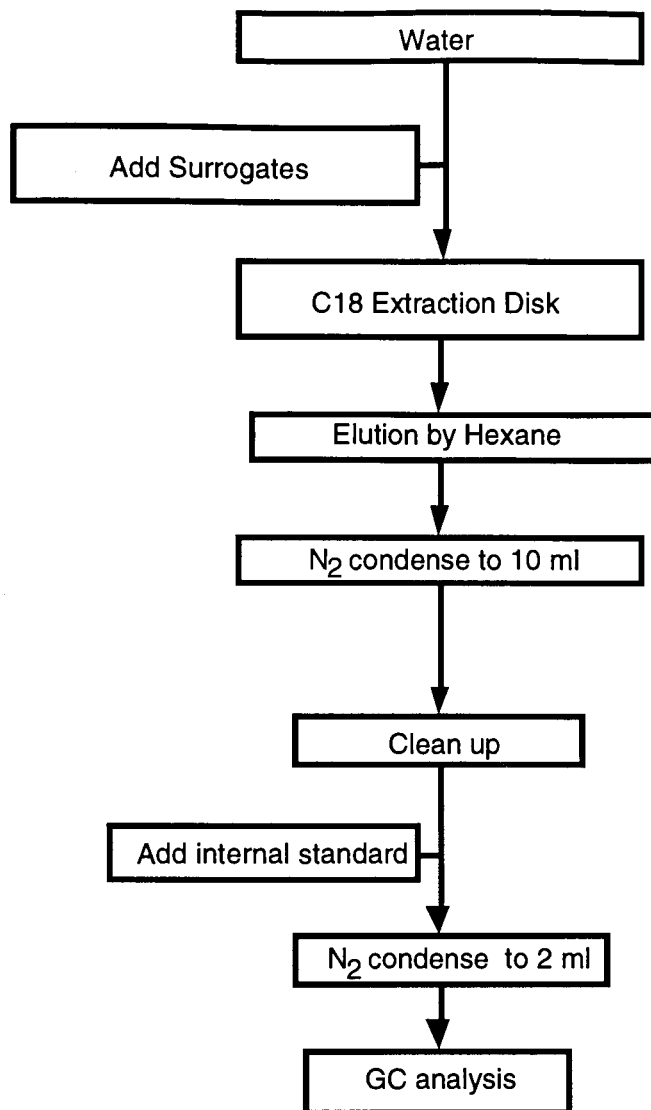
Figure 3.2 Chemical extraction protocol for water samples.
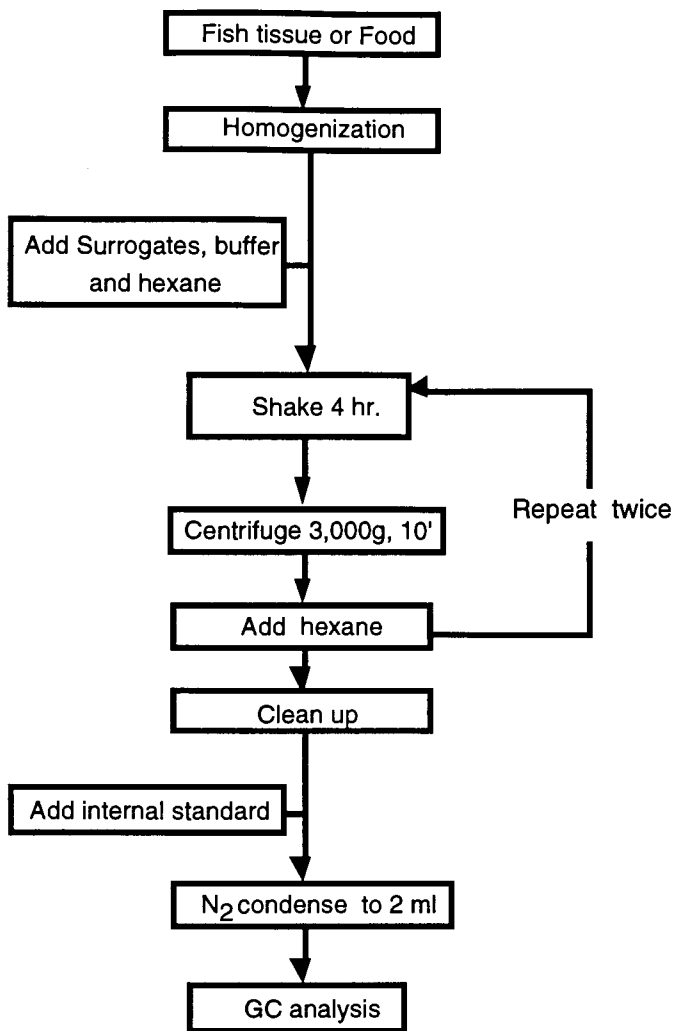
Figure 3.3 Chemical extraction protocol for test food and fish tissue.
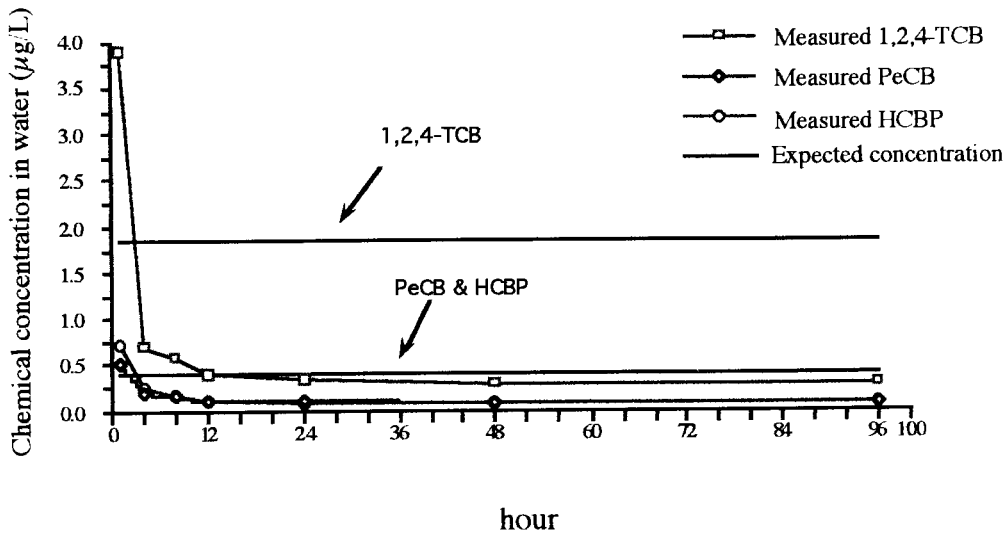
Figure 3.4 Measured chemical concentrations of 1,2,4-TCB, PeCB and HCBP in water during juvenile rainbow trout exposure experiment. Each point represents water concentration when fish was sampled. Each point is the mean of 2 samples.
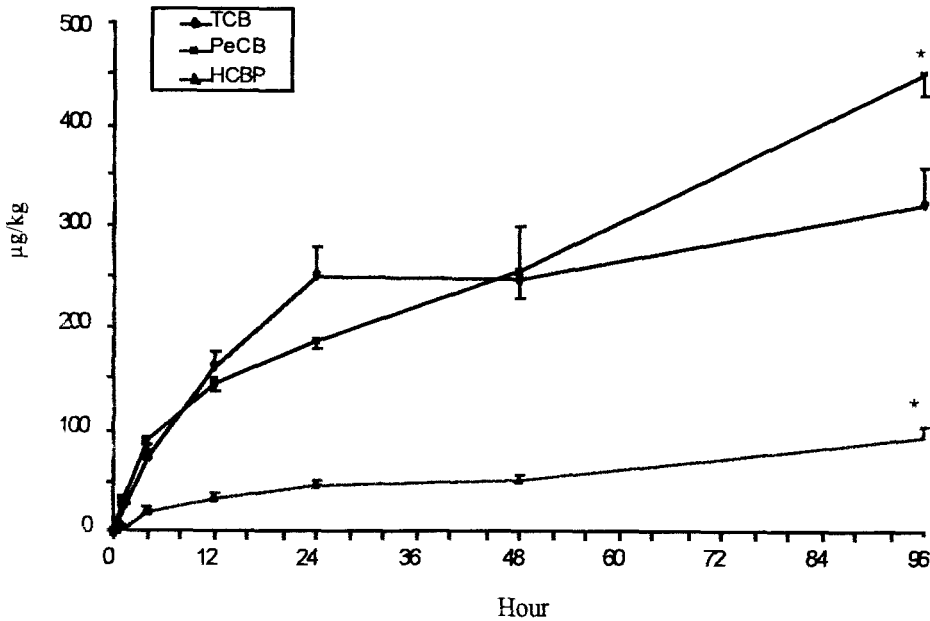
Figure 3.5 Concentration of test chemicals, 1,2,4-TCB, PeCB and HCBP in rainbow trout *(Oncorhychus mykiss)* muscle samples during a 4-day water exposure. Each point is the mean of 8 fish. * : significant difference (P <0.05) between the 24-hour and 96-hour exposure.
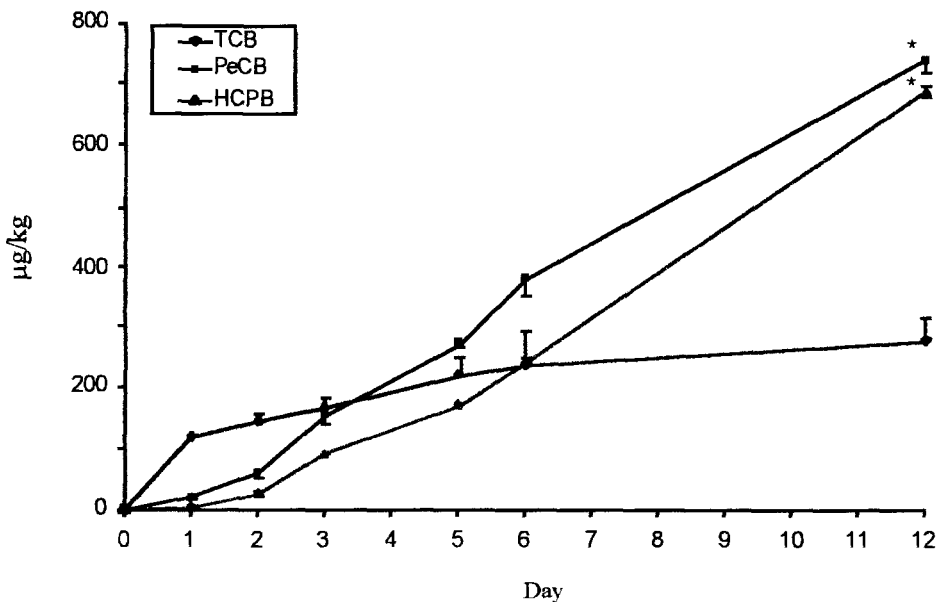


Figure 3.6 Concentration of test chemicals, 1,2,4-TCB, PeCB and HCBP in rainbow trout *(Oncorhychus mykiss)* muscle samples during a 12-day feeding experiment with chemically spiked food. Each point is the mean of 10 fish. * : significant difference (P < 0.05) between the 5-day and 12-day exposure.
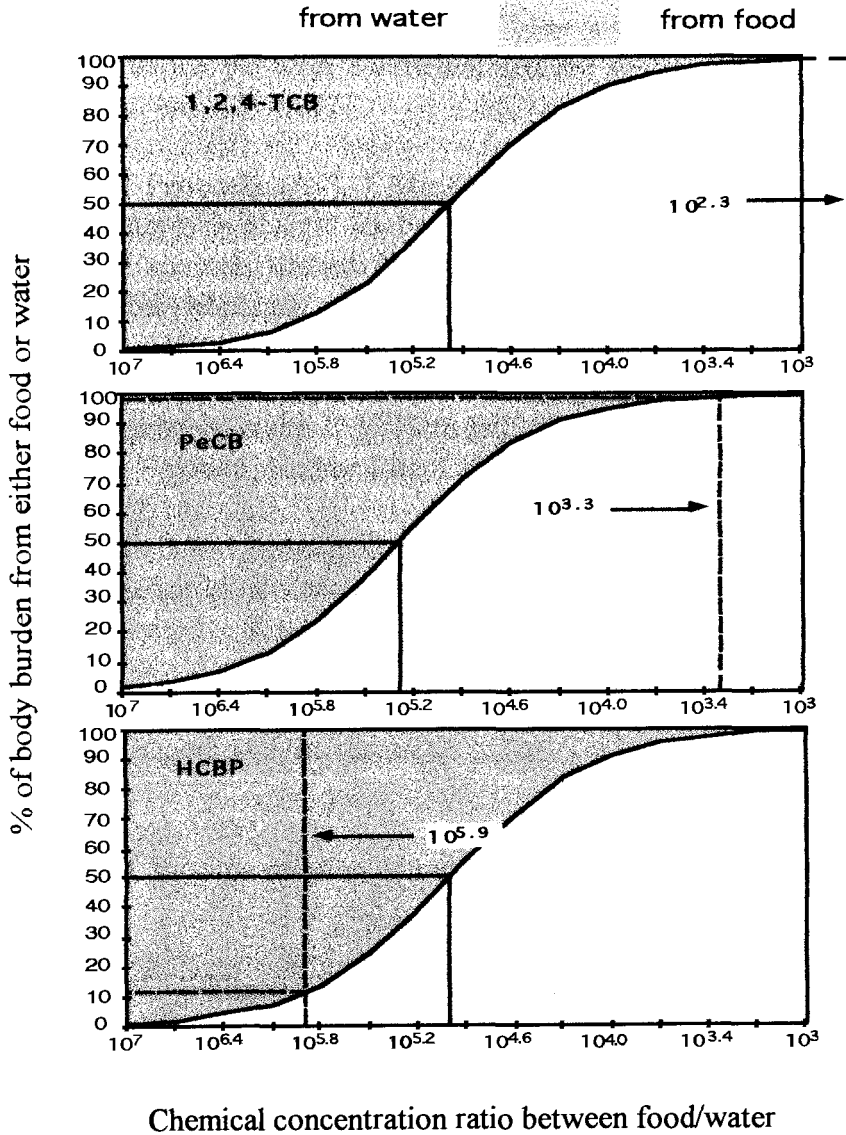
Figure 3.7 Calculated percentage contribution of test chemicals, of 1,2,4-TCB, PeCB and HCBP in food/water in fish body from gill/GI tract uptake as a function of the ratio of the assumed chemical concentrations ratio in food/water. The solid line represent the 50% of uptake from food and water from the correspondent the ratio of the chemical concentration in food/water. The vertical dash line represents the possible ratio of chemicals in food/water by using the equation $Cd/Cw = K_{ow}.L(0.02)$. The horizontal dash lines represent the predicted chemical distribution in fish based on the predicted chemical ratio in food/water and the $k_1$ and $k_d$ values from the experiment.

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# CHAPTER 4

# UPTAKE OF LIPOPHILIC XENOBIOTICS BY FISH

# IN SEDIMENT - LADEN WATER

# FROM THE FRASER RIVER VIA GILL UPTAKE

**ABSTRACT**

This study investigates the uptake of three lipophilic chemicals, 1,2,4-TCB, PeCB and HCB, with log $K_{ow}$ values from 3.98 to 7.55, by unfed juvenile rainbow trout (_Oncorhynchus mykiss_) in test aquaria containing sediments from the Fraser River. A comparison of centrifugation and filtration methods to obtain concentration of free dissolved chemicals in water was conducted to obtain the truly dissolved chemicals. The results showed filtration to be the better of the two methods. The test chemicals and sediments were introduced into aquaria 9 days before the fish were introduced and concentrations in the bottom sediments, suspended sediments and filtered (0.45 μm) water were measured. Concentration levels reached a quasi-steady state by day 9. A 6-day exposure resulted in a significant bioaccumulation of the test chemical in the fish tissues and significant reductions in the concentrations of chemicals in the bottom sediments, suspended sediments and filtered water. However, the body burden of HCB and PeCB appearing in the fish after 6 days could not be accounted for solely by the amount of chemical dissolved in the water when the fish were introduced. Mass balance analysis was not possible for 1,2,4-TCB because of fish metabolism. The conclusion was that lipophilic chemicals such as PeCB and HCBP associated with Fraser River sediment are bioavailable.

# 1. INTRODUCTION

## 1.1 SUSPENDED SEDIMENTS AND CHEMICAL BIOAVAILABILITY

In a recent report from the B.C. Ministry of Environment, Lands and Parks, high rates of fish morbidity were found in the Fraser River when they contacted deposited sediment containing numerous toxicants from industry and storm water (Newcombe et al. 1996). This finding implies that either the sediments cause mortality, or the chemicals associated with the sediment cause the mortality, likely as a result of bioaccumulation of chemicals in fish body.

While researchers agree that lipophilic xenobiotics are taken into fish via the food chain and water, the role of suspended sediments is less certain. This uncertainty is of particular concern with regard to the Fraser River because it has a very high suspended sediment load (10-1,400 µg/L, depending on season) (Environmental Canada) and adsorption of lipophilic xenobiotics to suspended sediments can produce concentrations that are orders of magnitude higher than those in water only (Karickhoff et al. 1979; Servos et al. 1989a; Sekela 1995). The Fraser River in British Columbia, Canada is one of the world's major fishery rivers. Naturally, there is concern about the uptake of toxicants by salmon as they pass through the river system. Therefore, the objective of this study was to provide preliminary information on the uptake by juvenile salmon of lipophilic xenobiotics associated with suspended sediments found in the Fraser River.

Routes of toxicant uptake in fish include the gut, gill and skin. Of these routes, exposure to suspended sediments is greatest at the gill because of the high water convection rate associated with gill ventilation. In addition the design of the gill is far more

efficient for diffusive exchange than either the gut or the skin. In fact, the fish gill is in much more intimate contact with the sediment-laden water than the gut is with sediment-laden food. The maximum diffusion distance in the interlamellar water is of the order of 10-20 microns and the diffusion distance between the water and lipid containing blood is around 1-2 microns in salmonids (Hughes 1984; Laurent 1984). These diffusion distances are all much larger in the gut. Nevertheless, whenever chemicals associated with bottom sediments are ingested (inadvertently or otherwise) by benthic fish, they are typically regarded as being available for uptake across the gut epithelium. Additional factors favoring gut uptake include digestive processes that enhance both the concentration gradient (Gobas et al. 1993b) and the disaggregation of material. Despite the highly effective gill exchange area in fish and a favorable concentration gradient, studies indicate that contaminants bound to organic sediments and particles in the water may not cross the gill. Adams et al. (1985) and Black et al. (1988), for example, found that benzo[a]pyrene and tetrachlorobiphenyl, when associated with particulate organic matter, did not diffuse across the gill membrane because of the high association constants between the contaminants and humic acid. Clearly, the bioavailability of lipophilic xenobiotics in sediment-laden water must also depend to a large degree on the ease with which the chemicals desorb from the suspended sediments and on the relative affinity for lipids in the fish gill. The sediments from the Fraser River have a low organic carbon content (around 1%), being characterized as glacial lacustrine deposits, and this fact may increase the ease with which lipophilic xenobiotics can desorb from the suspended sediments in the Fraser River.

The hypothesis to be examined is that lipophilic chemicals associated with the suspended particles containing low organic carbon (from Fraser River) are available for uptake by juvenile rainbow trout. To test this hypothesis, the chemical mass balance of chemical from water, sediment, suspended sediments and fish were measured and a technique to block the GI tract was used to preclude gut exposure. The uptake of chemicals in the bottom sediments via GI tract was also compared.

## 1.2 OBTAINING THE TRULY DISSOLVED CHEMICAL CONCENTRATIONS

To obtain an accurate mass balance, water chemical concentration (truly dissolved chemicals) had to be measured. As part of my quality control, a reliable analysis method was needed. Centrifugation and filtration techniques have been widely used previously for separating solids from the sediment-loaded water but both of these methods have merits and limitations, as described below.

### 1.2.1 Centrifugation

Centrifugation has been frequently used to study sediment/water partition coefficients. Experiments have investigated centrifugation time and speed. At low suspended sediment concentrations (<10 g/L), Gshwend et al. (1985) reported that centrifugation at 1700g for 60 minutes had no effect on the partition coefficient value between suspended particles and water (Kp) compared with 760g for 20 minutes. However, Servos et al. (1989 b) showed the "free water concentration" was about 1 to 1.35 times higher from centrifugation at 6,000g compared with 20,000g. Centrifugation for

a relatively long period of time (i.e. a few hours) was considered as possibly reducing chemical concentration (Karickhoff et al. 1978). On the other hand, particles were still not completely eliminated from the supernatant, even with a centrifugation speed of 27,100g and a duration of 4 hours (Voice et al. 1983). These non-settling particles presumably had a density similar to water. The chemicals associated with non-settled particles in the supernatant therefore, may lead to overestimating the dissolved chemical concentration in water. There is, unfortunately, no accepted centrifugation and duration time available to be used as universal methods for water/suspended sediments separation. In this study, the water chemical concentrations from either centrifugation or filtration method were compared. The centrifugation method employed a speed of 20,000g for 30 minutes (R-5 Super-speed Refrigerated Centrifuge - Truments Sorvall, Dupont Canada) using 25-mL Corex centrifuge glass tubes.

1.2.2 Filtration

Filtration is another common method to remove the particles from water. However, variable results of water chemical concentration using filtration have caused discussion over the size of the membrane filter that should be used to obtain the true dissolved chemical concentration. Controversy over the size of dissolved particles (from 0.015 μm to more than 0.45 μm) has caused debate in chemical extraction studies (Karickhoff et al. 1978; Nielsen 1994; Horowitz et al. 1996). The most commonly accepted pore size filtration film for extract the truly dissolved chemical from water is 0.45 μm (Mackay 1991; Horowitz et al. 1996; USEPA 1983; Office of Water Data

Coordination 1984; APHA 1989; ASTM 1995) which was applied (Schleicher & Schuell Co. Keene, NH) in this study.

To solve the problems of colloidal association of chemicals and the clogging of the membrane by colloidal particles, sediment-laden water should not include a very high content of sediments. Sorption of chemicals onto the filter membrane may underestimate the true dissolved concentration in water. To calculate the loss by sorption of chemicals on the filter membrane, portion of chemical adsorbed in the membrane must be tested and used for future calibration for chemical concentrations.

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS

#### 2.1.1 Test animals and chemicals

Approximately 200 juvenile rainbow trout (*Oncorhychus mykiss*) weighing from 3.0 g to 3.5 g were obtained from Westcreek Fish Farm, Langley, B.C, and were used for the experiments. Details of the acclimation of fish, water quality and the three test chemicals, 1,2,4-TCB, PeCB and HCBP are described in Chapter 2. The hardness of the water in the aquaria was around 17.1 mg/L as $CaCO_3$, and the alkalinity was 17.1 mg/L as $CaCO_3$. By comparison the Fraser River has a hardness of 50 to 100 mg/L as $CaCO_3$ and an alkalinity of 40-90 mg/L as $CaCO_3$, depending upon the season and geographical location.

#### 2.1.2 Sediments

The top 5 cm of sediments were collected from the shore line of the Fraser River at Annacis Island during a low water period. The entire sample was well mixed to create an homogeneous mixture before being used. The sediments were analyzed with a gas chromatograph (GC) for the presence of the test chemicals (see below), and none of test chemicals were detected. The organic matter content of these sediments was determined by the method of loss on ignition in triplicate (Klute 1986); a 30 g sample of air-dry sediments was placed in a ceramic crucible, and combusted in an ignition oven at 500 °C until constant weight. The organic matter content was 2.73 ± 0.65%. Since organic matter usually contains about 50% organic carbon (Mackay 1991), I estimated that the

sediments contained around 1.4% organic carbon. Sediment subsamples weighing 100 g were added to the test aquarium (2.2 g/L sediment). The composition of the sediments was 35.25% fine sand, 57.25% silt and 7.5% clay.

## 2.2 EXPERIMENTAL METHODS

### 2.2.1 Comparison of centrifugation and filtration

The procedure used to compare dissolved chemical concentrations in sediment-laden water samples by filtration and centrifugation is summarized in Figure 4.1. Two 50 L glass aquaria were filled with 45 L of aerated dechlorinated tap water containing sediments. Three test chemicals, dissolved in 1 mL methanol, were added in each aquarium. Water, sediments and chemicals were vigorously mixed by a Teflon stir bar for about 10 minutes and the system was left to allow chemical distribution between water and sediments, and for the sediments to settle. Water was carefully sampled, without stirring, after 2, 6, and 9 days. Chemical concentrations were determined for the whole water sample, and for the water after the suspended sediments were removed by either filtration with a 0.45 μm filter membrane (Schleicher & Schuell Co. Keene, NH) or centrifugation at 2,000g for 30 minutes.

The adsorption of chemicals on the filter membrane was assessed in a separate experiment (Fig. 4.2). To prevent chemical adsorption to the particles existing in the tap water, the water was pre-filtered (0.45 μm) prior adding the test chemicals. The chemical analysis then was conducted before and after a second filtration with a 0.45 μm membrane filter. The test was performed in triplicate.

## 2.2.2 Fish exposure protocols

All exposures were performed in 50 L glass aquaria filled with 45 L of aerated dechlorinated tap water (Fig. 4.2). The water temperature was $12 \pm 1°$ C. A plastic lid covered each tank. The study period for each experiment consisted of a 9 day equilibration period followed by a fish exposure period. At the outset of the chemical equilibration period (day zero), 100 g of Fraser River sediments were added to the test aquaria and stirred vigorously. The three test chemicals were added at this time, dissolved in 1 mL methanol. The amounts of the chemicals dissolved in the methanol were as follows: 1415 μg of 1,2,4-TCB, 70.3 μg of PeCB and 102.3 μg HCBP. The test chemicals were allowed to partition between the water, suspended sediments and bottom sediments for 9 days. The majority of chemical partitioning occurred during the first 24 h after addition (data not shown) with much smaller changes occurring over the next 24 h (see Fig. 4.4). Analysis of water and sediments (sampled periodically during this 9 day period) showed a quasi-equilibrium was reached between the concentrations of the test chemicals in the water, the suspended sediments and the bottom sediments by day 9. Fish were introduced to the aquaria on day 9 in each of the two fish exposure protocols.

### 2.2.2.1 Protocol 1

The first experimental protocol examined whether or not fish access to bottom sediments affected chemical bioaccumulation. To do this, replicate aquaria were horizontally and equally divided by a stainless steel mesh on day 9 (Fig. 4.3). Ten fish were introduced below the horizontal mesh of each aquarium. These "benthic" fish therefore had access to the test chemicals associated with the bottom sediments. An

additional 10 fish were introduced to the upper half of the aquarium, above the horizontal mesh. These "pelagic" fish did not have access to the chemicals associated with bottom sediments. After a 10-day exposure period (on day 19), five fish from each replicate group were sampled for chemical analysis. Although these fish were not fed, there was no way of reliably monitoring sediment ingestion by the fish. Therefore, the experimental design included another group of fish that had had their pharynx blocked. The pharynx was blocked by surgically implanting a small, premolded plug (0.5 mm diameter, 0.8 mm length) of silicone rubber (Dow Corning) into the pharynx with hemostats. In fish with a pharyngeal plug, chemical exposure via the gut was completely eliminated. Fish were allowed to recover for 4 days before being introduced into the test aquarium. The pharyngeal plug was reexamined on sampling at the end of the exposure period. The occasional fish that had lost the pharyngeal plug was excluded. Pilot experiments showed that fish with a pharyngeal plug did not take food when it was introduced into the aquarium and no feces were excreted. Control fish were handled in a similar manner prior to exposure, but a pharyngeal plug was not inserted. Although fish normally do not have a blocked pharynx, these test fish did not appear visibly distressed. There were no erratic behaviors compared with the control fish. Oxygen consumption was measured in separate groups of control and test fish, placed in a 2 L container filled with dechlorinated water saturated with oxygen. The container was covered with a black plastic sheet and an oxygen electrode was sealed into the top of the container. The water was stirred by a stir bar and oxygen consumption was measured over a 30 minute period. There was no major difference in the oxygen consumption values for the test (0.141 mg $O_2$/g.h) and control

(0.131 mg $O_2$/g.h) fish, suggesting that any level of stress in the test fish was small when compared with the control fish.

Fish were individually weighed before the experiment to ensure a reasonably narrow range of body mass between 3.0 and 3.5 g. It was not possible to individually tag each fish, but each fish was reweighed at the end of the experiment. The average body mass at the end of the experiment was still in this range (pelagic control fish = 3.36 g; pelagic test fish = 3.20 g; benthic control fish = 3.17 g; benthic test fish = 3.25 g). Therefore, any loss in body mass during the experiment was likely small. Control fish with access to the bottom sediments were never observed foraging in these nutrient-poor sediments.

*2.2.2.2 Protocol 2*

The second experimental protocol was aimed at more closely following the disappearance of test chemicals from the water and sediments, as well as following the appearance of the test chemicals in the fish. The aquarium design, the loading of sediments, the loading of test chemicals, and the equilibration period were the same as those described above except that there was no horizontal mesh. Thirty fish were added to each of two replicate aquaria. Half of the fish in each aquarium were controls and half had their pharynx plugged (visually distinguished by a fin clip). Five fish from each replicate group were removed for chemical analysis on day 15 (after 6 days of exposure), on day 21 (after 12 days of exposure), and at the end of the experiment on day 27 (after 18 days of exposure).

Fish removed from the test aquaria were killed by a sharp blow to the head and immediately frozen at -80 °C for no longer than 1 month until the tissues were analyzed. Water and bottom sediments samples were taken at the same time for chemical analysis.

# 3. CHEMICAL EXTRACTION AND ANALYSIS

## 3.1 CHEMICAL EXTRACTION OF WATER SAMPLES

Water samples of 50 to 500 ml were taken on each sampling day. The untreated and treated (centrifugation and filtration) water samples were then processed for chemical extraction as described in Chapter 3. Each water sample from sediment-laden exposure experiment was divided: one part was analyzed unfiltered and the other was analyzed after filtration through a 0.45 μm filter. The extraction procedure was the same as that described in Chapter 3.

## 3.2 CHEMICAL EXTRACTION OF FISH

Immediately prior to tissue analysis, the fish were thawed and the body surface was washed gently with distilled water and the skin was dried carefully. For the first exposure protocol, chemical extraction was performed on a 0.3-0.7 g skeletal muscle. Whole fish were sampled for chemical analysis in the second exposure protocol. The entire fish was first minced and about 0.5 g of the homogenate was used for analysis. All fish tissue samples were homogenized and chemical extraction was the same as that described in Chapter 3.

## 3.3 CHEMICAL EXTRACTION OF SEDIMENTS

Each sediment sample was weighed. A weighed subsample of around 1.5 g wet sediment was placed in a centrifuge test tube with acid buffer and the surrogate chemical standard. The extraction procedure was the same as for fish tissue except there was no

homogenization step as referred in Chapter 3. The remaining sediments were weighed and dried in a fume hood until a constant weight was reached. The ratio of dry to wet sediments was calculated.

3.4 CLEAN UP AND GC ANALYSIS

The clean up of extracts followed the same procedure as described in Chapter 3 prior to GC analysis (described in Chapter 2). The concentrations of chemicals associated with the suspended sediments were calculated by subtracting the value for the filtered water sample from that for the unfiltered water sample. The chemical concentrations were statistically compared with ANOVA using two factor model for the first protocol and one factor model for the second protocol with $p < 0.05$ as an indication of statistical significance.

# 4. RESULTS

## 4.1 THE COMPARISON OF CENTRIFUGATION AND FILTRATION METHODS

Chemical loss was found in the filter membrane adsorption test. It was discovered that the chemical recovery efficiency after passing through a filter membrane was inversely proportional to the $K_{ow}$ value of test chemicals, i.e., 94.0% for 1,2,4-TCB, 85.0% for PeCB and 30.0% for HCBP. In the later experiments, all filtered water samples were adjusted according to these percentages.

The results of the filtration and centrifugation tests are shown in Table 4.1. In all cases, the total chemical concentration in sediment-laden water decreased by two- to four-fold from day 2 to day 9. This reflects either settling of suspended sediments or loss (by either volatilization or adsorption). However, these time-dependent changes did not affect the comparison of centrifugation and filtration methods.

For 1,2,4-TCB, the ratio of the chemical concentration determined by centrifugation and filtration was similar for all sampling dates (Table 4.1). Thus, either method seemed appropriate for this test chemical. For PeCB, the ratio of the chemical concentration determined by centrifugation and filtration increased with time from near unity at day 2 to 1.38 at day 9. However, for HCBP, this ratio was consistently greater than unity, with the centrifugation method yielding water concentrations about twice those obtained by filtration. This situation could have occurred because either the centrifugation method overestimated the chemical concentration, or the correction for chemical adsorption onto the filter was inaccurate. To determine which problem was most likely, three water samples were filtered that had been previously centrifuged. The

discoloration of the filter clearly showed that centrifugation had been inefficient in removing all the fine particles. From this, I concluded that the filtration method gave the more reliable estimate of true water concentration of the high log $K_{ow}$ compound, HCBP, in sediment-laden water.

## 4.2 THE SEDIMENT-LADEN EXPOSURE

### 4.2.1 Physical partitioning of the test chemicals

Figure 4.4 illustrates the concentrations of 1,2,4-TCB, PeCB, and HCBP in the filtered water, suspended sediments and bottom sediments during the first 9 days of the exposure protocols. The concentrations in the bottom sediments increased slightly with time after day 1, whereas the water and suspended sediment concentrations decreased somewhat with time. By day 9, it appears that the physical partitioning of the chemicals was approaching a quasi-equilibrium.

### 4.2.2 First exposure protocol

All three test chemicals were detected in the skeletal muscle biopsies following a 10-day exposure period (Fig. 4.5). For PeCB and HCBP, the experimental blockage of the pharynx had no significant effect on the muscle chemical concentrations. These results suggest that the gills were the major site of uptake of the test chemicals in these unfed juvenile rainbow trout.

There was also significant 1,2,4-TCB uptake via the gills (Fig. 4.5) but in this case 1,2,4-TCB uptake into the muscle was significantly higher ($P < 0.05$) in the test fish with

the pharyngeal plug. This higher uptake of the more water soluble of the three test compounds could reflect a slightly higher stress level in the test fish. However, this difference was not seen in the second exposure protocol (see below).

Access to the bottom sediments in the benthic fish group had no significant effect on the muscle xenobiotic concentrations. Each of the three test chemicals reached similar muscle concentrations after the 10-day exposure period whether or not the fish had access to the bottom sediments (Fig. 4.5). Consequently, the horizontal mesh was not used to partition the aquaria for the second exposure protocol.

4.2.3 Second exposure protocol

The second exposure protocol provided a greater resolution of the disappearance of the test chemicals from the water and sediments and of the appearance of the test chemicals in the exposed fish (Figs. 4.6 & 4.7).

*4.2.3.1 Chemical concentration in fish*

Figure 4.6 illustrates the concentration of the test chemicals in the pooled tissue sample as a function of exposure time. For PeCB and HCBP, the tissue concentrations after 18 days exposure were not significantly different to those observed after 6 days exposure (Fig. 4.6). These data suggest that the fish had reached a quasi-equilibrium with PeCB and HCBP after a 6-day exposure to the test chemicals. There was no significant difference between control fish and those with the pharynx blocked, confirming the observations made with the first exposure protocol. Interestingly, the concentrations of PeCB and HCBP were significantly lower for the pooled tissue samples (Fig. 4.6) than

those for the muscle biopsies (Fig. 4.5). Potential reasons for this maybe 1). the adsorption of the test chemicals in fish skin may occur during exposure and the water wash was not able to remove the adsorbed chemicals from skin, which caused the higher chemical concentration when the whole fish body, including skin, was analyzed. 2). the number of fish in each aquarium was different in each exposure protocol, i.e. 20 fish from the first exposure protocol and 30 fish from the second exposure protocol.

Compared with the 6-day exposure, tissue 1,2,4-TCB concentrations were significantly lower ($p < 0.05$) after 12 days of exposure and lower still after 18 days of exposure (Fig. 4.6). These data suggest that the fish were metabolizing 1,2,4-TCB, as discussed in Chapter 3, and so a quasi-equilibrium was not reached for 1,2,4-TCB. Unlike the first exposure protocol, there were no significant differences between the control fish and those with the pharynx blocked.

### 4.2.3.2 Losses of Chemicals

In the second exposure protocol, the concentrations of the test chemicals in the water and sediments were monitored during the fish exposure period (day 9 through day 27). Coincident with the appearance of the test chemicals in the fish on day 15, there were pronounced decreases in the xenobiotic concentrations in the filtered water and the suspended sediments (i.e., after 6 days of fish exposure) (Fig. 4.7). For 1,2,4-TCB, PeCB and HCBP, the concentration in the bottom sediments and suspended sediments changed very little on day 21 and day 27 compared with day 15. The water concentrations were below the detection limit. These data further suggest a quasi-equilibrium existed for PeCB and HCBP after 6 days of exposure.

*4.2.3.3 Mass balance of test chemicals*

On day 9 in the second exposure protocol, the mass balance of the test chemicals was calculated from the measured concentrations in water, suspended sediments and bottom sediments and the initial volumes of water and sediments (minus any loss due to sampling). Figure 4.8 illustrates the mass distribution of the three test chemical within the three compartments. As expected, based on the log $K_{ow}$ values for the test chemicals, around 80% of the HCBP and around 50% of the PeCB partitioned into the bottom sediments (Fig. 4.8). Less than 6% of the HCBP and 14% of the PeCB was in the water compartment. In contrast, less than 13% of the 1,2,4-TCB was partitioned into the bottom sediments and approximately equal amounts were distributed between the water and suspended sediments (Fig. 4.8).

Of the 102.3 µg HCBP added to the test system, 76% (78 µg) could be accounted for in the water and sediment samples taken at day 9. Of the 70.3 µg of PeCB added, 45% (31.5 µg) could be accounted for in the water and sediments samples taken at day 9. Of the 1,415 µg of 1,2,4-TCB, 35% (491.4 µg) was accounted for in the water and sediment samples at day 9. Chemical losses were likely to the atmosphere and possibly adsorption to the glass walls of the aquaria.

A similar mass balance calculation was made on day 15, following a fish exposure period of 6 days (Fig. 4.8). For both PeCB and HCBP, the appearance of the test chemicals in the fish was accompanied by a substantial decrease in the chemical mass associated with the water and suspended sediments, and to a lesser degree with the bottom sediments (as expected by the data presented in Figures 4.6 & 4.7). What Figure

4.8 illustrates is that the chemical mass in both the water and suspended sediments was reduced to a very low level after the fish were introduced. Moreover, the mass of HCBP originally associated with the water compartment just prior to introducing the fish could not account by itself for the mass of HCBP in the fish. In fact, the 21.9 µg of HCBP found in the fish tissues is 28% of the total HCBP measured at day 9 and slightly more than the combined masses for water and suspended sediments. These results clearly suggest that HCBP bound to suspended sediments from the Fraser River desorbed and were taken up across the gills of juvenile rainbow trout. A similar conclusion can be reached for PeCB since 59% (18.6 µg) of PeCB measured on day 9 was found in the fish on day 15, but on day 9 only 55% of the PeCB was associated with the suspended sediments and dissolved in the water (Fig. 4.8).

Any interpretation of the mass balance for 1,2,4-TCB is difficult because the test system was not in a quasi-equilibrium. There were significant losses of 1,2,4-TCB from the test system prior to day 9 (around 7.2% per day) and the rate of loss was higher still during the 6 day fish exposure (around 9.2% per day).

# 5. DISCUSSION

## 5.1 THE METHOD OF PARTICLES/WATER SEPARATION

Both the centrifugation and filtration methods were valid for use with 1,2,4-TCB, possibly because this chemical is unlikely to adsorb either to fine particles or the filter membrane. For both PeCB and HCBP the error associated with centrifugation is not easy to correct since the amount of chemical associated with unsettled particles is difficult to estimate by any other method than filtration. In other solid/liquid separation experiments, Voice et al. (1983) found a similar result, i.e., after centrifugation at 27,100g, absolute turbidity was found. He then concluded that the presence of turbidity in the water sample, even after high-speed centrifugation, suggests that some of the residual particles are extremely small and resistant to separation. This resistance to separation possibly affects the measurement of concentration of higher log $K_{ow}$ chemicals.

## 5.2 UPTAKE OF LIPOPHILIC CHEMICALS

### 5.2.1 Bottom sediments

Results from first exposure protocol showed that, for each of the three test chemicals, similar muscle concentrations were reached after the 10-day exposure period whether or not the fish had access to the bottom sediments, or whether or not the fish had its pharynx blocked. These results proved that the GI tract was not responsible for chemical uptake from either the bottom sediments or from the suspended particles. This finding is supported by Kolok (1996) who found that gizzard shad exposed to sediments for 22 days showed no higher body burden of benzo[a]pyrene-equivalents in control fish

compare to fish with the gut surgically ligated. Thus, like the present study, sediment ingestion did not appear to significantly influence body burden in unfed fish. In summarizing other experiments in which the bioaccumulation factor had decreased when (suspended) sediments were added to water, Schrap (1991) concluded that chemicals sorbed on sediments are not available for uptake in the GI tract, or if the sorbed chemicals are available for uptake in the GI tract, this route is of minor importance, or negligible, compared with gill uptake. My study agrees with this conclusion.

## 5.2.2 Suspended sediments

Most previous studies on the effect of particles and sediments in water on xenobiotic uptake have focused on those that are rich in organic carbon (Adams et al. 1985; ASTM 1985). The Fraser River sediments are typically poor in organic carbon. The sediments used in this study had about 1.4 % organic carbon. The low organic carbon content of the Fraser River sediments should, therefore, affect the ease with which xenobiotics desorb from the suspended sediments because of lower chemical association constants for these types of sediments (Di Toro et al. 1983, Sept.; Black et al. 1988). My data support such a prediction. Non-feeding juvenile rainbow trout were found to take up xenobiotics of differing log $K_{ow}$ values from test aquaria containing sediment-laden water. The uptake of HCBP and PeCB was largely completed after a 6-day exposure period. Also, the uptake occurred primarily through the gill since similar results were generated for fish in which the gut uptake route was completely eliminated by a pharyngeal plug.

My study was consistent with Opperhuizen's (1988) study on the influence of low organic matter on the bioaccumulation of lipophilic chemicals, in which a higher bioaccumulation of some chlorinated chemicals was observed in test guppies (*Poecilia reticulata*) than control fish for those with log $K_{ow}$ values between 5 to 6 (such as penta and hexachlorobenzene, and tri and tetrachlorobiphenyls). In my study, the uptake of chemicals with log $K_{ow}$ over 7 was enhanced by the presence of suspended sediments and this needs further study for clarification. Thus, the conclusion that particles can act as a source of the lipophilic chemicals to fish should be given more attention in aquatic toxicology.

## 5.2.3 Mass balance

Taking the chemical extraction error into account for filtered water and fish tissues (Fig. 4.8), the chemical mass of 1,2,4-TCB in fish was less than that in filtered water. However, the chemical mass of PeCB and HCBP in fish exceeded the total chemical mass in filtered water. An important observation was that the amounts of HCBP and PeCB present in fish tissue after a 6-day exposure could not be accounted for solely by the amounts of the chemicals dissolved in the water at the time when the fish were first introduced. The total amount was closer to the combined amounts initially present in the water and the suspended sediments. The implication of this finding is that lipophilic chemicals with similar properties as HCBP and PeCB readily desorb from suspended sediments typical of the Fraser River and are taken up across the gills.

The mass balance analysis which leads to this conclusion can be challenged in two ways. Firstly, there were unaccounted losses of chemical between day 9 and day 16. In the cases of PeCB and HCBP, these were relatively small compared with the acquired body burden of the fish on day 16 (see Fig. 4.8) and do not affect the main conclusion. The losses for 1,2,4-TCB, however, were so large that this study discounted the accuracy of the mass balance analysis for 1,2,4-TCB. Even so, I suspect that most of the TCB loss was due to fish metabolism. Second, it is possible that chemical desportion from the glass could have accounted for some of the chemical uptake into the fish. The present experiments did not assess the sorption and desorption of chemicals to. the glass walls of the aquaria. It is likely that a portion of the unaccounted PeCB and HCBP during the 9-day equilibration period was adsorbed to the glass, the remainder being lost to the atmosphere. However, I have no data on the relative desorption rates of these test chemicals from glass versus Fraser River sediments. Nonetheless, it is my contention that this was not a major contributor to the fish burden since I could not then explain the rapid removal of quantities of PeCB and HCBP from the filtered water and suspended sediment compartments. Furthermore, fish exposure rates to the chemicals associated with the suspended sediments were always considerably higher than the exposure to the aquaria walls. The fish continuously irrigated their gills with water containing these suspended sediments.

## 5.3 1,2,4-TCB

The choice of 1,2,4-TCB as the test compound with a log $K_{ow}$ around 4.0 seems to have been unwise. 1,2,4-TCB was only dosed once. With a higher Henry Law Constant (up 590 Pa.m$^3$.mol-1) compared with HCBP (139 Pa. m$^3$.mol-1), 1,2,4-TCB was lost from the test aquaria at the highest rate of the three test chemicals. Loss of 1,2,4-TCB to the atmosphere from the test aquaria seems the most likely explanation for the majority of this change before fish input. When fish were introduced to the aquaria, the rate of 1,2,4-TCB loss increased and the tissue 1,2,4-TCB concentrations decreased with time (see Fig. 4.7), unlike the situation for PeCB and HCBP. The decline in 1,2,4-TCB tissue concentrations could reflect metabolism and elimination of the parent compound from the fish. An additional problem with 1,2,4-TCB was observed in the first, but not the second exposure protocol. The 1,2,4-TCB concentration in muscle biopsies of fish with a pharyngeal blockage was significantly higher than the controls. Although this observation does not affect our main conclusion, it suggests that the toxicokinetics of 1,2,4-TCB were altered by this surgical procedure even though this study allowed a 4-day recovery period. It is possible that the additional stress increased gill 1,2,4-TCB uptake, but it is also possible that 1,2,4-TCB metabolism and elimination were retarded.

## 5.4 BCF

There is no question that chemicals dissolved in water are bioavailable for uptake across the gill (OECD 1981a; Carlberg et al. 1986; Servos et al. 1989c; Schrap et al. 1990;

Gobas 1993a). The bioconcentration factor (BCF) can be calculated from the chemical concentration in the fish divided by the bioavailable chemical concentration in the water at equilibrium (ASTM 1985). Using such a calculation the BCFs were at least 73,400 for PeCB and 36,900 for HCBP (These are minimum values for the BCF because the chemical concentrations in filtered water were actually below the GC detection limit.). Ideally if there is no chemical uptake via the gut and the chemicals associated with suspended sediments do not desorb and get taken up across via the body surfaces (gill and skin), the bioaccumulation factor (BAF) should equal the BCF. Such an agreement between BCF and BAF is not apparent in this study (e.g., BAF = fish lipid content x $K_{ow}$ which yields a value of 5,000 for PeCB). I believe that this discrepancy arises because of the difficulty in accounting for the desorption and gill uptake of chemicals associated with suspended sediments of a low organic carbon content, the very low water concentration is also a factor in this result. Consequently, BCFs calculated using only the dissolved water chemical concentration may be misleading for sediment-laden waters such as those from the Fraser River. If the chemical concentration of unfiltered water is used to calculate the BCF in our experiments, the BCF values are much lower (6,600 for PeCB) and closer to the BAF prediction. Obviously the situation will be different for xenobiotics bound to sediments rich in organic carbon (Black et al. 1988), since chemical desorption from organic-rich sediments seems very limited in the context of the water transit time through gill lamellae. Owens et al. (1994), studied the fate and distribution of polychlorinated dibenzodioxins and polychlorinated dibenzofurans in a northern Canadian river system. They suggested that simple water column bioconcentration models may not always

predict biotic levels of lipophilic compounds. They noted that suspended sediments were an important vector for environmental transport and an important entry point into the food web.

Consistent with the results from Chapter 3, the BCF of PeCB was higher than that of HCBP, which proved that PeCB is more readily to be taken by fish than HCBP. In addition, the calculated BCF values in this study were much higher than those in Chapter 3. There is a possibility that the dechlorinated tap water in former experiments contained fine particles that were not filtered out. Thus, the detected chemical concentration in unfiltered water appeared to be higher than that in filtered water. This introduced an inconsistency between the two experiments such that the chemical concentration from unfiltered water may have led to underestimation of BCF values.


## 5.5 ENVIRONMENTAL IMPLICATION IN THE FRASER RIVER

The applicability of the present data to the Fraser River system is difficult to address at this time. The suspended sediment loading densities were at the low end of the range found in the Fraser River, because there was not the extensive mixing of the water that would have kept higher densities of these fine sediments suspended, as is the case over most of the river's length. (Vigorous agitation of the aquarium water would have excessively stressed the fish in this laboratory setting.) Arguments could also have been made that the test chemicals were more readily desorbed because of sediment disaggregation. Alternatively, the lower suspended sediment load may have decreased gill uptake by decreasing gill exposure to chemical-laden suspended sediments. Likewise,

mixing would likely have prevented some of the decreases in water and suspended sediment chemical concentrations that occurred when fish were added to the aquaria. Further studies are needed therefore to transfer the present findings to a real environmental situation. There is, however, emerging evidence that the suspended sediments in the Fraser River are a major transport vector for organochlorine compounds (M. Sekela, pers. comm.). This means that the bioavailability of chemicals associated with suspended sediments in the Fraser River warrants further scrutiny. At present this study envisages that lipophilic chemicals associated with suspended sediments in the Fraser River can readily desorb as chemicals are removed from the water phase by uptake into fish gill tissues. Whether or not there is direct desorption from suspended sediments to the gill tissues, rather than via the water phase (Di Toro et al. 1983), will require further study and will involve a consideration of the water boundary layers associated with fish gills. At this time I do not feel that an uptake of suspended sediment particles per se into the gill epithelium (Martens et al. 1993) would be significant enough to account for the rapid uptake of these lipophilic chemicals.

The primary uptake route of the xenobiotics in these experiments is suggested to be via the gills. However, this study did not assess the role of skin uptake. Instead, our suggestion is based on the known partitioning of oxygen uptake between the skin and the gills. Uptake of oxygen, which is also lipophilic, is predominantly via the gill rather than the skin in salmonids of this size (Rombough 1988). Nonetheless, in fish of a smaller size and with poorly developed gills, the skin uptake would be of greater significance.

# 6. CONCLUSION

In summary, this study provided evidence that lipophilic xenobiotics associated with fine suspended sediments from the Fraser River can become readily bioavailable for gill uptake in juvenile rainbow trout, probably because of desorption from these sediments having a low organic carbon content. Therefore, since all sediments are not equal in terms of the ease of desorption of associated lipophilic xenobiotics, it would be unwise to conclude that fish exposed to contaminants in sediment-laden water are only at risk via the food chain.

Table 4.1 Concentrations of test chemicals, 1,2,4-TCB, PeCB and HCBP, in sediment-laden water when measured before and after either filtration (0.45μm) or centrifugation (20,000 g for 30 min).

1,2,4-TCB (μg/L)

| | whole water | filtration | centrifugation | ratio of centrif./fitra. |
|---|---|---|---|---|
| day | Mean | Mean | Mean | |
| 2 | 8.343 (n=2) | 6.442 (n=2) | 5.801 (n=3) | 0.90 |
| 6 | 3.168 (n=2) | 2.458 (n=2) | 2.614 (n=2) | 1.06 |
| 9 | 2.045 (n=2) | 1.202 (n=3) | 1.173 (n=3) | 0.97 |

PeCB (μg/L)

| | whole water | filtration | centrifugation | ratio of centrif./fitra. |
|---|---|---|---|---|
| day | Mean | Mean | Mean | |
| 2 | 1.316 (n=2) | 0.993 (n=2) | 0.918 (n=3) | 0.92 |
| 6 | 0.579 (n=2) | 0.383 (n=2) | 0.412 (n=2) | 1.08 |
| 9 | 0.464 (n=2) | 0.231 (n=3) | 0.312 (n=3) | 1.35 |

HCBP (μg/L)

| | whole water | filtration | centrifugation | ratio of centrif./fitra. |
|---|---|---|---|---|
| day | Mean | Mean | Mean | |
| 2 | 0.564 (n=2) | 0.120 (n=2) | 0.224 (n=3) | 1.87 |
| 6 | 0.253 (n=2) | 0.043 (n=2) | 0.081 (n=2) | 1.88 |
| 9 | 0.221 (n=2) | 0.053 (n=3) | 0.095 (n=3) | 1.79 |
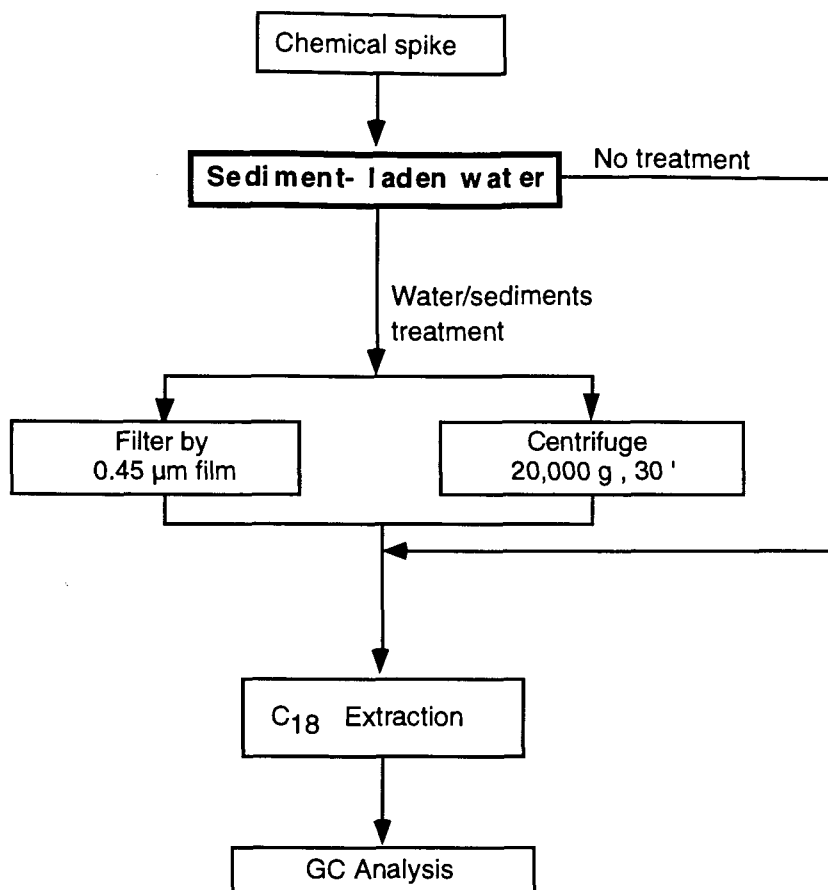
Figure 4.1 The chemical analysis methods used to compare filtration and centrifugation of sediment-laden water samples.
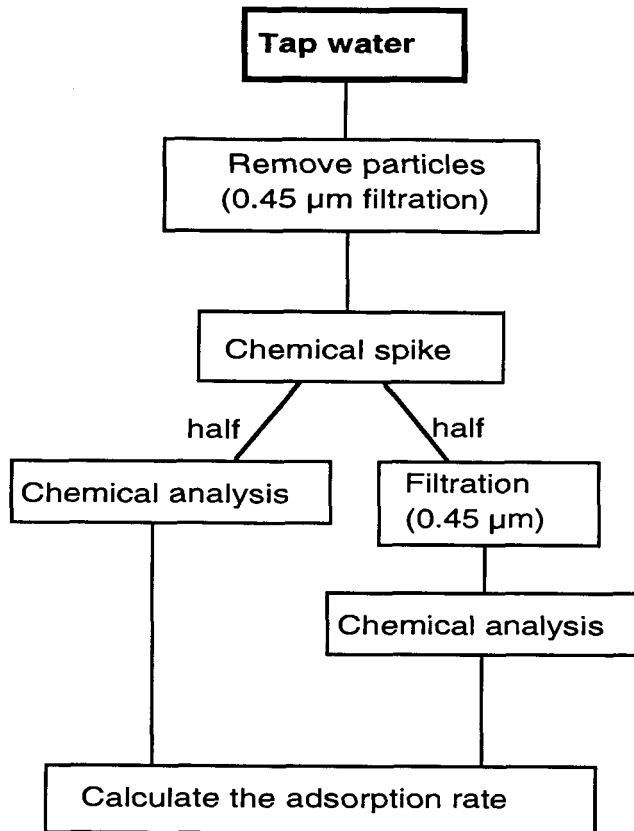
Figure 4.2 Protocol used to quantify the chemical adsorption to 0.45 μm membrane filter.
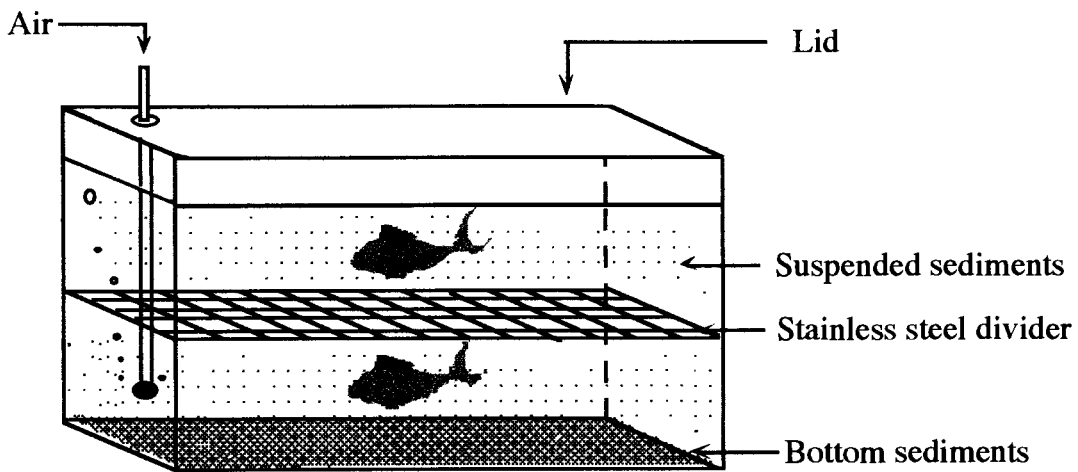
50 L aquarium

Figure 4.3 A schematic diagram to illustrate how juvenile rainbow trout were exposed to water containing either bottom sediments plus suspended sediments or only suspended sediments. Suspended sediments divider used only in first protocol.

Figure 4.4 Concentrations of 1,2,4-TCB, PeCB and HCBP in filtered water, suspended sediments and bottom sediments contained in the test aquaria prior to the introduction of fish for the first and second exposure protocols. Values are presented as an average and range (vertical bars) duplicate aquaria and duplicate samples for each protocol.

Figure 4.5 Concentrations of 1,2,4-TCB, PeCB and HCBP in rainbow trout (*Oncorhychus mykiss*) muscle samples after a 10-day exposure to sediment-laden water .

"P" refers to pelagic fish exposed to suspended sediments only above the steel mesh.

"B" refers to benthic fish exposed to suspended sediments and bottom sediments below the steel mesh.

There was no significant differences between any of the comparable "P" & "B" groups.

Each point is the mean of 10 fish. * denotes a significant difference ($p < 0.05$) between respective P, B & control fish groups.

**Figure 4.6** Concentrations of 1,2,4-TCB, PeCB and HCBP in rainbow trout (*Oncorhychus mykiss*) whole body samples during the 18-day exposure protocol. Each point is the mean of 10 fish. * : denotes a significant difference ($P < 0.05$) compared with the value for a 6-day exposure and a double asterisk denotes a significant difference ($P < 0.05$) compared with the value for a 12-day exposure (second exposure protocol). There was no significant differences between respective control and pharynx-blocked fish.

Figure 4.7 Concentrations of 1,2,4-TCB, PeCB and HCBP in filtered water, suspended sediments and bottom sediments before and after fish ($N = 30$) input for the 18-day exposure experiment. Values are presented as an average and range (vertical bars) duplicate aquaria.
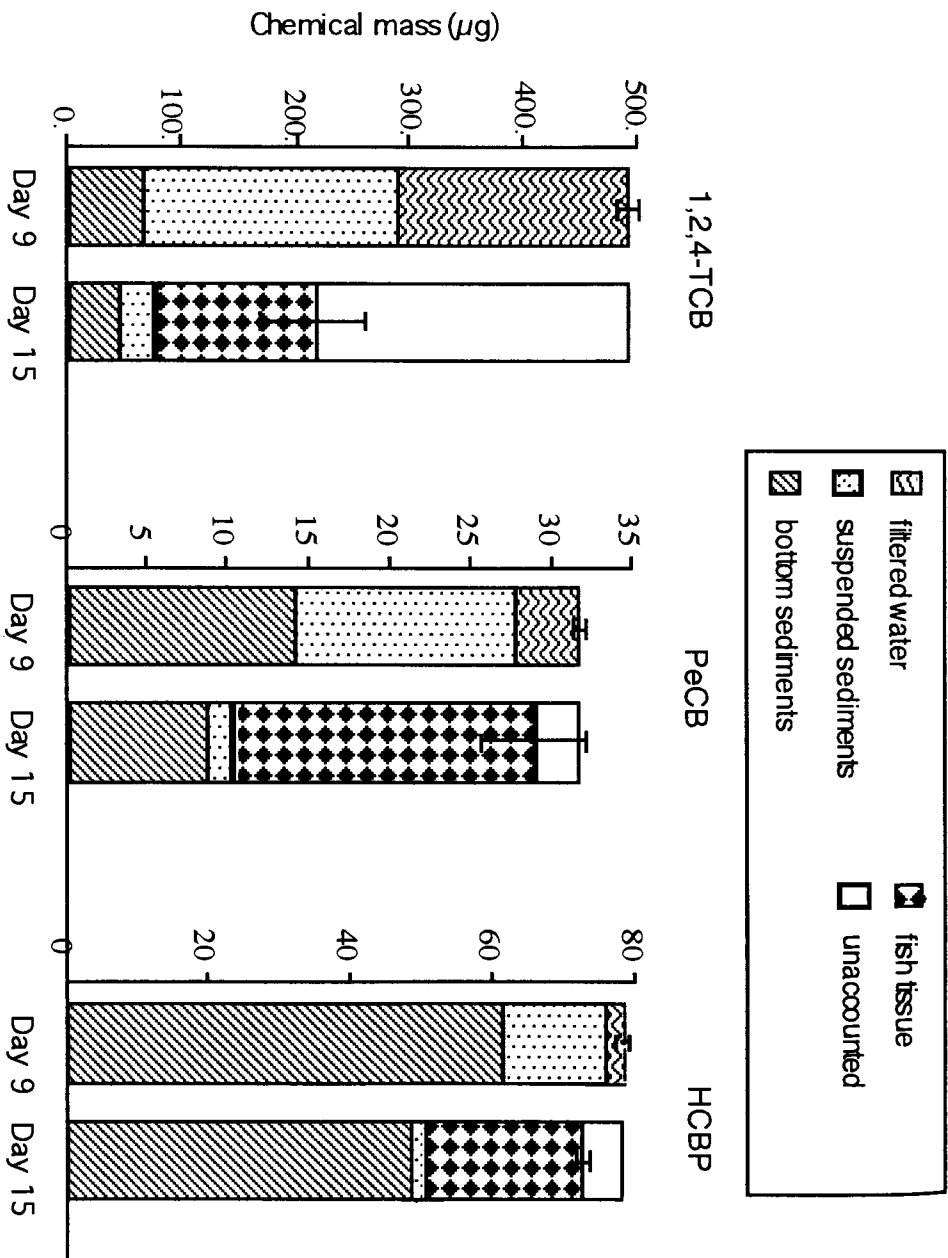
Figure 4.8 Chemical mass balance of 1,2,4-TCB, PeCB and HCBP in water, suspended sediments, bottom sediments and fish whole body) on day 9 (pre-fish introduction) and day 15 (after a 6 day fish exposure) for the second exposure protocol. The error bars represent the standard error of test chemicals in filtered water or in fish, respectively.

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# CHAPTER 5

# THE IMPACT OF DISSOLVED ORGANIC MATTER

# ON THE BIOAVAILABILITY AND UPTAKE

# OF

# LIPOPHILIC XENOBIOTICS

# ABSTRACT

In this study, the impact of humic acid on the bioavailability of three chemicals, 1,2,4-TCB, PeCB and HCBP with log $K_{ow}$ values ranging from 3.95 to 7.55, was examined in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed for 2 and 4 days to the test chemicals in water containing 0 to 14.32 mg/L Aldrich humic acid. A significant reduction was found for fish uptake of HCBP in fish exposed in the humic acid concentration above 4.81 mg/L. There was no significant change in the fish body burdens of PeCB and 1,2,4-TCB in groups exposed to 0, 4.81 and 14.32 mg/L of humic acid. These results indicated that humic acid may only affect uptake of a chemical with a very high log $K_{ow}$ value, e.g., 7.55. In contrast, the uptake of the chemicals PeCB and 1,2,4-TCB was found to increase significantly in fish exposed to low concentration of humic acid (1.54 mg/L). This study concluded that the $K_{ow}$ value and humic acid concentration are two factors which affect the lipophilic chemical uptake in fish.

# 1. INTRODUCTION

Dissolved organic matter (DOM) is an important component of suspended particles particularly in freshwater systems. In natural water systems, DOM is largely composed of humic acid. The average concentration of the organic (humic) substances in the surface water is 4.4 mg/L of humic substances, or 2.2 mg organic carbon /L (Suffet et al. 1994). Humic acid is refractory in the environment (McCarthy 1983a; Servos et al. 1989d) and its strong binding of lipophilic chemicals influences chemical partitioning in aquatic ecosystems. The presence of DOM is generally considered to reduce the amount of freely dissolved lipophilic chemicals in water (Landrum et al. 1984; McCarthy et al. 1985b; Servos et al. 1989d; Gobas 1994). Studies have also shown that bioaccumulation of lipophilic chemicals is reduced when dissolved and particulate organic matter is present in the water (McCarthy et al. 1985a; McCarthy et al. 1985c; Landrum et al. 1987; Kukkonen et al. 1989; Servos et al. 1989d; Schrap et al. 1990; Day 1991; Muir 1994; Rowan 1994; Twiss et al. 1995). However, the bioavailability and bioaccumulation of lipophilic chemicals in pelagic fresh water fish exposed to water containing DOM are not fully understood because the role of DOM was not well studied. Moreover, there is a lack of knowledge about the impact of varying concentrations of DOM on bioaccumulation.

The role of DOM concentration is important for two reasons: 1. Most lipophilic chemicals associated with DOM can desorb into water. Studies have shown strong evidence that the sorption to and desorption from the DOM is an inherent kinetic property of hydrophobic, even super hydrophobic, chemicals, (Gschwend et al. 1985;

McCarthy et al. 1985a&b). Using equilibrium dialysis and fluorescence techniques, McCarthy (1985b) observed the reversible binding of five PAHs to humic acid, and noted that the binding of BaP to dissolved humic material was completely reversible, although the desorption process was slower than the association process. 2. Increasing the DOM concentration was found to reduce the toxicity (by reducing the free-dissolved chemical concentration) (Gilderhus 1982; McCarthy et al. 1985a; Muir et al. 1985; Black et al. 1988; Cary et al. 1987; Kukkonen 1991; Day 1991; Schrap et al. 1991) and bioconcentration factor (BCF) (Bruggeman et al. 1984; Muir et al. 1985; Opperhuizen et al. 1985) of lipophilic chemicals. The concentrations of DOM in these studies were mostly greater than 2 mg/L; the impact of a lower concentration of DOM may differ. For example, Muir et al. (1994) showed no difference of chemical uptake, described as $k_1$ and BCF at lower levels of DOC, indicating that a lower concentration of DOM may not affect the bioaccumulation of lipophilic chemicals.

The impact of DOM on uptake of 1,2,4-TCB, PeCB and HCBP in pelagic fish has not been studied previously. In this study, the relationship between the concentration of humic acid and chemical body burden in fish over time was investigated for lipophilic chemicals with log $K_{ow}$ values from 3.98 to 7.55. My working hypothesis was that the bioavailability of these three lipophilic chemicals is inversely related to the concentration of DOM and to their log $K_{ow}$ values, but low concentration of DOM does not necessary reduce the bioavailability of three test chemicals. Humic acid was used as the surrogate DOM in these bioavailability studies. The possibility of GI tract uptake is considered

unlikely because freshwater fish basically do not drink water, therefor, the gill uptake was proposed as dominant route for chemical uptake.

# 2. MATERIALS AND METHODS

## 2.1 MATERIALS

### 2.1.1 Test animals and chemicals

Juvenile rainbow trout (*Oncorhychus mykiss*) weighing from 2.5 g to 3.5 g were used in this study. Details of the acclimation of fish and water quality are outlined in Chapter 2. The decision about the number of test fish placed in each aquarium was based on observations made in early experiments that fish uptake of chemicals via the gill was so fast so that the chemicals would be depleted in a short time if a large number of fish were introduced. Thus, as small a number of fish as possible (24) were used in this study. Since the GI tract was assumed not to be an uptake route for chemical bioaccumulation, no pharyngeal blockage was used in this study.

Humic acid (Aldrich Chemical Co., Inc.) and the same three test chemicals as used in Chapter 3 (1,2,4-TCB, PeCB and HCBP) were used.

## 2.2 EXPOSURE PROTOCOL

### 2.2.1 Fish exposure

The humic acid and test chemicals were spiked into 4 x 50 L aquaria, filled with 45 L of aerated dechlorinated water, and were vigorous mixed by a Teflon stir bar for about 10 minutes. The humic acid concentrations in each aquarium were 0 (control), 1.54, 4.81 and 14.32 mg/L respectively, which represents the concentration range of DOM in most water systems. The water temperature was $12 \pm 1$ $^0$C and pH was 6.25 to 6.9. The amount of test chemical concentrations were nominally 1450, 455 and 462 µg/L for 1,2,4-

TCB, PeCB and HCBP, respectively. The HCBP concentration was deliberately higher than its water solubility (0.4 µg/L Mackay et al. 1992) because of a consideration that HCBP association with DOM may have greatly eliminated the free HCBP concentration because of its very high log $K_{ow}$ value. The chemicals in water were allowed to partition between humic acid and water for two days. Six fish were then introduced into each aquarium which was covered with a plastic lid. Three fish were sampled from each aquarium after two days of exposure, based on the observed rapid gill uptake of these compounds (see Chapter 3 and 4). The remaining fish were sampled after another two days of exposure. This second sample was to estimate whether uptake continued. Also, the short exposure period was intended to avoid unacceptable levels of 1,2,4-TCB metabolism experienced with longer exposure durations (see Chapter 3 and 4).

The chemicals extracted from fish tissue (whole body) were analyzed according to the methods described in Chapters 2 and 3. The average fish body burden of the three test chemicals was determined for 6 fish from 2 aquaria. ANOVA was used to test the impact of the different concentrations of humic acid within the groups (comparing the chemical body burden in fish for varying humic concentrations) and between the test groups (comparing the chemical body burden in fish for the 2- and 4-day samples.

## 2.2.2 Chemical analysis of water sample

The chemical concentration in water was analyzed by passing a 20 mL water samples through a $C_{18}$ cartridge. The cartridge was used in an attempt to distinguish between free-dissolved and humic acid-associated chemicals. Landrum et al. (1984)

showed that humic acid-bound compounds would pass though the $C_{18}$ cartridge because the humic acid was considered to sorb poorly to $C_{18}$ at pH > 5. Thus, the chemical trapped by a $C_{18}$ cartridge and eluted later by hexane would represent the dissolved chemical concentration. Since the water concentration from the control group (humic acid = 0 mg/L) was considered as 100% of the free chemical concentration, and since the chemicals passed through the $C_{18}$ cartridge were not measured, the humic acid-bound chemicals in other groups would be obtained from the difference between the concentration in the control group. Once the free dissolved and humic acid-associated chemical concentrations are known in the test group, the chemical partition between the water and humic acid, and between the water and fish can be derived. The method of chemical extraction was conducted in the same manner as the method mentioned described in Chapter 3. The GC analysis followed the procedures described in Chapter 2.

Chemical mass balance in the test system was determined after the 2-day and 4-day exposures (see section 3.2).

# 3. RESULTS

## 3.1 CHEMICALS IN FISH BODY

The apparent chemical body burdens in fish are shown in Figure 5.1 and the statistical comparisons are shown in Table 5.1.

Increasing the humic acid concentration to 4.81 mg/L significantly reduced bioavailability of chemical HCBP in 2-day exposure by about 2.5-fold (Figure 5.1). A similar 1.7-fold reduction occurred in the 4-day exposure. However, 14.32 mg/L humic acid did not further reduce HCBP bioavailability, compared with 4.81 mg/L humic acid.

In contrast, the PeCB and 1,2,4-TCB chemical body burden in fish were not significantly different compared with control fish when fish were exposed to either 4.81 or 14.32 mg/L humic acid (Figure 5.1).

Interestingly, the addition of 1.54 mg/L humic acid significantly increased the body burden of 1,2,4-TCB and PeCB (Fig. 5.1). For 1,2,4-TCB, the increase was more than 100%, and for PeCB, it was over 50% in the 2-day exposures (Fig. 5.1). Similar increases of these two test chemicals were seen in the 4-day exposure. In contrast, HCBP bioavailability was unaffected by 1.54 mg/L humic acid.

The data for 4-day exposures should be treated with caution because the rate of chemical uptake had slowed for PeCB and HCBP. Unlike PeCB and HCBP, the body burden of 1,2,4-TCB in the 4-day exposure groups were lower than the 2-day exposure groups. This probably occurred because of rapid TCB metabolism and elimination as observed in earlier experiments (see Chapter 3 and 4).

Among three test chemicals, only HCBP increased significantly in day 4 against day 2 (Fig. 5.1). The fact that 4-day exposure was less than double that from the 2-day exposure group suggests that the uptake rate had slowed.

## 3.2 WATER CHEMICAL AND MASS BALANCE ANALYSIS

The chemical concentrations in water obtained with the $C_{18}$ method are shown in Table 5.2. Chemical concentrations were similar in all test groups no matter what the concentration of humic acid was. Therefore, it is suggested that the $C_{18}$ failed to separate the DOM-associated chemicals from the truly dissolved chemicals.

The mass balance of the chemicals after 2-day exposure and 4-day exposure (Fig. 5.1 a&b) showed that the amount of chemicals in the water compartment was much greater than that in fish. As in sediment-laden exposure experiments (Chapter 4), chemical loss from the water compartment was measurable after addition of fish into the aquarium. Of the 1450 μg TCB, 70% remained after 2 days following fish input and 30% remained in the water after 4 days. Of the 512 μg of PeCB, 80% remained after 2 days and about 50% was left after 4 days. Of 482 μg of HCBP, 70% remained at 2 days and between 35% and 60% remain after 4 days.

# 4. DISCUSSION

## 4.1 THE WATER ANALYSIS

According to a study which reported that free-dissolved lipophilic chemicals would be retained by a reverse phase $C_{18}$ cartridge and humic acid-bound chemicals would pass through the cartridge (McCarthy et al. 1985b), it was expected that the measured dissolved chemical concentration in the humic-acid laden water would decrease. However, the free concentration of each test chemical in all samples was similar regardless of the humic acid concentration in the test water. Therefore, these results suggest that the $C_{18}$ cartridge did not separate the free and bound test chemicals from DOM-laden water in this study. Many factors, such as the water quality, DOC content, extraction volume of water and flow rate may influence obtaining the free dissolved chemicals, and these factors need to be examined in a future study. In addition to these factors, another possibility for the unsuccessful separation of freely dissolved chemicals from humic-acid-laden water may be that the chemical associated with humic acid may be retained in the cartridge. Humic acid is also organic matter and has an affinity for polymeric structures (Schnitzer 1976), such as the $C_{18}$ cartridge. In fact, in this study, a brownish color was seen in the cartridge which indicated that humic acid was retained in the $C_{18}$ column possibly with complexed chemicals. Another method to separate the humic acid-associated chemicals, a dialysis bag, was used by Landrum (1984). The result of dissolved chemical concentration obtained using the $C_{18}$ cartridge was significantly higher than using dialysis when partition coefficient of chlorinated chemicals between humic acid

and water was evaluated. The above explanation may apply to Landrum's results, but was unfortunately not considered in his discussion.

An alternative method for estimating the dissolved chemical concentrations in humic-acid-laden water is with the following equations (Gobas 1994):

$$C_{wd} + \varnothing_H \cdot C_H = C_w \tag{5.1}$$

where $C_{wd}$ is dissolved chemical concentration in water with unit of µg/L and $\varnothing_H$ is humic acid concentration with unit of mg/L. $C_H$ is chemical concentration associated with humic acid with unit of µg/mg and $C_w$ is total chemical concentrations in water. Since $C_H$ can be obtained by $K_{ow} \cdot 0.5 \cdot C_{WD}$, where 0.5 is the estimated proportion of DOC as humic acid. The equation therefore can be rearranged as:

$$C_W = C_{WD} + \varnothing_H \cdot C_{WD} \cdot (K_{ow} \cdot 0.5 \cdot C_{WD}) \tag{5.2}$$

$$C_W/C_{WD} = 1 + \varnothing_H \cdot K_{ow} \cdot 0.5 \tag{5.3}$$

$$C_{WD}/C_W = 1/(1 + \varnothing_H \cdot K_{ow} \cdot 0.5) \tag{5.4}$$

The solutions to these equations are presented is in Figure 5.3 and Table 5.3, in which the water concentration is set at 4 µg/L for all three chemicals. The dissolved 1,2,4-TCB showed virtually no change with an increase of humic acid concentration of more than 90% even if the humic acid concentration was as high was 14.32 mg/L. Dissolved PeCB decreased about 20% in 4.81 mg/L of humic acid and lost almost 50% when humic acid is 14.32 mg/L. There was very little HCBP (3.5% to 0.4%) dissolved in water, even when humic acid was as low as 1.54 mg/L.

4.2 CHEMICAL MASS BALANCE

Although mass balance analysis was hampered by the inability to separate the humic acid, it does show that the chemical supply in the water was more than sufficient for fish uptake. Unaccounted loss of chemicals might also result from adsorption to the aquarium wall and evaporation. This kind of loss occurred in Chapter 4 with sediment-laden water. Also, in both studies, the unaccounted loss of 1,2,4-TCB was more than the other two chemicals, again suggesting metabolism by the fish as well as evaporation from aquarium as discussed in Chapter 3.

## 4.3 EFFECT OF HUMIC ACID ON UPTAKE OF TEST CHEMICALS

For the short-term exposure experiments used here (a 2-day and 4-day exposure) where bioaccumulation is known to occur, humic acid at a concentration of 4.81 mg/L, or greater, significantly decreased uptake of HCBP by 1.7- to 2.5-fold. This result implies that humic acid effectively adsorbs HCBP as a function of increasing humic concentration. This must involve the reduction of dissolved chemicals in water and consequently, reduces gill bioavailability. The high affinity of HCBP for humic acid must be greater than that for gill membranes. In addition, the large size of the complex of humic-associated HCBP may not be able to cross the gill epithelium. A similar result was shown by MaCarthy (1985a), when uptake of 3- methylcholanthrene (log $K_{ow}$ 7.11) was reduced from 32 to 82% when the humic acid is 1.5 to 15 mg C/L.

The finding that the chemical mass of HCBP in fish (2 to 6%) was more than that estimated to be dissolved in water (< 1% at 4.81 mg/L of humic acid) suggests that the

uptake of HCBP may be not only from the dissolved chemicals but also from the chemicals associated with humic acid. Possible explanations for this are as follows: 1. The humic-bound chemicals may be loosely associated to (Schnitzer 1976) DOM, and therefore, easily desorbed at a low concentration of DOM and be taken up by fish. Thus, it is possible that the chemicals may be taken up by the fish gills when this loose particle/chemical complex contacts or approaches the gill epithelial membrane. The lipid content of biological tissue would facilitate this type of desorption process since the humic acid particles do not cross the gill membrane, though the chemicals do. 2. There is a possibility of gill uptake of small part of humic acid-associated chemicals. MacCarthy et al. (1985b) refer to the humic material as "dissolved' since it is resistant to centrifugation and can pass through a 0.3 μm filter. This functional definition may indicate that the humic acid is bioavailable to the fish gills. It is reported in another study that small particles were found to be taken up by fish gills when the particle-chemical complex contacts the gill epithelial cells (Martens et al. 1993). Small suspended particles, identified as metal or mineral, were in the gills of four species of under-yearling salmon sampled from Fraser River. The size of the intracellular particles seen in the gill epithelial and filamental cells ranged from 0.32 to 0.54 μm (Martens et al. 1993). A similar theory was also reported by Newcombe et al. (1996), namely, that phagocytosis by cells of the fish's gill may transport suspended particles into the fish body. Further study is needed to assess it properly. Since the humic acid is not only water soluble, but is also rich in organic carbon, passive diffusion may occur in the fish gill membrane. In an study that chemicals were associated with humic acid, MacCarthy et al. (1985b) discovered that the

binding affinity ($P_a$) of BaP, benzanthracence and anthrancene with humic acid decreased slightly as the concentration of DOM increased (to 40 to 80 mg/L). Further observation of the chemical uptake by fish in the same study found that the uptake coefficiency of BaP-humic acid complex for bluegills, was estimated as positive, which indicates that some of the complex is incorporated by the fish gills.

In contrast, PeCB and 1,2,4-TCB concentration in fish was unchanged by a humic acid concentration of 4.81 mg/L or greater. The inability of humic acid to reduce bioaccumulation of 1,2,3-TCB and PeCB can be explained by the fact that humic acid did not limit the chemicals dissolved in water, or the dissolved chemicals in water were high enough for fish uptake. From Figure 5.3, the predicted amount of 1,2,4-TCB and PeCB dissolved in water can be as high as 93% and 56%, respectively, even at a concentration of 14.32 mg/L humic acid. The affinity of these two chemicals (with lower log $K_{ow}$ ) for DOM is clearly less than that for HCBP. Similar finding was reported in Muir et al. (1994)'s study, in which, no difference in $k_1$ and BCF in rainbow trout (*Oncorhynchus mykiss*) exposed to synthetic water with 145 $\mu$M DOC, filtered lake water with 550 $\mu$M DOC, and filtered humic acid solutions from a 0.45 $\mu$m filter with 263 $\mu$M DOC containing four pyrethroid insecticides (log $K_{ow}$ 5.5 to 6.5). These data indicated that the presence of a low concentration of dissolved organic materials may not necessarily affect the bioavailability of lipophilic chemicals.

The 60% and 100% increases of 1,2,4-TCB and PeCB in fish body, respectively, at the lowest humic acid concentration tested (1.54 mg/L) comparing with that in the control group, were unexpected. This result seems to contradict the general conclusion

that the presence of DOM will reduce the uptake of lipophilic chemicals into aquatic organisms (McCarthy 1983a; Landrum et al. 1987; Mackay 1991; Servos et al. 1992b). This result, however, is not without precedence in the literature that some studies found that adding humic acid can increase the bioaccumulation of chemicals in aquatic organisms. For example, Leversee et al. (1983) reported that humic acid enhanced the bioaccumulation of 3-methylcholanthrene (log $K_{ow}$ 7.11) more than 2-fold when *Daphnia magna* were exposed to Aldrich humic acid at a concentration of DOC 2.0 mg/L, compared with 0.2 mg/L. In a study where the Empore™ disk, composed of $C_{18}$ solid phase, was used to simulate the bioaccumulation of lipophilic pollutants, Freidig's group (1995) reported that the Empore™ disk absorbed hexachlorobenzene in a higher amount (80 ng/disk) than had been found by other groups (0, 4.23 and 13.80 mg/L) when the disk was exposed to water with a humic acid concentration of 1.41 mg/L. Chemical adsorption was the lowest with 13.80 mg/L humic acid. These results are consistent with the results in this study, although in Freidig's study, the chemical adsorbed in the Empore™ disk was considered as free-dissolved chemical. However, using a disk to simulate the mechanisms of fish gill uptake of DOM-associated lipophilic chemicals may not be fully representative. In addition, Servos et al. (1989d) also discovered that in the presence of humic acid (1.2 & 2.0 mg/L), the apparent uptake rate constant showed a slight increase for both 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (H7CDD, log $K_{ow}$ 9.8) and 1,2,3,4,5,6,7,8-octachlorodibenzo-*p*-dioxin (O8CDD, log $K_{ow}$ 9.8) in rainbow trout compared with the same chemicals in water which had less than 0.7 mg/L of organic carbon. When humic acid was higher (up to 8.6 mg/L), 1,3,6,8-T4CDD (T4CDD, log $K_{ow}$

8.7) and 1,2,3,4,7,8-H$_6$CDD (H$_6$CDD, log K$_{ow}$ 9.8) had a reduced uptake rate. This suggested that the increase in bioconcentration of lipophilic chemicals may involve factors such as the DOM concentration and the solubility of chemicals.

The apparent increased aqueous solubility of lipophilic chemicals under presence of DOM was reported by some studies. Adding humic material extracted from soil (500 mg/L) was found to increase the solubility of DDT by 20- to 40-times (Wershaw et al. 1969). Boehm and Quinn (1973) demonstrated that n-alkanes (hexadecane, eicosane) were more soluble in untreated natural water (2 to 7 mg C/L) than in water that had been treated by either carbon adsorption or ultraviolet irradiation to remove organic matter. The water solubilities of other chemicals, such as PCB isomers (Hassett et al. 1979, Chiou et al. 1986), chlorinated benzene (Chiou et al. 1986), lindane (Caron 1985) and chlorinated-dioxins (Webster, et al. 1986, Servos et al. 1989c) was also found to have higher water solubilities in the presence of DOM. This increase in solubility may be restricted to concentrations of DOM lower than 2 mg/L.

Chiou et al. (1986) reported that the extent of solubility enhancement depends on the type of chemical as well as on the concentration and source of the chemical, and on the concentration and source of DOM (in which the DOM size, polarity, and molecular configuration are considered to be the important factors). Once the water solubility of a chemical increases, it may promote the bioconcentration of lipophilic chemicals in fish.

Although a study of the impact of both humic acid and sediments together was not included in this thesis, the results from other studies suggested that the presence of humic acid, or other sorptive components of the dissolved pool, may affect binding to

sediment or suspended particles, i.e., the humic acid binding to chemicals would appear to be more rapid than its comparable binding to sediment (McCarthy et al. 1985b), and that this alters the fate and transport of organic contaminants in aquatic systems. However, in another study, Lores et al. (1993) exposed fish to sediments and humic acid-laden water (sediments were collected from three locations in separate exposures and humic acid concentrations were 0, 3 and 30 mg/L), they found that the ratio of body burden of 2,2',4,4',5,5'-hexchlorobiphenyl in sheepshead minnows (*Cyprinodon variegatus*) and chemical concentration in sediments was 7.5 in 0 mg/L humic acid and 9.3 in 30 mg/L humic acid. This result indicated that the addition of humic acid did not reduce accumulation of sediment-bound toxicants, which is consistent with the major finding in this study. Since the partition of a chemical between DOM, sediments and suspended sediments is a kinetic partition, fish living in between this partitioning should be affected continuously.

# 5. CONCLUSION

The results of this study demonstrated that dissolved organic particles complicate the bioavailability and bioaccumulation of lipophilic chemicals in fish and indicated that the humic acid a concentration and log $K_{ow}$ value are two key factors, i.e., high concentration of humic acid would reduce the bioavailability of chemical with very high log $K_{ow}$ values, such as HCBP but does not influence the chemicals with lower log $K_{ow}$ values, such as 1,2,4-TCB and PeCB. However, a very low concentration of humic acid may promote chemical bioavailability in fish. Since most water systems have dissolved organic matter no more than 14 mg/L, the results of this study provide important information regarding the impact of the humic acid on the chemical bioaccumulation in fish. The influence of DOM on bioavailability and uptake of lipophilic chemicals can be considered as complicated and further study on large groups of lipophilic chemicals should be continued.

Table 5.1 Statistical analyses of the impact of the humid acid on the bioavailability of test chemicals, 1,2,4-TCB, PeCB and HCBP between the groups (ANOVA).

TCB

| Humic acid (mg/L) | 0 2 day (4 day) | 1.54 2 day (4 day) | 4.81 2 day (4 day) |
|---|---|---|---|
| 1.54 | +(+) | | |
| 4.81 | - (-) | + (+) | |
| 14.32 | - (+) | + (+) | - (-) |

PeCB

| Humic acid (mg/L) | 0 2 day (4 day) | 1.54 2 day (4 day) | 4.81 2 day (4 day) |
|---|---|---|---|
| 1.54 | +(+) | | |
| 4.81 | - (-) | + (+) | |
| 14.32 | - (-) | - (+) | - (-) |

HCBP

| Humic acid (mg/L) | 0 2 day (4 day) | 1.54 2 day (4 day) | 4.81 2 day (4 day) |
|---|---|---|---|
| 1.54 | - (-) | | |
| 4.81 | + (+) | + (+) | |
| 14.32 | + (+) | + (+) | - (-) |

+ : $P < 0.05$ (student t-test)

Table 5.2 Water concentrations of the three test chemicals, 1,2,4-TCB, PeCB and HCBP, as a function of humic acid concentration and obtained after $C_{18}$ extraction.

| Chemicals | Humic acid µg/L | 2 day µg/L | 4 day µg/L |
|---|---|---|---|
| 1,2,4-TCB | 0 | 21.59 | 10.7 |
| | 1.4 | 19.02 | 9.44 |
| | 4.2 | 22.52 | 5.75 |
| | 14.3 | 20.71 | 9.13 |
| PeCB | 0 | 9.47 | 5.32 |
| | 1.4 | 8.97 | 4.52 |
| | 4.2 | 9.95 | 5.35 |
| | 14.3 | 9.66 | 4.46 |
| HCBP | 0 | 7.32 | 3.44 |
| | 1.4 | 7.39 | 4.2 |
| | 4.2 | 7.59 | 6.03 |
| | 14.3 | 7.56 | 5.86 |

Table 5.3 The proportion of truly dissolved test chemicals, 1,2,4-TCB, PeCB and HCBP, in humic-laden water estimated using equation $C_{WD}/C_W = 1/(1+Ø_H \cdot K_{ow} \cdot 0.5)$.

| Humic acid concentration (mg/L) | 1,2,4-TCB % | PeCB | HCBP |
|---|---|---|---|
| 0 | 100 | 100 | 100 |
| 1.54 | 99.3 | 92.4 | 3.5 |
| 4.81 | 97.8 | 79.5 | 1.2 |
| 14.32 | 93.6 | 56.6 | 0.4 |
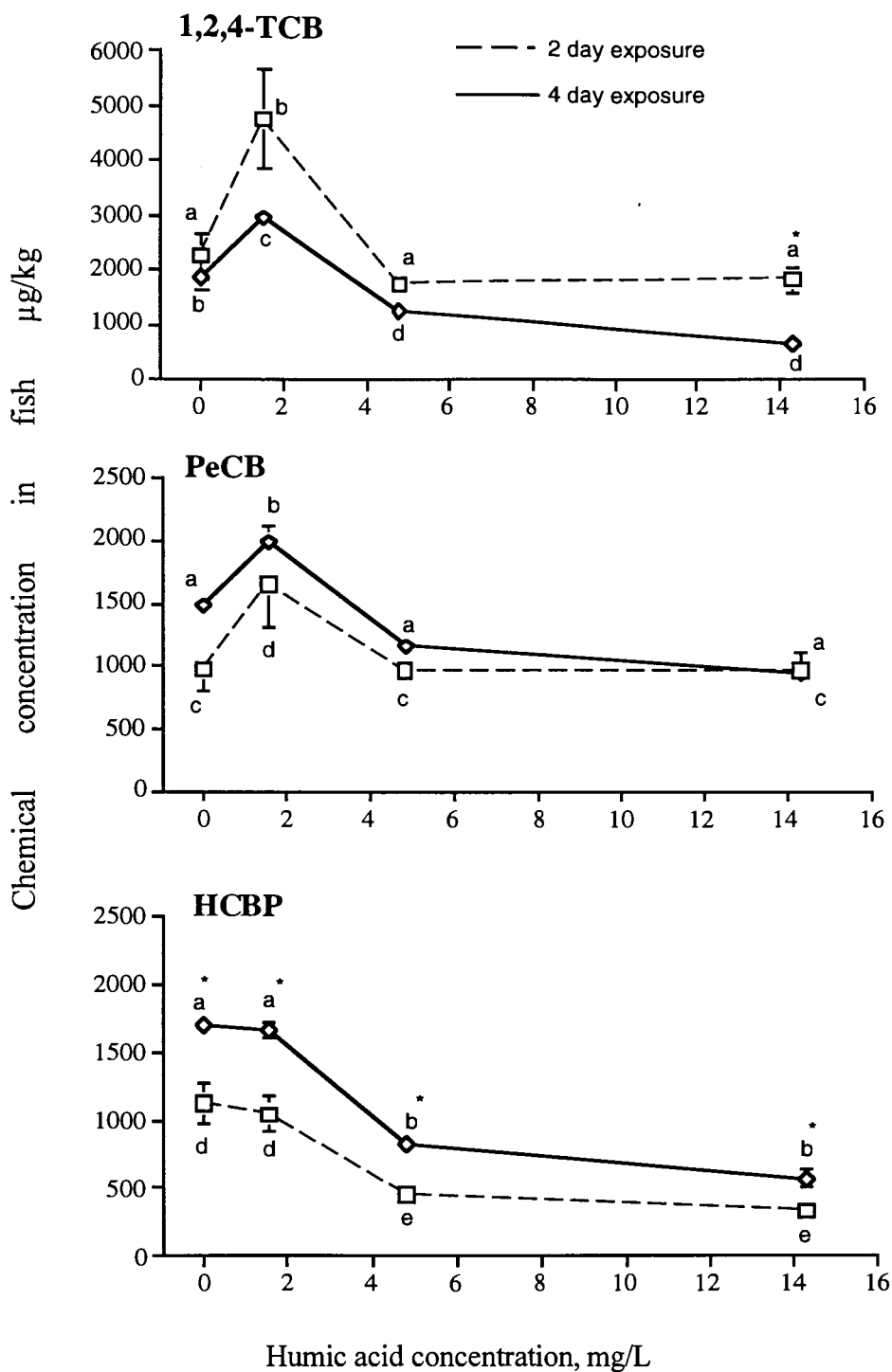
Figure 5.1. The concentrations of test chemicals, 1,2,4-TCB, PeCB and HCBP in rainbow trout (*Oncorhychus mykiss*) muscle samples after 2-day and 4-day exposure with different concentrations of humic acid. Different letters show significant difference between humic acid concentrations for a given exposure groups (p < 0.05). Each point is the mean of 3 fish. denotes the significant difference between 2-day and 4-day exposure in same humic concentration.
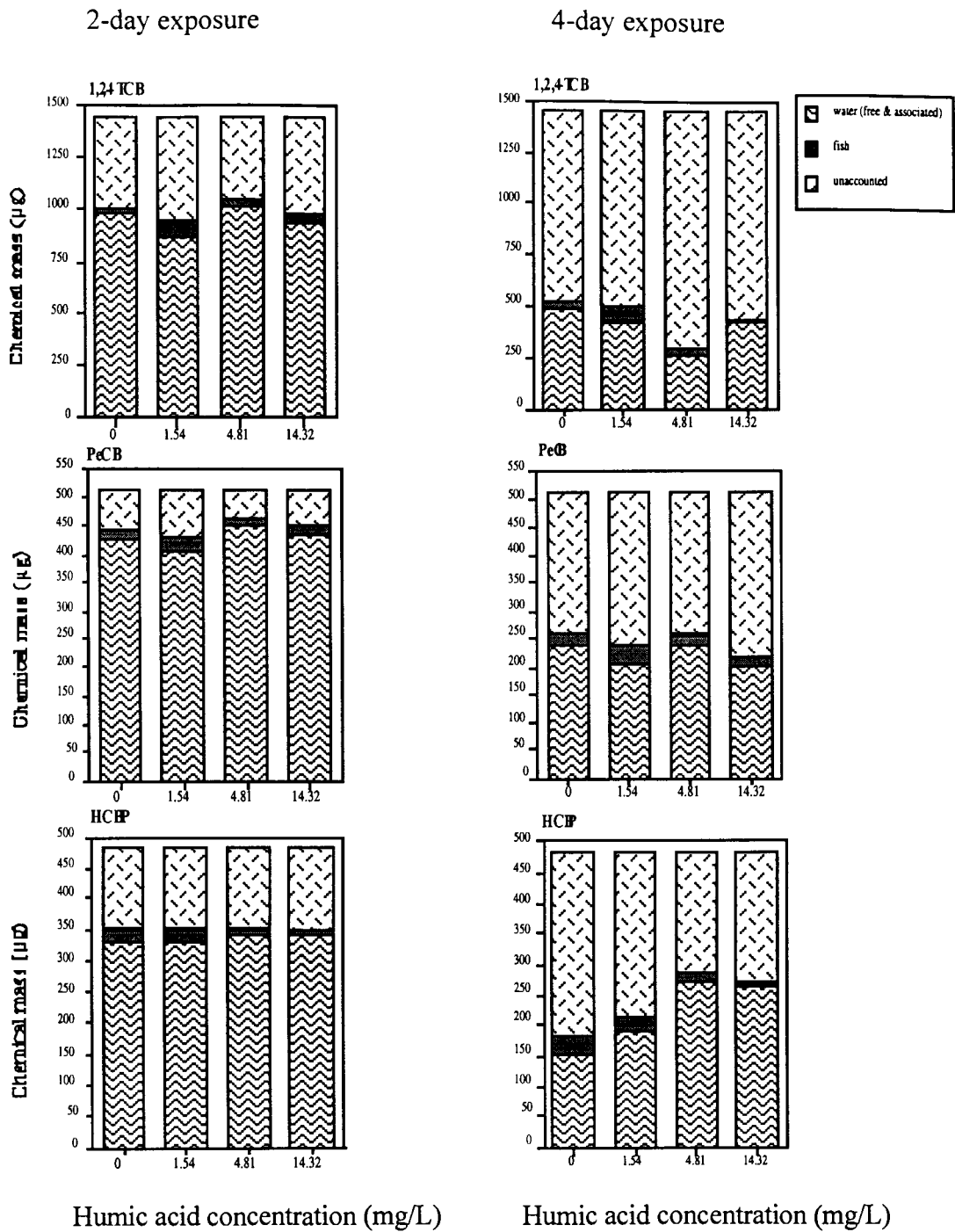
Figure 5.2 The mass balance of three test chemicals, 1,2,4-TCB, PeCB and HCBP in water and rainbow trout (*Oncorhychus mykiss*) after 2-day and 4-day exposure in humic-laden water.
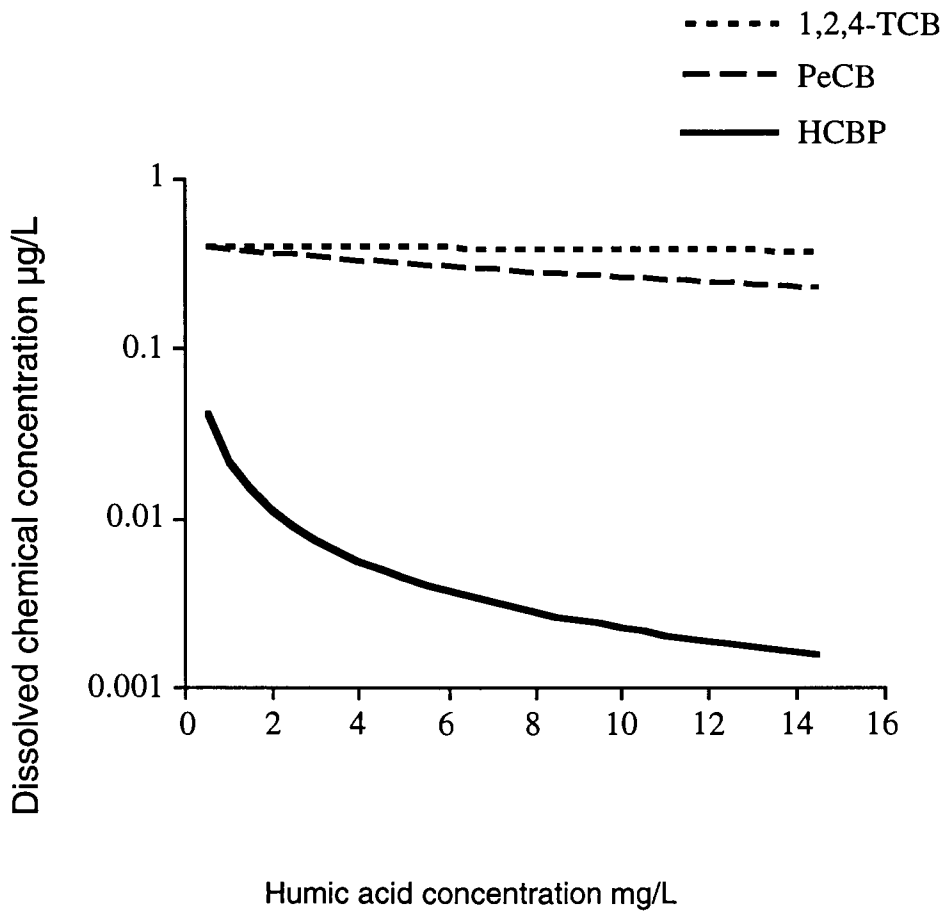
Figure 5.3 An estimate of the dissolved test chemicals, 1,2,4-TCB, PeCB and HCBP, in humic-laden water using the equation $C_{WD}/C_W = 1/(1+\emptyset_H \cdot K_{ow} \cdot 0.5)$.

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# CLOSING CONCLUSION AND FUTURE DIRECTION

For this thesis, I tested two uptake routes, gill and GI tract, for three lipophilic chemicals, 1,2,4-TCB, PeCB and HCBP, and investigated the impact of the Fraser suspended sediment and dissolved organic matter, humic acid, on the bioavailability, uptake and bioaccumulation of these chemicals.

The results of this study suggest that both gill and GI tract are important uptake routes for lipophilic chemical accumulation in fish. Gills play a more important role in the case of log $K_{ow}$ values up to 5.0.5 while absorption by the GI tract is the major pathway for chemicals with log $K_{ow}$ as high as 7.5 when fish are exposed to both contaminated water and food. The study also demonstrated that suspended particles can serve as the chemical source for fish uptake. Gill uptake was found to be the most likely pathway for the uptake of lipophilic chemicals from these suspended particles.

The results suggest that the conventional equation for predicting chemical concentration in fish body, i.e.,

$$dC_f/dt = Cw.k_1 + Cd.k_d - C_f(k_2 + k_e + k_m), \tag{6.1}$$

does not fully represent the sources of chemicals found in fish body in water systems with contaminated suspended sediments, because it does not include the gill uptake of chemicals from the suspended particles. Thus, we may need to construct a new kinetic model in which the gill uptake from the contaminated suspended particles would be integrated as follow

$$dC_f/dt = C_w . k_1 + C_d . k_d + C_{ss}. k_{ssf} + C_{DOM} . k_{DOM} - C_f(k_2 + k_e + k_m), \tag{6.2}$$

where the $C_{ss}$ and $C_{DOM}$ represent the chemical concentration in suspended sediments and DOM, respectively. The uptake rate constants, $k_{ssf}$ and $k_{DOM}$, represent gill uptake rate from suspended sediments and DOM, respectively. Since the gill uptake rate constant from water is orders of magnitude greater than that of the GI tract, the proportion of chemicals associated with suspended particles taken via gills maybe meaningful in prediction of chemicals in the fish body.

Beyond the three lipophilic chemicals tested for this study there is a large group of other lipophilic chemicals being released into aquatic system that undoubtedly interact in a complex pattern with various aquatic compartments. Further study is needed to explore (1) the kinetic distribution of lipophilic xenobiotics as between sediment, suspended sediment and DOM as well as (2) the relationship between the chemical distribution in these compartments, and (3) how this influences on the bioavailability and bioaccumulation of such chemicals.

# GLOSSARY

**Bioaccumulation:** the general term describing the increase of chemicals (usually nonessential ones) by any or all of the possible routes (i.e., respiration, diet, dermal) from any source in the aquatic environment where chemicals are present (i.e., water, dissolved, colloidal or particulate organic carbon, sediments, or other organisms), and regardless of the mechanism of uptake (Spacie et al. 1995).

**Bioavailability:** The degree to which a contaminant in a potential environmental source is available for uptake (Hamelink et al. 1994). In this study, the definition of bioavailability refers to environmental bioavailability instead of pharmacological definition which involves absorption and reaction at the target site.

**Bioconcentration:** The process by which an organism increases the tissue concentration of a chemical to many times that of the environment through transfer across surfaces not involved in digestion (Gobas et al. 1997).

**Bioconcentration factor:** Ratio of tissue chemical residue to water chemical concentration (Connell 1990; Spacie et al. 1995).

**Biomagnification:** The process by which an organism increases the tissue concentration of a chemical higher than that in the food it consumes (Spacie et al. 1995).

**Biomagnification factor:** Ratio of tissue chemical residue to the chemical concentration in food (Gobas et al. 1997).

**Fugacity (f, Pa):** Thermodynamic activity or "escaping tendency" of a chemical in a particular phase. It is a measure of the activity of the chemical in that phase and can be viewed as the partial pressure a chemical exerts as it attempts to escape from one phase and migrate to another (Mackay 1991; Spacie et al. 1995).

**Fugacity capacity (mol/m³Pa):** Analogous to heat capacity and is a proportionality constant. It can be derived from the equation C/Z.

**Uptake rate constant: (also called kinetic rate constant):** The amount (real or hypothetical) of environmental medium (water or food) cleared of chemicals by uptake into the organism per unit mass of organism per unit time expressed as a flow rate (e.g., L/kg.day or kg/kg.day, time unit can be hour, too) (Spacie et al. 1995)

**Xenobiotic chemicals:** a chemical that is foreign to the human or another organism's body (Sipes et al. 1991).

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# ABBREVIATIONS USED IN TEXT

**1,2,4-TCB:** 1,2,4-trichlorobenzene

**1,3,5-TCB:** 1,3,5-trichlorobenzene

**BCF:** Bioconcentration factor

**BMF:** Biomagnification factor

**DOC:** dissolved organic carbon

**DOM:** dissolved organic matter

**GI:** gastrointestinal (tract)

**HCB:** 1,2,3,4,5,6-hexachlorobenzene

**HCBP:** 2,2'4,4'6,6'-hexachlorobiphenyl

**PCB:** polychlorinated biphenyl

**PeCB:** 1,2,3,4,5-pentachlorobenzene

**QSAR:** Quantitative structure-activity relationship

**TCBP:** 2, 2', 5, 5'- hexachlorobiphenyl

**TeCB:** 1,2,4,5-tetrachlorobenzene

# ABBREVIATIONS USED IN EQUATIONS

$\varnothing_H$: humic acid concentration with unit of mg/L.

**A:** the cross-sectional area through which solute is diffusing

**Ac:** GC peak area of chemicals

**As:** GC peak area of surrogates

**Ai:** GC peak area of internal standards

**C:** Concentration

$\quad$ $C_d$ (μg/kg): concentration of a chemical in food

$\quad$ $C_f$ (μg/kg): concentration of a chemical in fish

$\quad$ $C_w$ (μg/L): concentration of a chemical in water

$C_c$: concentration of chemicals

$C_H$: chemical concentration associated with humic acid with unit of μg/kg and

$C_i$: concentration of internal standards

$C_s$: concentration of surrogates

$C_{ss}$: chemical concentration in suspended sediments

$C_{DOM}$: chemical concentration in dissolved organic matter

$d_x$: diffusing distance

$D_s$: the diffusion coefficient of a chemical

$K_{ow}$: the partition coefficiency between octanol and water, used as a measure of hydrophobicity.

$\quad$ **k:** uptake clearance and elimination rate constant

$\quad$ $k_1$ (L /kg .day): gill uptake rate constant

$\quad$ $k_d$ (kg /kg .day): Gastrointestinal (GI) tract uptake rate constant

$k_2$ (1/d): gill elimination rate constant

$k_e$ (1/d): GI tract elimination rate constant

$k_{ssf}$: gill uptake rate from suspended sediments

$k_{DOM}$: gill uptake rate from suspended sediments

$L_d$ : lipid content in fish food

$Q_s$: chemical diffusion mass

RF(C): response factor for test chemicals

RF(S): response factor for surrogates

$U_{gills}$: chemical mass in fish via gill uptake

$U_{GI}$: chemical mass in fish via GI tract uptake route

$V_f$: volume of fish (L or kg, assuming 1 L $\cong$ 1 kg)