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Relative Contributions of Aqueous and Dietary Uptake of Hydrophobic Chemicals to the Body Burden in Juvenile Rainbow Trout

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Abstract. This study assessed the relative contributions of aqueous versus dietary uptake of three hydrophobic chemicals, 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,3,4,5-pentachlorobenzene (PeCB), and 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCBP). Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed separately to chemically spiked water and food for 4 days and 12 days, respectively. Chemical concentrations were measured in the food, water, and tissues, and this allowed calculation of uptake rate constants (k_1 from water exposure, k_d from food exposure). The k_1 values for the three test chemicals were approximately five orders of magnitude greater than the k_d values. Using these measured uptake rate constants, a simulation model was used to predict the relative aqueous versus dietary uptake when fish were exposed simultaneously to water and food contaminated with these hydrophobic chemicals. The model predicted for all three test chemicals that the two uptake routes would contribute equally to the chemical body burden in fish whenever the food:water chemical concentration ratio was near 10^5. However, using food:water chemical concentration ratios that might be expected in nature, the model predicted that gill uptake could account for over 98% of fish body burden for both 1,2,4-TCB and PeCB uptake (log K_{ow} values of 3.98 and 5.03, respectively). For HCBP (log K_{ow} of 7.55), the model predicted that the dietary uptake could contribute over 85% of the body burden. Thus, depending on the actual food:water chemical concentration ratio, aqueous uptake via the gills can predominate even when the chemicals have a log K_{ow} value greater than 5.0. In addition, we confirmed that dietary uptake of hydrophobic xenobiotics increases with increasing log K_{ow}.

Persistent lipophilic chemicals tend to bioaccumulate in fish to a concentration greater than that in either the food or the water. Bioaccumulation can be directly affected by the octanol-water partition coefficient (K_{ow}) of the chemical in a number of ways. Foremost, high log K_{ow} chemicals bioaccumulate to a greater degree than less lipophilic ones (Connell 1990). In addition, because uptake of lipophilic toxicants can occur from contaminated food as well as contaminated water, log K_{ow} can affect the relative role of dietary (biomagnification) versus aqueous (bioconcentration) uptake. Heath (1995) summarized our state of knowledge on the bioaccumulation of hydrophobic chemicals in the following way. "Chemicals with a 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 with a log $K_{ow} > 6$ are probably taken up entirely by gut uptake." Since the transition from predominantly aqueous to predominantly dietary uptake occurs over a fairly broad range for log K_{ow}, the purpose of the present study was to better define this transition point.

There is strong evidence that dietary uptake is the major route for chemicals with a very high log K_{ow} (Rudd 1964; Monod and Keck 1982; Thomann and Connelly 1984; Muir *et al*. 1985; Crossland *et al*. 1987; Gobas *et al*. 1988; Batterman *et al*. 1989; Servos *et al*. 1992). However, there are varying results and conclusions regarding the log K_{ow} at which dietary uptake predominates. While some authors have suggested that dietary uptake is a factor only when log K_{ow} is greater than 5 (Connell 1990; Opperhuizen 1991), others have suggested that aqueous uptake dominates when fish are given simultaneous aqueous and dietary exposure (Ferguson *et al*. 1967; Robinson *et al*. 1967; Chadwick and Broocksen 1969; Reinert 1972; Jarvinen *et al*. 1977; Fowler and Elder 1978; Tulp *et al*. 1979; Shaw and Connell 1982; Leblanc 1995). Certainly, bioconcentration factors increase with log K_{ow} values above 2 (Bruggeman *et al*. 1984; Oliver and Charlton 1984; Sabljic 1987; Connell 1990; Hawker 1990; Randall *et al*. 1990; Smith *et al*. 1990; Gobas 1990; Nenza 1991). Nevertheless, this relationship breaks down when log K_{ow} exceeds 6 (Gobas and Morrison 2000), indicating an increasing importance of dietary uptake at very high log K_{ow} values. Even so, Opperhuizen (1991) predicted that uptake of hydrophobic chemicals by the gills and gut were of equal importance because the efficiency of gill uptake of xenobiotics from the water and gut uptake from food are both approximately 50% regardless of K_{ow} values (see also Norstrom *et al*. 1975; Jarvinen and Tyo 1978; Macek *et al*. 1979).

Given the established practice of using log K_{ow} as a predictive tool in environmental decision making, improved definition of the transition point between dietary and aqueous uptake

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can only assist in better predicting chemical body burdens of fish under field conditions. Therefore, we used separate laboratory exposure experiments to derive uptake rate constants for aqueous and dietary exposures for three lipophilic chemicals with a range of log K_{ow} values from 3.98 to 7.55. The derived uptake rate constants were then used in a mathematical model to simulate field situation conditions (Gobas and Zhang 1992) and predict the relationship between the relative roles of dietary and aqueous uptake as a function of log K_{ow}, and at what log K_{ow} value the transition occurred.

Materials and Methods

Exposure Protocols

Fish: Juvenile (2.5–3.5 g) rainbow trout (*Oncorhynchus mykiss*) were obtained from Westcreek Fish Farm in Langley, BC, and were held for at least 3 weeks in flow-through tanks (500 L) receiving dechlorinated municipal water at a rate of 3 L/min. Continuous aeration achieved a dissolved oxygen concentration of $>$ 8 mg/L at 12°C. Water pH was 6.1–6.3. Water hardness was 17.1 mg/L as $CaCO_3$, and alkalinity was 17.1 mg/L as $CaCO_3$. The fish were fed daily with Clark's dry extruded fish feed until 2–4 days prior to the experiments. The food ingredients included fishmeal, canola meal, fish oil, whole wheat, feather meal, can molasses, ethoxyquin, and vitamins. Total crude protein was 47%, total fat 14%, crude fiber was 2.5%, calcium actual 2.0%, phosphorus actual 1.5%, vitamin A 25,000 IU/kg, vitamin D_3 2,400 IU/kg, and vitamin E 125 IU/kg.

Chemicals: The chemicals to which the fish were exposed were 1,2,4-trichlorobenzene (1,2,4-TCB, Aldrich Chemical Co.; 99%), 1,2,3,4,5-pentachlorobenzene (PeCB, Aldrich; 98%) and 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCBP, AccuStandard; 100%). 1,3,5-Trichlorobenzene (1,3,5-TCB, Aldrich; 99%), 1,2,3,4,5,6-hexachlorobenzene (HCB, Aldrich; 99%) and 2,2′,5,5′-tetrachlorobiphenyl (TCBP, AccuStandard; 100%) were used as internal standards for chemical extraction and clean-up in quantifying the loss of the test chemicals during chemical analysis. 1,2,4,5-Tetrachlorobenzene (TeCB, Aldrich; 99%) was used as the "internal standard" for gas chromatographic analysis. Some of the physical properties of these chemicals are listed in Table 1. The concentrations of the test chemicals in water and food were more than 100 times lower than the 96-h LC_{50} value.

Aqueous Exposures: Aqueous stock solutions were prepared immediately before use and contained a mixture of 925 μg 1,2,4-TCB/L, 205 μg PeCB/L, and 208 μg HCBP/L. The chemicals were first dissolved individually in methanol. The exposure apparatus was a 65-L aquarium, and this was primed by stirring in 130 ml of the stock solution at the start of the experiment, resulting in nominal concentrations of 1.85 μg 1,2,4-TCB/L, 0.41 μg PeCB/L, and 0.42 μg HCBP/L. Thereafter, the aquarium water was partially replaced every hour with a computer-controlled pump and solenoid to control the delivery of the stock solution and the aerated dilution water (see Wood *et al*. 1996 for full details of the exposure apparatus). Every hour chemical stock was delivered to the test aquarium at a rate of 5 ml/min for 1.2 min, and water was delivered at the rate of 1 L/min for 3 min, for a water turnover time of 22 h. A second, control aquarium was set up in the same manner but receiving water containing 2 ml/L methanol. Water temperature was 13 ± 1°C and oxygen concentration was $>$ 8 mg O_2/L.

Forty-eight fish (weighing 2.6 ± 0.3 g) were placed in both aquaria. Water samples (50 to 500 ml) and fish samples (eight fish each time) were then taken periodically (1, 4, 8, 12, 48, and 96 h) for chemical analysis. After removal, fish were killed by a sharp blow to the head and immediately frozen at −80°C. Storage was no longer than 1 month prior to chemical analysis.

Dietary Exposures: A one-step food chain was used to facilitate the evaluation of biomagnification (Connell 1990). Analysis of the commercial fish food (Moore-Clark Co., Vancouver, BC) prior to use confirmed the absence of the test chemicals. Chemically spiked food was prepared 1 week before the experiment using a household blender. Food was first minced in the blender and then softened by adding water and mixing until wet to the touch. Test chemicals were dissolved in methanol and mixed with the food for 1 h before making favorable-sized food pellets using a 3/32″ hamburger die. The food pellets were dried in a fume hood. The concentration of the test chemicals in the food was confirmed by chemical analysis as: 49.2 ± 1.6 mg 1,2,4-TCB/kg, 17.9 ± 0.5 mg PeCB/kg, and 24.3 ± 0.1 mg HCBP/kg.

Seven 45-L glass aquaria were used to hold six groups of 10 fish and one group of 4 fish. These aquaria received 13 ± 1°C dechlorinated water at a rate of 1.5 L/min that was aerated to maintain the oxygen concentration $>$ 8 mg/L. Fish were fed daily approximately 2% of their body mass. To minimize chemical contamination of the water by the feces and food, the wastes on the bottom of the aquarium were removed twice daily using a siphon tube. In addition, the water was continuously filtered with an activated charcoal/foam filter (Aquaclear Mini, Rolf C. Hagen Inc., Montreal). On each of the first 6 days of the feeding trial, the 10 fish in one aquarium were sampled for chemical analysis. On the 12th day, the last four fish were sampled. Fish were killed by a sharp blow to the head and immediately frozen at −80°C. Storage was no longer than 1 month prior to chemical analysis.

Chemical Analysis

Chemical Extraction from Water Samples: Chemicals were extracted from water samples with a solid reverse-phase method that used either an octadecyl (C_{18}) (a nonpolar sorbent 18 carbon straight-chain hydrocarbon) Bond Elute cartridge (Varian Co.) or C_{18} EmporeTM disk (J.T. Baker), depending on sample volume. Both were preconditioned with methanol. The chemicals were then eluted with hexane, as described in detail by Blevins *et al*. (1993). The extracts were cleaned up before GC analysis.

Chemical Extraction from Food and Tissue Samples: Chemicals were extracted from subsamples of fish tissue (0.5 g) and food (0.2 g). Fish were 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 about 0.5 g skeletal muscle, including skin, was removed, minced, and homogenized in a 15-ml hand-held homogenizer (Pyrex Co., England) using 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 containing 5 ml hexane and 3–5 ml buffer solution. The homogenate was centrifuged at 3,000 *g* for 10 min after 4 h of shaking (American Optical Co., Richmond, CA). The supernatant was collected and the procedure was repeated with another 5 ml of hexane. The supernatants were pooled and cleaned up before GC analysis.

Clean-up: Water, tissue, and food extracts were cleaned up before GC analysis by transferring them into a 15-cm glass clean-up column. The column contained (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 (0.3 g, Caledon). The column was prewashed with hexane before clean-up. After the extracts had passed through the column, 5 ml of hexane was used to elute the column. The

Table 1. The physical-chemical properties of the test chemicals, surrogate chemicals, and the internal standard chemicals and the acute toxicity of the test chemicals

	Molecular Weight	log K_{ow}	Water Solubility (mg/L)	Vapor Pressure (Pa)	96-h LC_{50} (mg/L)	Test Species
1,2,4-TCB	181.45	3.98[a]	46.09[a]	60.6[b]	3.36-21.4[g]	Bluegill Fathead minnow
PeCB	250.3	5.03[a]	0.83[a]	0.219[b]	0.25-0.83[g]	Bluegill Fathead minnow
HCBP	360.9	7.55[a]	0.00041[a]	0.012[c]	61[h]	Cutthroat trout Yellow perch
1,3,5-TCB	181.45	4.49[a]	4.1[a]	77[e]		
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[f]		

[a] Miller and Wasik (1985).
[b] Mackay and Shiu (1981).
[c] Shiu and Mackay (1986).
[d] Miller *et al*. (1984).
[e] Mackay *et al*. (1982).
[f] Mackay *et al*. (1985).
[g] US EPA (1980).
[h] US Dept. of Interior/Fish and Wildlife Service (1986).

elutate was concentrated to 1.5 ml with N_2 at room temperature. Internal standard (0.5 ml) was added to the extracts immediately before GC analysis.

GC Analysis: The extracts were analyzed by gas chromatography (GC), using surrogate and internal standard chemicals for calibration. Surrogate chemicals were added into the sample at the start of the extraction procedure and the internal standard was injected at least once into the GC before sample analysis. 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 to increase from 100 to 300°C in 24.5 min. 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 used for each analysis. The mean of these values represented one value for each tissue, food, or water sample. GC retention times 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, 10.77 min for HCB, 13.2 min for TCBP, and 14.90 min for HCBP. The chemical recovery was estimated from the surrogates and internal standards and the following values were used: 94% to 96% for TCB and 98% to 101% for PeCB and HCBP. The precision had more than a 98% confidence limit.

Modeling Theory

A mathematical description of the uptake and elimination of chemicals in fish is given by the following model (Gobas 1993):

$$dC_F \div dt = C_w \cdot k_1 + C_d \cdot k_d - C_F \cdot (k_2 + k_e + k_m + k_g) \quad \text{(Eq. 1)}$$

where C_F (μg/kg fish), C_w (μg/L water), and C_d (μg/kg diet) are the chemical concentrations in fish, the freely dissolved concentration in the water, and the concentration in the diet, respectively. k_1 [L/(kg fish · day)] and k_d [(kg diet)/(kg fish · day)] are the uptake rate constants (also sometimes referred to as uptake clearance constants) for chemical uptake via the gills and from the diet, respectively. k_2, k_e, k_m, and k_g are elimination rate constants (1/day) of the chemical via the gills, feces, metabolic transformation, and growth dilution, respectively. While k_1 incorporates processes such as gill ventilation, transport in blood, membrane transfer, and internal distribution, k_d incorporates processes such as ingestion rate, gut wall permeation, assimilation efficiency, and internal transport via the blood (Landrum *et al*. 1994; Gobas and Morrison 2000). Because the chemical body burden in a fish under field conditions is the result of both dietary and aqueous uptake, the relative contribution of aqueous and diet uptake can be assessed as:

$$U_{\text{gills}} \div U_{\text{GI}} = (k_1 \cdot C_w) \div (k_d \cdot C_d) \quad \text{(Eq. 2)}$$

where U_{gills} and U_{GI} (μg/day) are the uptake rates of chemical in the fish via the gills and gastrointestinal (GI) tract, respectively.

To solve these equations, the uptake rate constants were determined in the laboratory experiments. The advantage of performing kinetic experiments is that once measured, the rate constants can be used to estimate the relative roles of dietary and aqueous uptake as long as the actual concentrations in the water and the food are known. This also means that the exposure concentrations used in this study did not have to match those in the actual environment.

Derivation of Uptake Rate Constants: Uptake constants were derived from observed concentrations by applying the BIOFIT model (Gobas and Zhang 1992), which is particularly useful in reducing the margin of error when deriving uptake rate constants when exposure is short and water concentrations vary over time during the exposure period. This method derives uptake and total elimination rate constants (*i.e*., the combined sum of k_2, k_e, k_m, and k_g) by fitting Equation 1 to observed water and fish tissue concentrations. If the chemical elimination rate (in g/day) from the fish is insignificant compared to the uptake rate (in g/day) and it takes a long time to achieve steady-state—as is typically the case at the beginning of the uptake experiment with high K_{ow} chemicals—the fitting methodology has insufficient information to derive the elimination rate constant. In those cases, only uptake rate constants can be derived.

Results

Aqueous Exposure

Chemical dosing was continuous during the entire aqueous exposure experiment. Nevertheless, chemical concentrations in water decreased sharply during the first 12 h (Figure 1) partly due to fish uptake. After 12 h, the measured water concentrations varied little.

Chemical body burdens resulting from the aqueous exposure are shown in Figure 2. The body burden of 1,2,4-TCB increased rapidly during the first 24 h to about 250 μg/kg. The accumulation rate of 1,2,4-TCB then slowed, and the body burden did not change significantly thereafter. The concentration of PeCB increased rapidly but did not reach steady-state during the exposure period. Bioconcentration of HCBP was slower than that of PeCB even though the exposure concentrations for the two chemicals were similar (Figure 2). In fact, the body burden of HCBP was more than three times lower than that for PeCB for both the 24-h and 96-h samples (Figure 1). Test chemicals were not detected in muscle samples taken from control fish.

Dietary Exposure

Chemical body burdens resulting from dietary exposure are shown in Figure 3. Bioaccumulation of 1,2,4-TCB occurred during the first 5 days of feeding, but thereafter body burden did not change significantly. Uptake of PeCB and HCBP during the first 2 days was initially slower than that of 1,2,4-TCB (Figure 3). Concentrations of PeCB and HCBP in the fish increased with time in a near linear fashion.

Uptake Rate Constants

The uptake rate constants for aqueous and dietary exposures are presented in Table 2. The k_1 values were about five orders of magnitude greater than the k_d values. Surprisingly, the k_1/k_d ratios were similar for 1,2,4-TCB and HCBP. PeCB showed the highest k_1/k_d ratio among the three test chemicals (Table 2).

Aqueous versus Dietary Chemical Uptake

To evaluate the relative contributions of aqueous and dietary uptake to chemical body burden, Equation 2 was used to model a simultaneous chemical exposure via water and food. This model incorporated the measured k_1 and k_d values and a wide range (10^3 to 10^7) for the food:water chemical concentration ratios. The results of the simulation model are shown in Figure 4. The fraction of chemical uptake from water was calculated as

$$k_1 \cdot C_w \div (k_1 \cdot C_w + k_d \cdot C_d) \qquad \text{(Eq. 3)}$$

whereas the fraction of chemical taken up from food was calculated as

$$k_d \cdot C_d \div (k_1 \cdot C_w + k_d \cdot C_d) \qquad \text{(Eq. 4)}$$

The model predicted that at a food:water concentration (C_w:C_d) ratio of around 10^5, chemical uptake via the gills and the GI tract would contribute almost equally to the chemical body burden. If the food:water concentration ratio was $> 10^7$, then dietary uptake would account for nearly 100% of chemical uptake. Conversely, if the food:water concentration ratio was $< 10^3$, then gill uptake would account for virtually 100% of the uptake.

Using an illustrative scenario where water and dietary concentrations are assumed to be at a chemical equilibrium, we estimated at an equilibrium, we estimated C_d/C_w as $K_{ow} \cdot L_d$ for the three chemicals, where L_d is the lipid content of the diet (a value of 2% lipid was used here). The C_d:C_w ratios were 191 (*i.e.*, $10^{2.3}$) for 1,2,4-TCB, 2,350 (*i.e.*, $10^{3.3}$) for PeCB, and 710,000 (*i.e.*, $10^{5.9}$) for HCBP. Substituting these C_d:C_w ratios into the model (Figure 4), gill uptake was predicted to account for over 98% of fish body burden of both 1,2,4-TCB and PeCB uptake. In contrast, the GI tract was predicted to contribute over 85% of body burden of HCBP.

Discussion

The present study confirmed that dietary uptake of hydrophobic xenobiotics increases in importance directly with log K_{ow}. In addition, we established that aqueous uptake was the predominant (98%) contributor to body burden for a lipophilic chemical with a log K_{ow} value of 5.05 and remained an important contributor (15%) for a lipophilic chemical with a log K_{ow} of 7.55. The implication of our findings relative to Heath's (1995) suggestion that the transition between aqueous and dietary uptake as the main contributor to lipophilic chemical uptake occurred at log K_{ow} between 3.0 and 6.0 is that the transition may be closer to log K_{ow} 6 than previously thought.

Our findings do not agree with Opperhuizen (1991), who predicted that the gills and GI tract were of equal importance for the uptake of hydrophobic chemicals. We agree with Opperhiuzen that gill uptake is important at high K_{ow} values, but we found a clear transition to dietary uptake at a log K_{ow} value somewhere between 5.0 and 7.5 for the C_d:C_w ratios that we modeled.

We are not alone in our suggestion that aqueous uptake in fish can predominate for certain hydrophobic chemicals with a log K_{ow} value close to 6.0. Support is provided by earlier studies that directly compared fish given only an aqueous exposure with fish given an aqueous plus dietary exposure. However, these earlier studies usually studied only one chemical rather than comparing several chemicals with differing hydrophobicity, as we did here. For example, Chadwick and Broocksen (1969) found that a 3-week aqueous plus dietary exposure to dieldrin (log K_{ow} 5.4) resulted in a similar dieldrin body burden as compared with aqueous exposure alone, suggesting a minor role for dietary uptake. Furthermore, bioaccumulation studies with dieldrin in guppies (Reinert 1972) and with DDT in fathead minnows (log K_{ow} 6.0) (Jarvinen *et al.* 1977) suggested that chemical body burden after aqueous exposure was greater than that after food exposure. In fact, the daphnia used to feed the guppies had accumulated dieldrin, presumably through bioconcentration, to a greater degree than the guppies (Reinert 1972). Also, there was no effect on the

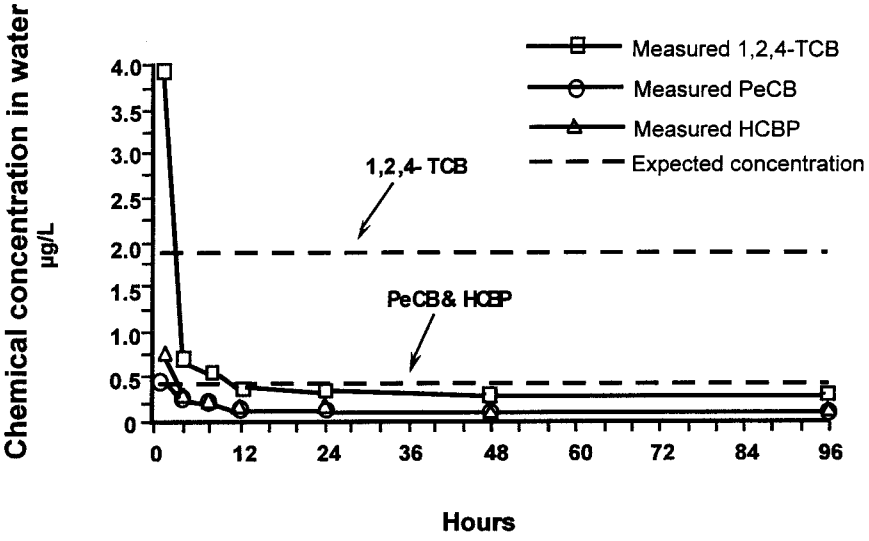

Fig. 1. Measured water concentrations of 1,2,4-TCB, PeCB, and HCBP during aqueous exposure of juvenile rainbow trout (*Oncorhynchus mykiss*). Each point represents water concentration when fish was sampled. Each point represents the average of two water samples

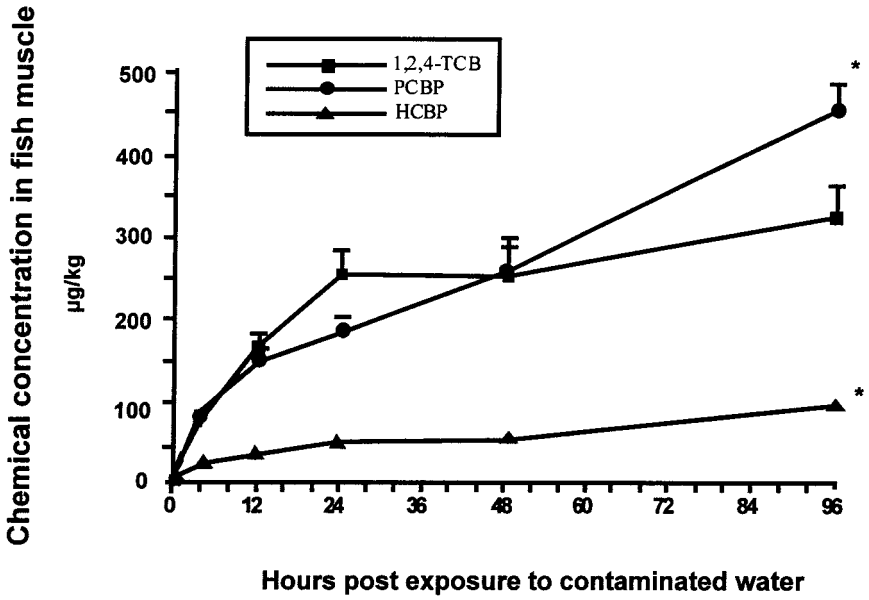

Fig. 2. Measured concentrations of 1,2,4-TCB, PeCB, and HCBP in juvenile rainbow trout (*Oncorhynchus mykiss*) muscle samples during a 4-day aqueous exposure experiment. Each point is the mean of eight fish. *denotes a significant difference ($p < 0.05$) between the 24- and 96-h values

DDT body burden when the contaminated food ration was doubled (Jarvinen *et al*. 1977). Other studies have come to a similar conclusion about the importance of aqueous uptake by taking advantage of the fact that marine fish drink 5–12% of their body weight daily whereas freshwater fish do not drink water (Murty 1986). Unfed Atlantic salmon exposed to ^{14}C-2,2′,4,5,5′-pentachlorobiphenyl (log K_{ow} 5.92) in fresh water had a higher chemical body burden compared with a sea water exposure under otherwise identical conditions (Tulp *et al*. 1979). When Ferguson and Goodyear (1967) compared endrin (50 µl/L, log K_{ow} = 4.53) uptake in black bullheads with and without their esophagus tied off (to prevent any exposure of the GI tract), there was no difference in mortality rates. This result implied that gill uptake of endrin, in the absence of gut uptake, was equally effective at killing the fish. Given this concurrence for a variety of chemicals, it is possible that the present finding of a transition point near to log K_{ow} 6.0 may have application beyond the three chemicals that were studied.

Given the large differences in k_1 and k_d for the three hydrophobic chemicals we studied, it is only when the C_d:C_w ratio becomes very large that dietary uptake can predominate. Servos *et al*. (1992) suggested that dietary contribution to body burden of polychlorinated dibenzo-p-dioxins (PCDDs) increased when water chemical concentrations declined to extremely low levels. Similarly, previous studies have stressed that low freely dissolved concentrations of highly hydrophobic chemicals (*i.e*., log $K_{ow} > 6$) precluded appreciable gill exposure and uptake (Thomann and Connelly 1984; Muir *et al*. 1985). In addition, 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 occurred primarily through food chain transfer because the water chemical concentration

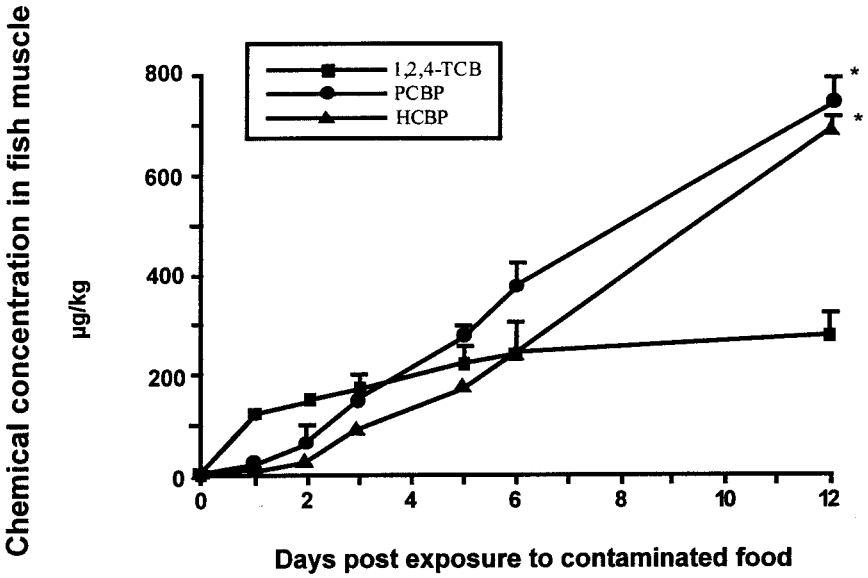

Fig. 3. Measured concentrations of 1,2,4-TCB, PeCB and HCBP in juvenile rainbow trout (*Oncorhynchus mykiss*) muscle samples during a 12-day feeding experiment. Each point is the mean of 10 fish, except for day 12 where n = 4 fish. *denotes a significant difference ($p < 0.05$) between the values for 5 days and 12 days

Table 2. Uptake constants (k_1 and k_d) for juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed separately to water and food containing the three hydrophobic chemicals, 1,2,4-TCB, PeCB, and HCBP

	1,2,4-TCB	PeCB	HCBP
k_1 (L/kg/day)	258	1360	257
k_d (kg/kg/day)	0.0028	0.0070	0.0026
k_1/k_d	93,478	194,285	98,846

was five to seven orders of magnitude lower than that in the diet. Even so, our model predicted that aqueous uptake would still contribute significantly at a log K_{ow} value as high as 7.55. This may be explained by the fact that factors other than extremely low water solubility (which produces a very high C_d:C_w ratio) contribute to this transition.

There are two ways to experimentally assess the relative contributions of aqueous and dietary uptake of hydrophobic chemicals to fish body burden. One is to experimentally vary exposure concentration at the gills and at the GI tract and then measure the resulting changes in body burden. This approach is labor intensive and costly. The alternative is to measure k_1 and k_d and then model the exposure concentrations using these values, as we did here. For such a model and a given set of uptake constants, the relative contributions of the two uptake routes will be determined primarily by the C_d:C_w ratio. This ratio will undoubtedly vary from situation to situation and between fish species. Therefore, the values we used here for C_d:C_w ratio may not apply to all natural situations. Nonetheless, other researchers could use our k_1 and k_d values to make predictions for their situation if they know the C_d:C_w ratio. It is also important to note that because k_1 is inherently much greater than k_d, the initial component of the chemical body burden of fish entering a contaminated water supply for the first time will result largely from aqueous uptake despite a high C_d:C_w ratio.

For the aqueous exposure experiment, measured water chemical concentrations decreased appreciably during the first 12 h of exposure even though there was intermittent chemical dosing and the stock solution was replaced daily. Several factors probably contributed to these losses. Rapid chemical absorption by the fish and adsorption of chemicals to aquarium walls likely predominated. In the case of TCB, volatilization probably contributed to the loss.

In the present study, k_1 values ranged from 257 to 1,360 L/kg · day, and k_d values ranged from 0.0026 to 0.0070 kg/kg · day. A difference of five orders of magnitude between k_1 and k_d values is consistent with previous studies of hydrophobic chemicals. Reported k_1 values range from 10^2 to 10^6 L/kg · day, while k_d values are mostly less than 1.0 kg/kg · day (Lieb and Bills 1974; Macek *et al.* 1979; Bruggeman *et al.* 1981; Skaar *et al.* 1981). For several classes of chlorinated hydrocarbons, k_1 values were between 100 and 10,000 L/kg · day, whereas k_d values were between 0.004 and 0.016 kg/kg · day (Opperhuizen and Sijim 1990; Opperhuizen 1991).

The expectation was that the k_1 for HCBP would be significantly greater than that for 1,2,4-TCB and PeCB. This was not the case. A possible reason for this might be that the measured HCBP water concentration was near to its water solubility limit (0.4 µg/L) for the majority of the experiment and slightly higher than the solubility limit at least for the first hour of exposure. Thus, it is possible that HCBP was not fully dissolved, *i.e.*, the HCBP concentration we measured was greater than the actual dissolved concentration. If this were the case, we would have underestimated k_1. What then follows is that the log K_{ow} for the transition from aqueous to dietary uptake would have been higher than our model predicted. Other researchers have used generator columns (Bruggeman *et al.* 1981; Opperhuizen and Stokkel 1988; Gobas *et al.* 1989) to limit the formation of crystals in water. Even so, Opperhuizen (1991) and Gobas *et al.* (1989) found that some chlorinated chemicals with a log $K_{ow} > 7$ had lower BCF values than chemicals with a lower log K_{ow}.

For the present simulation model, dietary lipid content was assumed to be 2%, a value probably close to a field situation.

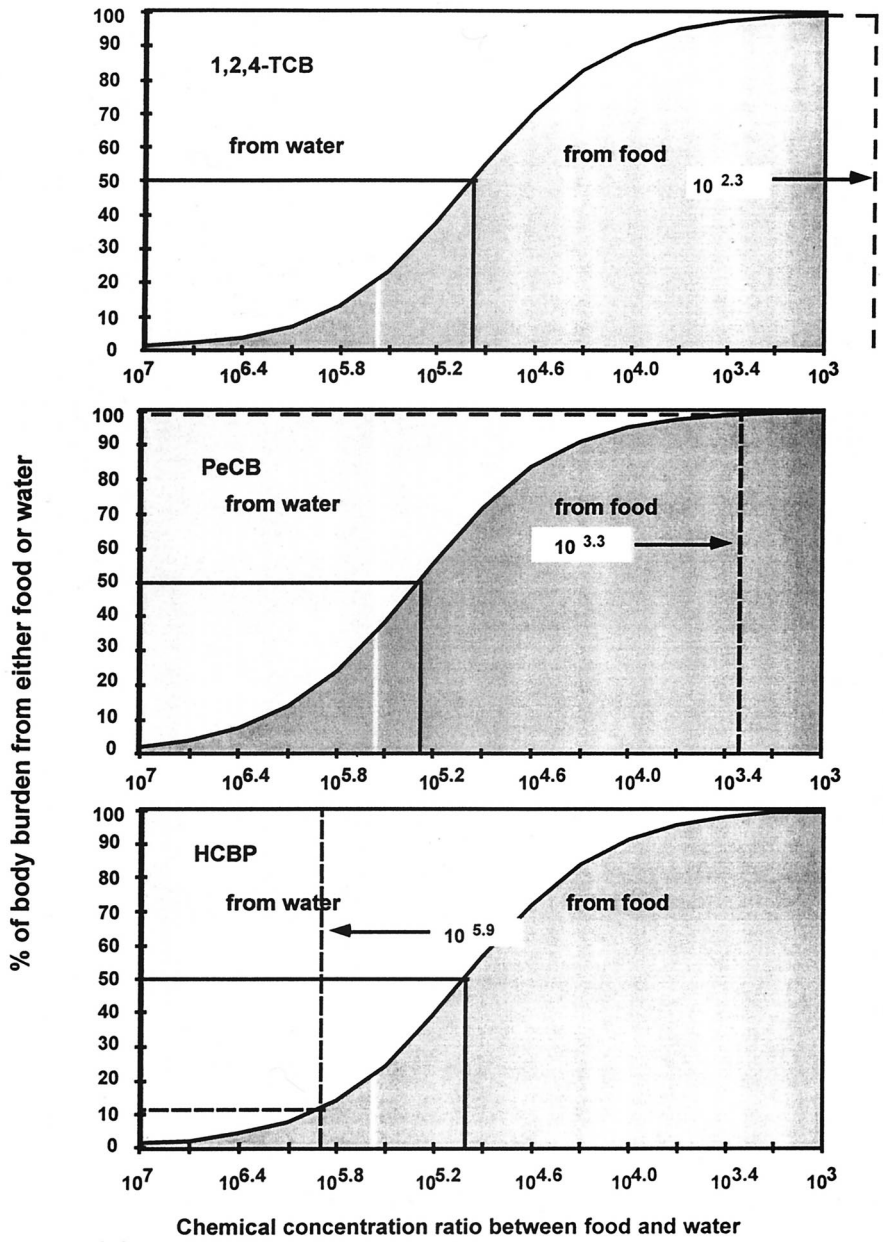

Fig. 4. Model predictions for juvenile rainbow trout (*Oncorhynchus mykiss*) of the percentage of chemical body burden for 1,2,4-TCB, PeCB, and HCBP derived from either the water via the gills or from the food via the gastrointestinal tract uptake. The model was based on measured values for the dietary and aqueous uptake rate constants for these chemicals and simulated simultaneous exposure to a wide range of food:water chemical concentration ratios. The food:water chemical concentration ratio at which food and water each contributed 50% the chemical body burden is indicated. The vertical dashed line represents an environmentally realistic prediction for the food:water chemical concentration ratio of these test chemicals, and from this ratio the relative contributions of aqueous and dietary uptake is indicated for each of the three test chemicals (the horizontal dash line)

However, a dietary lipid content of 14% was used to facilitate chemical dosing while measuring k_d. In theory, differences in food lipid content could affect dietary uptake. However, in practice this does not appear to a large effect. The influence of dietary fat on dietary uptake efficiency was examined for chemicals with a log K_{ow} of 4.51–6.10 and was found to be the same for high (13.5%) and low (<0.2%) fat food (Gobas *et al*. 1993). However, dietary uptake efficiency was 30–50% higher for low fat food with chemicals having a very high log K_{ow} (6.3–8.0). Therefore, our simulation model could have underestimated the dietary contribution to HCBP uptake to a similar degree. If this were the case, then (a) the modeled contribution of aqueous uptake would have been lower than predicted for HCBP, and (b) the transition from aqueous to dietary uptake with increasing K_{ow} would have been more abrupt than predicted.

The body burden of 1,2,4-TCB reached a plateau with both dietary and aqueous exposure experiments. A reasonable explanation for these results is that the uptake and the loss (metabolism and excretion) of 1,2,4-TCB reached a balance after 1 day of exposure. Fast elimination of 1,2,4-TCB through metabolism was suggested as an important factor in other studies, including one of our earlier studies (Qiao and Farrell 1996). In fact, 1,2,4-TCB has a half-life of 1 to 3 days in bluegill sunfish and American flagfish (Barrows *et al*. 1980; Smith *et al*. 1990) and is rapidly metabolized by rats and monkeys (Lingg *et al*. 1982). We did preliminary tests on 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, personal communication). However, its higher toxicity to fish and its lower sensitivity to GC analysis precluded further use.

In summary, we examined the relative importance of aqueous and dietary uptake routes for hydrophobic chemicals by measuring k_1 and k_d, and then modeling uptake as a function of the chemical concentration ratio between food and water. The k_1 values were considerably higher than the k_d values. For the C_d:C_w ratios that we modeled for simultaneous aqueous and dietary exposure, aqueous uptake via the gills predominated at log K_{ow} values up to 5.05. However, dietary uptake via the GI tract predominated at a log K_{ow} value of 7.5, with a small contribution from aqueous uptake.

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