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DIETARY UPTAKE KINETICS OF 2,2′,5,5′-TETRACHLOROBIPHENYL IN RAINBOW TROUT

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ABSTRACT:

The disposition of [UL-^{14}C]2,2′,5,5′-tetrachlorobiphenyl (TCB) in rainbow trout (*Oncorhynchus mykiss*) was studied in acute dietary exposures using TCB-contaminated fathead minnows (*Pimephales promelas*). Trout were sampled at several postfeeding time points and TCB-derived radioactivity was measured in gut contents and selected tissues. Gastric evacuation was exponential with time and was 95% complete within 36 h of feeding. The ratio of activity in upper intestinal tissue to that in blood declined between 6 and 48 h, as did the lumenal contents/tissue ratio. Stomach content lipid declined between 0 and 24 h, while the lipid content of chyme remained relatively constant. These observations are consistent with liquid phase emptying of lipid and TCB to the upper intestine followed by rapid coassimilation. Tissue/blood activity ratios for the stomach, lower intestine, muscle, liver, and kidney were constant and probably represented near equilibrium conditions. The fat/blood activity ratio increased through 96 h, indicating that TCB was redistributing to fat. The lower intestinal tissue/feces activity ratio increased between 6 and 24 h and then declined rapidly. Fecal lipid content also increased between 6 and 24 h, but the amount of this increase was insufficient to explain observed changes in the distribution of TCB-derived activity. A small amount of 3-hydroxy TCB was detected in feces. Generally, however, metabolism had little or no impact on the uptake, distribution or elimination of TCB. Measured assimilation efficiencies exceeded 90% and are the highest ever reported in fish feeding studies with TCB.

Accurate predictions of chemical accumulation by fish are needed for human health and ecological risk assessment. Chemical attributes that contribute to accumulation include environmental persistence, generally reflecting low rates of abiotic and biotic transformation, and hydrophobicity, often characterized as the log of a compound's octanol/water partition coefficient (log K_{OW}[1]). It is generally accepted that the diet is the primary route by which fish accumulate compounds with log K_{OW} values greater than 5 (Bruggeman et al., 1984). Compounds with log K_{OW} values greater than 6 have been shown to biomagnify in fish, resulting in whole-body chemical concentrations greater than those in prey items (after correcting for differences in lipid content) and higher than levels predicted from an equilibrium chemical distribution between the fish and water (Oliver and Niimi, 1988; Russell et al., 1999). Dietary uptake is thought to be facilitated by processes that accompany digestion, including the absorption of dietary lipid and reductions in meal volume. According to the "digestion hypothesis", these processes create an activity gradient within the GI tract that strongly favors chemical diffusion into fish (Gobas et al., 1993b). Recent work with several fish species confirms that chemical activity in digesta (expressed in fugacity units) can exceed that of an ingested meal (Gobas et al., 1993b, 1999).

The efficiency with which fish assimilate chemicals from dietary sources is of special interest because this parameter is critical for models of trophic transfer in aquatic food webs (Thomann, 1989; Gobas, 1993). Several studies have shown that assimilation efficiency declines with chemical log K_{OW} at values greater than 7 (Gobas et al., 1988; Opperhuizen and Sijm, 1990). This observation led Gobas et al. (1988) to propose a two-phase "resistance" model for dietary uptake. A resistance term was subsequently incorporated into mechanistic models of digestion and techniques were devised to estimate its value from experimental data (Gobas et al., 1988, 1993a,b). In contrast, Burreau et al. (1997) reported that there was no decline in assimilation efficiency with log K_{OW}. This latter study is notable for its use of live prey items to provide a "naturally contaminated" contaminant source.

Techniques used to estimate assimilation efficiency have varied. In several studies, assimilation efficiencies have been estimated from whole-body chemical residues after feeding fish contaminated food for an extended period of time. Assimilation efficiencies calculated in this manner represent the net effect of uptake and elimination, including biotransformation, and are generally termed "net" values. Kinetic approaches may also be applied to this type of data yielding estimates of "true" assimilation at early time points (generally denoted ϵ); that is, the amount of compound actually taken up from the diet (Bruggeman et al., 1981, 1984). In other studies, assimilation efficiencies have been estimated after a one-time dietary exposure by backward extrap-

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[1] Abbreviations used are: log K_{OW}, log of a compound's octanol/water partition coefficient; GI, gastrointestinal; TCB, 2,2′,5,5′-tetrachlorobiphenyl; LSC, liquid scintillation counting; GC/MS, gas chromatography/mass spectrometry; HCB, hexachlorobenzene.

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olation of depuration data (Niimi and Oliver, 1983). Values determined in this manner can be related directly to estimates of ϵ obtained in longer-term dosing studies. Most of the studies conducted to date have used formulated diets spiked with the compound of interest. Chemical uptake from these diets may differ from that which occurs with live prey items due to differences in chemical association with the food matrix and the time required to digest and pass the meal.

Despite this progress, current understanding of the biochemical and physiological factors that control dietary uptake of ingested chemicals by fish remains limited. In particular, detailed kinetic studies that would permit an evaluation of competing theories of dietary uptake are essentially absent. The purpose of the present study, therefore, was to describe the disposition of [UL-^{14}C]2,2′,5,5′-tetrachlorobiphenyl (TCB) in rainbow trout fed a meal of TCB-contaminated fathead minnows. The feeding rate and TCB concentrations in minnows were designed to produce an "environmentally relevant" exposure. The gut was then partitioned to determine the chemical time course in tissues and contents of the stomach, upper and lower intestine, as well as other tissues and organs. These data are interpreted in the context of current theories of chemical uptake by fish and mammals from dietary sources.

Materials and Methods

Chemicals. TCB (>95% pure, 13.3 mCi/mmol in toluene) was obtained from Sigma (St. Louis, MO). The toluene was evaporated off and the TCB was diluted to 1.1 mg/ml (50 μCi/ml) in acetone to facilitate its addition directly to water. Unlabeled 2,2′,5,5′-tetrachlorobiphenyl, 3-methylsulfonyl-2,4,5,5′-tetrachlorobiphenyl, and 3- and 4-methylsulfonyl-2,2′,5,5′-tetrachlorobiphenyl were purchased from Cambridge Isotope Laboratories (Andover, MA). 4-Hydroxy-2,2′,5-trichlorobiphenyl was obtained from Ultra Scientific (North Kingstown, RI). Pesticide grade organic solvents were purchased from Fisher Scientific (Fair Lawn, NJ).

Animals. Rainbow trout (*Oncorhynchus mykiss*) weighing 60 to 100 g were purchased from Seven Pines Trout Hatchery (Lewis, WI). The fish were treated upon arrival with a dilute formalin solution (0.016% v/v) to control ectoparasites. A week later they were lightly anesthetized with tricaine methanesulfonate (MS-222, 100 mg/l; Argent Laboratories, Redmond, WA) and gavaged with praziquantel (Droncit, 50 mg/kg of body weight in a gelatin capsule; Bayer, Shawnee Mission, KS) to eliminate tapeworms (Post, 1987). The fish were held in sand-filtered Lake Superior water and fed a commercial trout chow (Silver Cup; Murray and Sons, Murray, UT) at a rate of 2% of body weight, three times weekly. Mean water chemistry characteristics were (range in parentheses) as follows: total hardness, 45 mg/l as $CaCO_3$ (45–46); alkalinity, 42 mg/l as $CaCO_3$ (41–44); and pH 7.7 (7.6–7.8). Water temperature was maintained at 11 ± 1°C and the lighting was adjusted to mimic the natural photoperiod.

Fathead minnows (*Pimephales promelas*) were obtained as 30-day-old animals from the Mid-Continent Ecology Division-Duluth laboratory hatchery. The fish were held at 25 ± 1°C and fed brine shrimp twice daily until they grew to the desired size. Experiments were conducted using minnows that were able to pass through a 1/4-inch nylon mesh sieve, but not through a 3/16-inch nylon mesh sieve. The average weight of these fish was approximately 0.5 g.

Fathead Minnow Dosing. Fathead minnows were dosed with TCB under static exposure conditions. TCB stock solution was added to a covered aquarium containing 18 liters of Lake Superior water and allowed to distribute for 2 h. Ninety minnows were then transferred to the tank and exposed for 96 h. Oxygen was provided throughout this period by gentle aeration. At the end of the exposure 5 to 10 minnows were analyzed individually by wet tissue oxidation and liquid scintillation counting (see "Tissue Sampling and Analyses") to determine TCB-derived radioactivity (TCB and metabolites). Each tank was then supplied with clean flowing water (150 ml/min) for the remainder of the holding period.

A subsample of 10 to 15 minnows was analyzed for total activity each time that they were removed and fed to trout. Altogether, 12 batches of minnows were exposed to a "high" dose of TCB (120 μl of spiking solution) resulting in a mean concentration (±S.D.) of 1663 (±230) ng/g of minnow, based on the assumption that all activity was present as the parent compound. Three batches of fathead minnows were exposed to a "low" dose of TCB (17 μl of spiking solution) giving a mean concentration of 244 (±44) ng/g of minnow.

Exposure System. Feeding studies were conducted using trout held individually in 33-liter glass aquaria. The mean (±S.D.) weight of all fish tested was 103.8 (±11.5) g. Each aquarium was supplied with 250 ml/min of Lake Superior water at 11 ± 1°C. Dissolved oxygen content was maintained at 85 to 100% of saturation and the photoperiod was controlled to mimic seasonal values.

Study Design. *Gastric evacuation and uptake of dietary lipid.* Trout were converted to a diet of live fathead minnows following their transfer to the aquaria. The feeding rate was 4% of body weight once every 48 h. Fish were eliminated as test subjects if, during this conversion period, they did not consume every meal within 5 min. Gastric evacuation was assessed by killing fish with an overdose of MS-222 at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, and 36 h after they had consumed their third prey meal. Stomach contents were dried at 60°C for 48 h and weighed to the nearest 0.001 g. Gastric evacuation as a percentage of the mass of the last meal fed was calculated on both a dry and wet weight basis. Similar methods were used to investigate temporal changes in the wet and dry weight of chyme (upper intestinal contents) and feces (lower intestinal contents). The lipid content of material in the stomach, upper intestine, and lower intestine was determined using the method of Bligh and Dyer (1959), adapted for use with small samples (0.2-g minimum). Material contained in the stomach after 24 h, and in the intestinal tract before 6 h, was insufficient for lipid analysis.

Disposition of TCB in rainbow trout following a single dietary exposure. Studies designed to characterize dietary uptake of TCB by trout were initiated in the same manner as the gastric evacuation studies. After three uncontaminated meals, however, the fish were fed a fourth meal consisting of the dosed minnows. Because the number of available aquaria was limited, it was not possible to collect data for all time points in a single experiment. Individual experiments were therefore designed to collect data at three sampling times (four replicates each). Trout that consumed the high TCB dose were killed at 6, 12, 24, 48, and 96 h ($N = 8$ at each sampling time). Those held for the 96-h sampling period were fed a meal of uncontaminated minnows 48 h after dosing. Experiments conducted at the low TCB dose were limited to the 12, 24, and 48-h sampling times ($N = 4$ fish).

Tissue Sampling and Analyses. Fish were processed to obtain samples of blood, bile, liver, kidney, muscle, fat, stomach, stomach contents, upper intestine (minus the pyloric caeca), upper intestinal contents, lower intestine, and lower intestinal contents. Blood was collected from the caudal vein using a heparinized syringe. Muscle tissue was obtained from above the lateral line, anterior to the dorsal fin, taking care to trim away subcutaneous fat. Adipose fat was obtained from abdominal fat deposits. The liver and kidney were removed in their entirety.

The GI tract was cut into four segments corresponding to the stomach, pyloric caeca (including a short length of upper intestine), the remaining upper intestine, and the lower intestine. The ends of each segment were clamped with hemostats before sectioning to minimize the loss of contents. When present, adipose fat was removed from the tissues, along with major blood vessels. The contents of the stomach, upper intestine (chyme) and lower intestine (feces) were collected by gentle stripping. These segments were then cut open and rinsed with a 0.9% (w/w) NaCl solution to remove any loose material from lumenal surfaces. Initially, an effort was made to strip the contents of the pyloric caeca; however, the small size of these structures made it impossible to collect this material. All gut content samples were homogenized by hand prior to subsampling.

TCB-derived radioactivity in all samples except bile was measured by wet tissue oxidation followed by liquid scintillation counting (LSC). An effort was made to obtain enough sample from each fish for three replicate determinations. In several instances, however, only one or two analyses could be performed due to insufficient sample mass (e.g., stomach contents 48 h after feeding) or low activity (e.g., lower intestinal contents 6 h after feeding). Samples were air dried overnight before being combusted in a Packard 307 Tri-Carb Oxidizer (Packard Instruments, Meriden, CT). Acceptable results were obtained with the following sample sizes: blood, ~0.5 g; muscle, liver, kidney, stomach, and intestinal tissues and contents, ~1.0 g. Fat samples (~0.3 g) were processed after adding 0.2 g of cellulose powder to slow

combustion. Radioactivity as $^{14}CO_2$ was trapped using Carbosorb (Packard Instruments) and counted using a Packard 2550 TR/LL scintillation counter. Bile samples (~0.1 ml) were added directly to 20 ml of scintillation cocktail (UltimaGold; Packard Instruments).

TCB Metabolism. *Hydroxylation by rainbow trout and rat liver microsomes.* Hepatic microsomes were obtained from male Sprague-Dawley rats weighing 150 to 200 g (Preston and Allen, 1980), and from a single male trout weighting 860 g (Dady et al., 1991). Each microsomal preparation was characterized to determine total protein and P450 content using methods described elsewhere (Kolanczyk et al., 1999).

Microsomal incubations were conducted in 1.5-ml microcentrifuge tubes containing 500 μl of trout or rat liver microsomes and $MgCl_2$ (100 μl, 100 mM), KCl (100 μl, 150 mM), EDTA (100 μl, 10 mM), and NADPH (100 μl, 50 mM), each in Tris-HCl buffer (20 mM, pH 7.0). Incubations were conducted for 1 h at 25°C (trout) or 30°C (rat) and were initiated by the addition of 250 ng of TCB in 50 μl of ethanol. Samples were extracted twice with 550 μl of ethyl acetate. The extracts were combined, washed with 1 volume of water, and taken to dryness under N_2. Reaction products were then redissolved in hexane for analysis by gas chromatography/mass spectrometry (GC/MS). A subset of samples was methylated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine using methods given by the manufacturer (Aldrich Chemical, Milwaukee, WI).

GC/MS analysis of fathead minnows, trout tissues, and digesta. Selected samples were analyzed by GC/MS for hydroxy-TCB metabolites. The focus of this effort was on the 24-h postfeeding time point. Trout were converted to a diet of fathead minnows as previously described and fed a single dosed meal. Fish were then processed to obtain samples of liver, chyme, and feces. A pooled sample of TCB-dosed fathead minnows was also collected and analyzed.

Extraction and analyte isolation procedures were based on those given by Hegstad et al. (1999). Samples weighing 1.0 to 2.0 g were homogenized with a small volume of water, transferred to a 100-ml round bottom flask, and refluxed with 80 ml of methanol for 2 h. Solids were removed by centrifugation at 1000*g* for 10 min, and 10% of the supernatant was taken for LSC. The remaining sample volume was concentrated to about 2 ml under N_2. Each sample was diluted with 2 volumes of water and extracted twice with 1 volume of hexane to partition unmetabolized TCB and hydroxy-TCB. Ten percent of each hexane extract was removed for LSC.

Methanol/water extracts of feces that remained after the hexane partitioning step were subjected to three digestive procedures: glucuronidase, sulfatase, or formic acid hydrolysis. Samples for glucuronide hydrolysis were diluted with phosphate buffer (20 mM, pH 6.5) and incubated with enzyme (Sigma) for 1.5 h at 37°C. Samples for sulfate hydrolysis were diluted with Tris-HCl buffer (20 mM, pH 7.4) and incubated with enzyme (Sigma) and the glucuronidase inhibitor D-saccharic acid-1,4-lactone (Sigma) for 1.5 h at 37°C. Additional samples were diluted with 500 μl of 0.5% formic acid and incubated for 1.5 h at 37°C. Methanol/water samples were then extracted again with 3 volumes of hexane. The hexane extracts for each digestion procedure were combined and 10% was removed for LSC. The remaining hexane was reduced to 0.1 ml under N_2 and analyzed by GC/MS.

Hexane extracts were exchanged into toluene prior to GC/MS analysis. Extracts from microsomal preparations and feces were analyzed on a Finnigan-MAT 95 high resolution mass spectrometer (San Jose, CA) interfaced to a Varian-Areograph 3400 gas chromatograph (Walnut Creek, CA) and fitted with a DB5 30-m fused silica column (J&W Scientific, Folsom, CA). The column was programmed from 150 to 300°C at 7°C/min. Extracts from fathead minnows, liver, and chyme were analyzed on a Hewlett Packard MSD equipped with a DB5 30-m fused silica column (J&W Scientific). The column was programmed from 70 to 310°C at 7.5°C/min.

Data Analysis and Presentation. Kinetic data from individual experiments within a given dose level (high or low) were compared by normalizing for differences in TCB concentration among batches of minnows. This was accomplished by multiplying the TCB concentration in each tissue by the ratio of the TCB concentration in minnows associated with that value to the weighted mean concentration for all minnows at that dosing level.

Net dietary assimilation efficiency was calculated as the mass of TCB in trout tissues divided by that in the diet. Tissues collected during the kinetic studies were used to calculate assimilation efficiency in those instances in which the carcasses had been frozen and saved (low-dose exposures only). The frozen carcasses were partially thawed, cut into 1-cm^3 pieces, and coarsely blended with an equal volume of 0.9% (w/w) NaCl solution. This material was then blended to homogeneity (~2 min) using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY) equipped with a PTA 20TS generator. Additional exposures were conducted to obtain assimilation efficiency estimates at the high-dose level. Trout were killed 12, 24, 48, and 96 h after dosing. The GI tract was cut into four segments as before and stripped of its contents. GI tract tissues were then combined with those of the carcass before processing.

A TCB mass-balance was also calculated by adding the TCB content of each trout to the amount of TCB contained in its gut contents, and dividing this value by the mass of TCB ingested. The amount of TCB in the diet was calculated as the product of meal size and the mean TCB concentration in the corresponding batch of minnows.

Statistical Analyses. A Student's *t* test (unpaired, $p > 0.05$) was used to evaluate dose-related differences in TCB concentration at each sampling time in the kinetic study.

Results

Gastric Evacuation and Uptake of Dietary Lipid. The pattern of food evacuation from the stomach, expressed as a decline in the percentage of ingested dry weight, is shown in Fig. 1. A similar pattern was obtained between 2 and 18 h when stomach contents data were expressed a wet weight basis (data not shown), due to the fact that the water content of these samples remained close to that of the ingested meal (approximately 80%). The water content of later samples increased to 96% at 36 h. The lag time before the start of gastric evacuation could not be accurately determined, but was less than 2 h. Approximately 60% of the meal passed to the upper intestine within 12 h of feeding, and greater than 95% was evacuated by 36 h. The fitted exponential equation describing these data was $Y = 113.9 \times 0.920^X$, where X represents time (h) and Y is the percentage of the ingested meal remaining in the stomach (Persson, 1986).

Changes in extractable lipid in contents of the stomach, and upper and lower intestine are shown in Fig. 2. The amount of lipid in stomach contents declined in a nearly linear manner between 0 and 24 h. In contrast, the lipid content of chyme and feces changed very little over time. The highest value in chyme was observed 12 h after feeding, while in feces the lipid content was greatest at 24 h. A rough

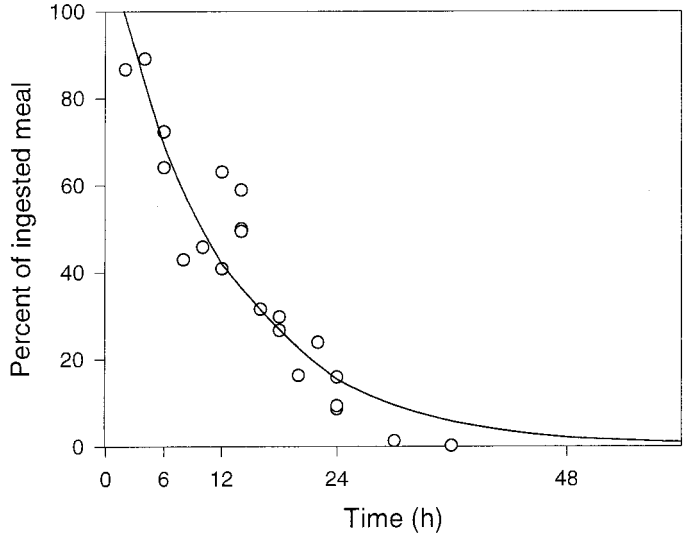

FIG. 1. *Gastric evacuation in rainbow trout following a meal of fathead minnows.*

Gastric evacuation on a dry weight basis was calculated as a percentage of ingested meal size. Each point represents an individual observation. The fitted exponential model: $Y = 113.9 \times 0.920^X$ ($r^2 = 0.859$), is shown as a solid line.

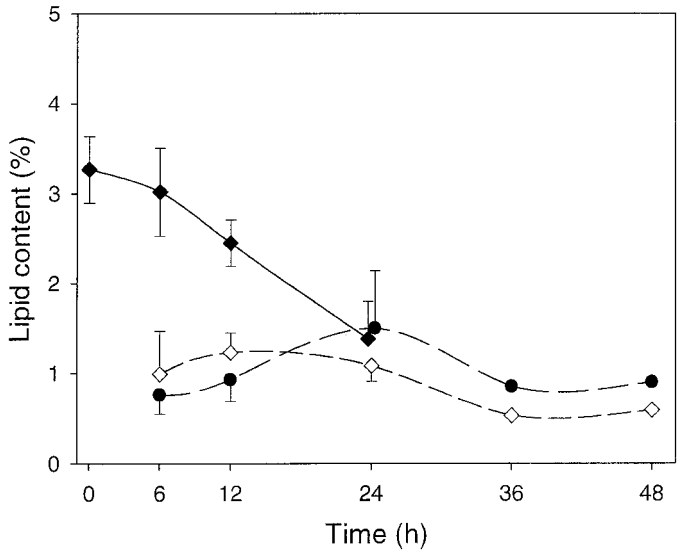

FIG. 2. *Dietary uptake of lipid in rainbow trout fed a meal of fathead minnows.*

The lipid content of material in the stomach at 0 h was set equal to that of fathead minnows prior to feeding (mean ± S.D., $N = 6$). Values given for contents of the stomach (♦), upper intestine (◇), and lower (●) intestine at 6, 12, and 24 h are sample means ± S.D., $N = 5$. Samples from three fish were combined to obtain values reported at 36 and 48 h.

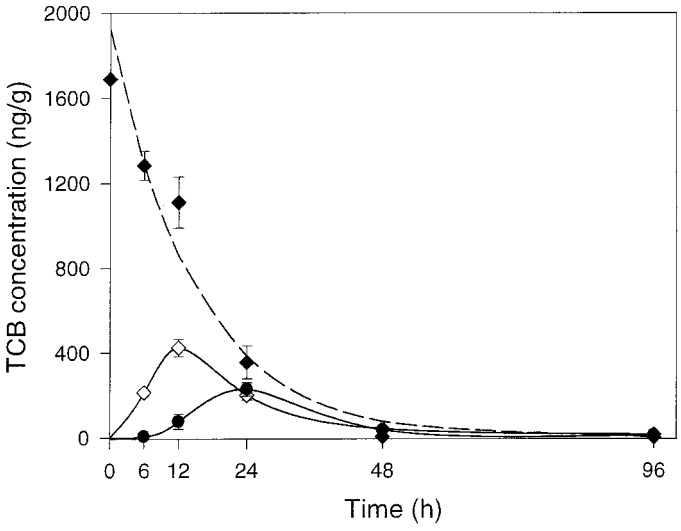

FIG. 3. *TCB kinetics in contents of the stomach, upper intestine, and lower intestine.*

Data were obtained from the high-dose exposure group. Values given for the stomach (♦), upper intestine (◇), and lower intestine (●) are sample means ± S.E., $N = 8$. The fitted exponential model: $Y = 1922.2 \times 0.935^X$ ($r^2 = 0.934$), is shown as a dashed line.

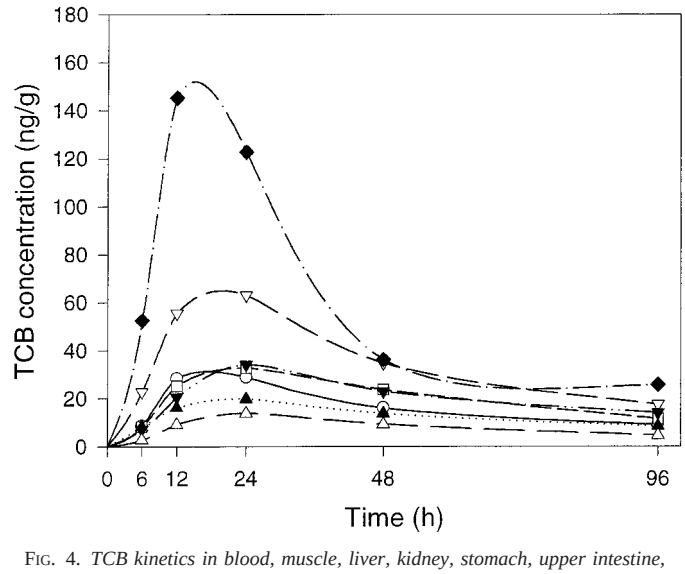

FIG. 4. *TCB kinetics in blood, muscle, liver, kidney, stomach, upper intestine, and lower intestine.*

Data were obtained from the high-dose exposure group. Values given for arterial blood (○), muscle (△), liver (▽), kidney (□), stomach (▲), upper intestine (♦), and lower intestine (▼) are sample means, $N = 8$.

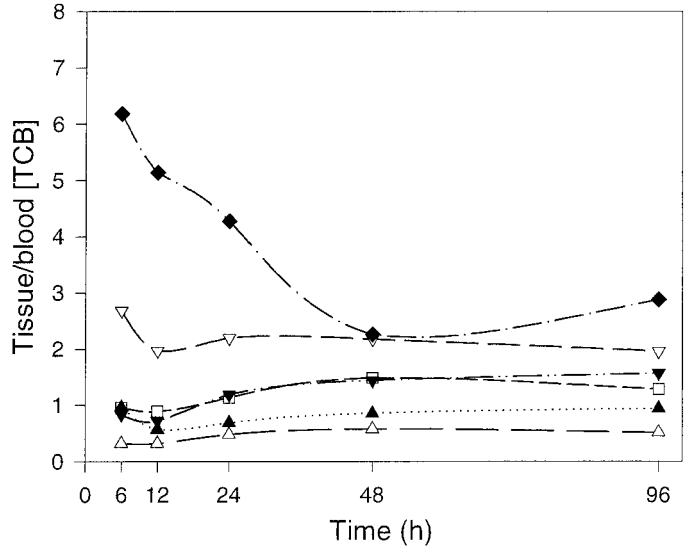

FIG. 5. *Tissue/blood TCB concentration ratios in lean tissues from the high-dose exposure group.*

Ratios for muscle (△), liver (▽), kidney (□), stomach (▲), upper intestine (♦), and lower intestine (▼) were calculated from mean concentrations in tissues and arterial blood.

estimate of lipid absorption efficiency was obtained using an average value for the total lipid content of feces and an estimate of fecal egestion as a fraction of ingested meal volume. In studies with pelleted diets, fecal egestion in rainbow trout averaged 30% of feeding rate when expressed on a dry weight basis (Atkinson et al., 1984). Because the water content of digesta in all gut segments changed very little over time, this same value was used in the present study to approximate fecal egestion on a wet weight basis. Adopting this value and an average lipid content in feces of 0.9%, the calculated lipid absorption efficiency was 91%.

Disposition of TCB. *TCB kinetics in tissues and gastrointestinal contents.* Kinetic studies were initiated by feeding trout a single meal of TCB-contaminated minnows. Concentration time course data for lumenal contents of the stomach, and upper intestine and lower intestine are shown in Fig. 3. All residue values are expressed on a wet weight basis and were calculated based on the assumption that radioactivity was present as unmetabolized TCB. In fish exposed to the high dose of TCB, the concentration of TCB in stomach contents declined from approximately 1660 ng/g immediately after feeding (nominal, based on measured concentrations in minnows) to 5 ng/g by 48 h. This decline was described by the exponential equation $Y = 1922.2 \times 0.935^X$, where X represents time (h) and Y equals TCB concentration. TCB concentrations in chyme and feces peaked at 12 and 24 h, respectively, at levels corresponding to 25 and 14% of the average TCB concentration in minnows. When multiplied by the mass of digesta present in each gut segment, these peak concentrations

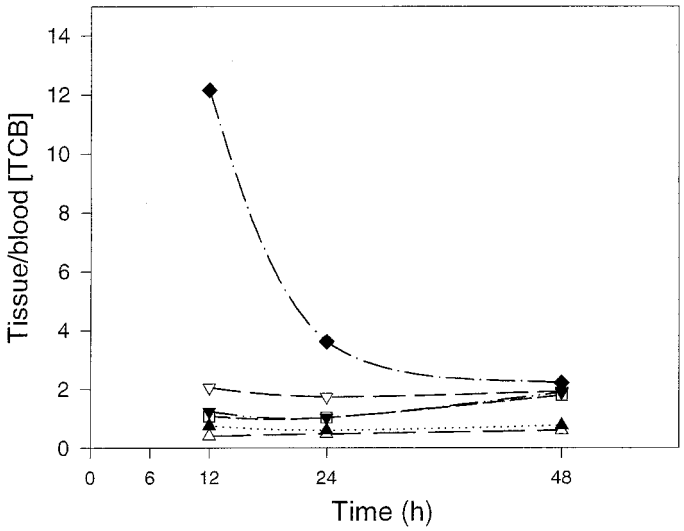

FIG. 6. *Tissue/blood TCB concentration ratios in lean tissues from the low-dose exposure group.*

Ratios for muscle (△), liver (▽), kidney (□), stomach (▲), upper intestine (♦), and lower intestine (▼) were calculated from mean concentrations in tissues and arterial blood.

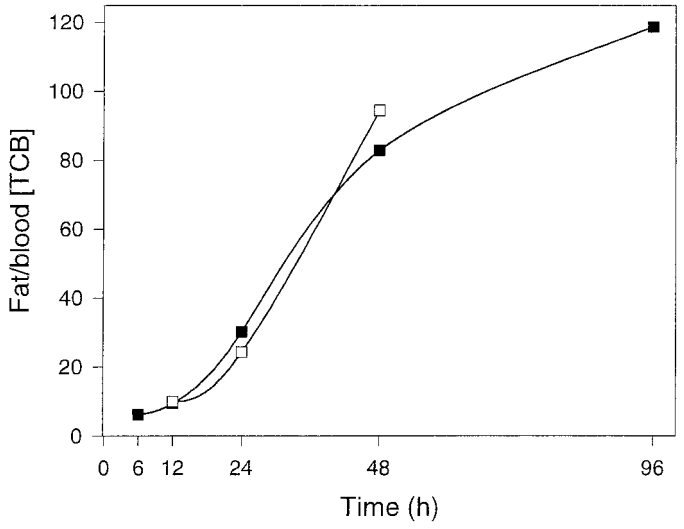

FIG. 7. *Fat/blood TCB concentration ratios from the high- and low-dose exposure groups.*

Ratios for the high- (■) and low (□)-dose exposures were calculated from mean concentrations in fat and arterial blood.

represented 2.2 and 0.8%, respectively, of the total mass of TCB ingested.

Tissue/blood concentration ratios. Concentrations of TCB peaked between 12 and 24 h in blood, and at 24 h in muscle, kidney, liver, stomach, and lower intestine (Fig. 4). In contrast, the TCB concentration in upper intestinal tissues peaked at around 12 h, coincident with the maximum concentration in chyme. A correspondence between blood and lean tissue TCB kinetics became apparent when the data were expressed as tissue/blood concentration ratios (Figs. 5 and 6). Ratios for all lean tissues except the upper intestine ranged from 0.3 to 3.0 and did not change appreciably with time. In the high-dose study group, the upper intestine/blood ratio declined from 6.2 at 6 h to 2.3 at 48 h and then stabilized at a value between 2.0 and 3.0. In the low-dose group, the upper intestine/blood ratio declined from 12.0 at 12 h to 2.2 at 48 h.

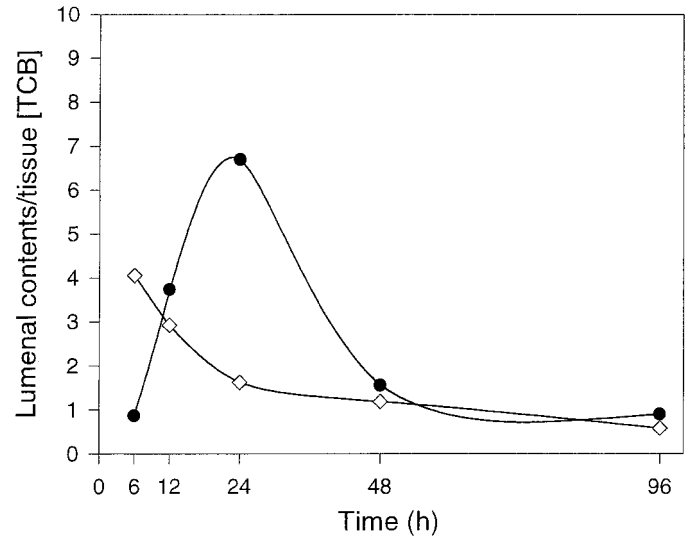

FIG. 8. *Lumenal contents/tissue TCB concentration ratios in the upper and lower intestine.*

Ratios for the upper intestine (◇) and lower intestine (●) were calculated from mean concentrations in the high-dose exposure group.

The TCB time course in adipose fat differed from that of all other tissues. In the high-dose group, TCB concentrations peaked at about 1300 ng/g, 48 h after feeding (data not shown). Concentrations of TCB in fat then declined slowly to about 1100 ng/g at 96 h, but because the rate of this decline was slower than that which occurred in blood, the fat/blood concentration ratio continued to increase (Fig. 7). A similar pattern was observed in the low-dose study group.

Lumenal contents/tissue concentration ratios. TCB concentrations in the upper and lower intestinal contents are expressed in Fig. 8 as lumenal contents/tissue ratios. The ratio for the upper intestine decreased from 4.1 at 6 h to 1.7 at 24 h and then slowly declined to a value less than 1.0. The ratio for the lower intestine increased sharply between 6 and 24 h, attaining a maximum value of nearly 7.0. This ratio then decreased just as rapidly before stabilizing at a value close to 1.0 between 48 and 96 h.

Biliary elimination of TCB. The gall bladders of fish sampled at 6 and 12 h were void of bile, presumably because fish were actively digesting the meal. Small amounts of bile were collected from several fish at 24 h. These samples were pooled to yield a total volume of about 0.5 ml. Based on total activity, the concentration of TCB in this sample was 40.6 ng/g (assuming a specific gravity of 1.0), or about two-thirds of that measured in liver at 24 h. Because of its small size, however, the mass of TCB present in this sample was less than 0.1% of the total amount ingested (for the fish from which bile was obtained). Unfortunately, the small amount of bile available precluded analysis by GC/MS.

Dose dependence. TCB concentrations exhibited dose dependence in all tissues except the upper intestine. This result is illustrated in Fig. 9, using muscle and blood as representative tissues. Comparisons between the two dose levels were made by multiplying TCB concentrations in tissues from the low-dose group by the high/low concentration ratio in fathead minnows (7.53). When adjusted in this manner, TCB concentrations in upper intestinal tissues from the low-dose group were significantly higher at 12 h than concentrations from the high-dose group (Fig. 10). Adjusted TCB concentrations in livers from the low-dose group were consistently lower than concentrations measured in high-dose animals, but because of variability around sample means, these differences were not statistically significant.

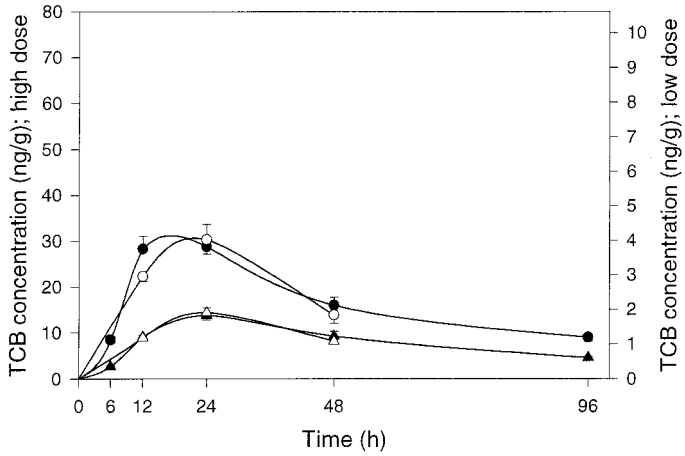

FIG. 9. *Dose dependence of TCB kinetics in muscle and blood.*

TCB concentrations in muscle (△) and arterial blood (○) are shown using solid (high dose) and open (low dose) symbols. Each point represents the mean ± S.E., $N = 8$ (high dose) or 4 (low dose). The low-dose scale was calculated by dividing the high-dose scale by the high/low dosing ratio of 7.53.

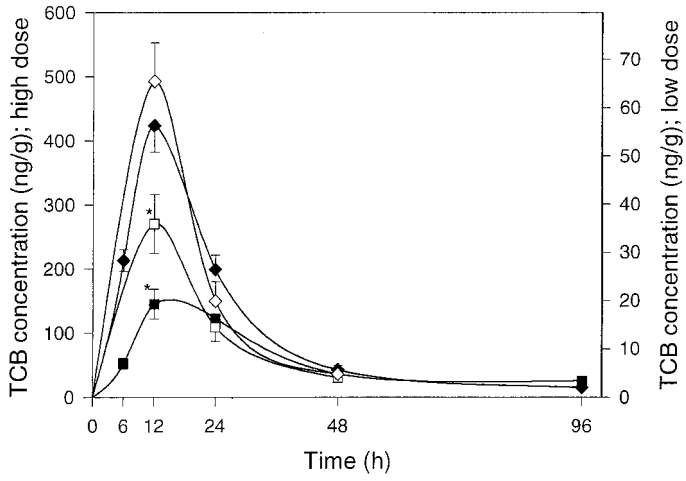

FIG. 10. *Dose dependence of TCB kinetics in upper intestinal contents and tissues.*

TCB concentrations in upper intestinal contents (△) and tissues (□) are shown using solid (high dose) and open (low dose) symbols. Each point represents the mean ± S.E., $N = 8$ (high dose) or 4 (low dose). The low-dose scale was calculated by dividing the high-dose scale by the high/low dosing ratio of 7.53. Significant differences among dose levels are denoted with an asterisk ($p < 0.05$).

TCB Metabolism. Microsomes isolated from rat livers possessed the following characteristics: protein content, 7.25 mg/ml of microsomes; P450 content, 1.97 nmol/mg of protein. The characteristics of trout liver microsomes were protein content, 6.56 mg/ml of microsomes; P450 content, 0.52 nmol/mg of protein. Incubations with rat liver microsomes were initially conducted at 37°C. At this temperature, however, a substantial amount (>50% at 30 min) of TCB was found to undergo reductive dechlorination, as well as hydroxylation. Reducing the incubation temperature to 30°C resulted in a shift in the metabolite profile from trichloro products to tetrachloro material. TCB, hydroxylated TCB, and the methylated derivative eluted at 11.2 and 12.1 min, respectively, for both trout and rat liver microsomes, indicating that only one hydroxylated product had been formed (Fig. 11). This product was identified as 3-hydroxy-TCB based on the electron ionization mass spectrum of the methylated derivative (Tulp et al., 1977). Rat liver microsomes converted approximately 35% of TCB to 3-hydroxy-TCB after a 1-h incubation at 30°C. Less than 1% of TCB was metabolized by trout liver microsomes after a 1-h incubation at 25°C.

Initial efforts to measure TCB metabolites in tissues and digesta were based on techniques given by Bergman et al. (1992). Briefly, this method involves sample extraction with hexane/acetone, fractionation on silica gel, and analysis by GC/MS. Using this procedure, the threshold for detection of 3-hydroxy-TCB was about 50 ng/g, which is greater than TCB concentrations achieved in blood and muscle of high-dose animals. A switch was then made to the methanol extraction method of Hegstad et al. (1999), which does not involve a silica gel fractionation step. The internal standards 4-hydroxy-2,2′,5-trichlorobiphenyl and 3-methylsulfonyl-2,4,5,5′-tetrachlorobiphenyl were easily detected when spiked into control samples of tissue, bile, chyme, and feces. 3- and 4-Methylsulfonyl-2,2′,5,5′-TCB and 3-hydroxy-TCB were also readily detected when spiked into control samples. The approximate threshold for detection of 3-hydroxy-TCB in samples extracted by methanol was 1.0 ng/g.

Methanol extracted essentially 100% of the radioactivity in samples from TCB-dosed animals compared with results obtained by sample oxidation and LSC. A pair of hexane extractions after the addition of water removed 91 to 94% of these counts, depending on sample type. This result suggested that some of the radioactivity extracted by methanol was either too polar to partition into the nonpolar solvent or was bound to dissolved organic material. A small amount of 3-hydroxy-TCB was detected in hexane following the extraction of feces by methanol (Fig. 12). An evaluation of relative peak areas suggested that the concentration of 3-hydroxy-TCB in feces was less than 1% that of TCB. The only analyte found in hexane extracts of other samples was unmetabolized TCB.

About one-third of the radioactivity remaining in methanol/water extracts of feces after the first two hexane extraction steps could be removed by additional (three) hexane extractions. Treatment of the methanol/water fraction with glucuronidase or formic acid increased the amount of hexane-extractable radioactivity to 50 and 70%, respectively, of the total remaining counts. This suggested that a small amount of radioactivity in the original sample was present as the glucuronide conjugate of 3-hydroxy-TCB. However, 3-hydroxy-TCB was not detected by GC/MS following treatment with glucuronidase or formic acid. Sulfatase hydrolysis did not increase the amount of hexane-extractable radioactivity in the methanol/water fractions and GC/MS was not performed on hexane extracts of these samples.

TCB Assimilation and Mass-Balance. Net assimilation efficiencies are illustrated in Fig. 13. Nearly all of the TCB ingested by fish was incorporated into tissue. Peak assimilation efficiencies of 94.5 and 99.8% were observed at 48 h for high- and low-dose exposures, respectively. At 48 h, only 0.3% of the administered dose was found in contents of the GI tract. Assimilation efficiencies calculated at 96 h were slightly lower than those calculated at 48 h, averaging 92.6 and 94.2% at the high and low doses. Averaged across both dosing levels, the summed mass of TCB in trout tissues and contents of the digestive tract accounted for (mean ± S.D.) 97.7 ± 6.9, 97.4 ± 7.4, 97.4 ± 3.6, and 93.5 ± 4.4% of the TCB mass consumed by these animals at 12, 24, 48, and 96 h, respectively.

Discussion

TCB is a common environmental contaminant of fish and has been used by numerous investigators as a model hydrophobic [log $K_{OW} \approx$ 5.84 (Hawker and Connell, 1988)] organic compound. TCB can be absorbed across fish gills with relatively high efficiency (McKim and Heath, 1983). In studies with goldfish (*Carassius auratus*), however, it was shown to biomagnify during simultaneous food and water exposures, suggesting that the diet is the primary route of uptake

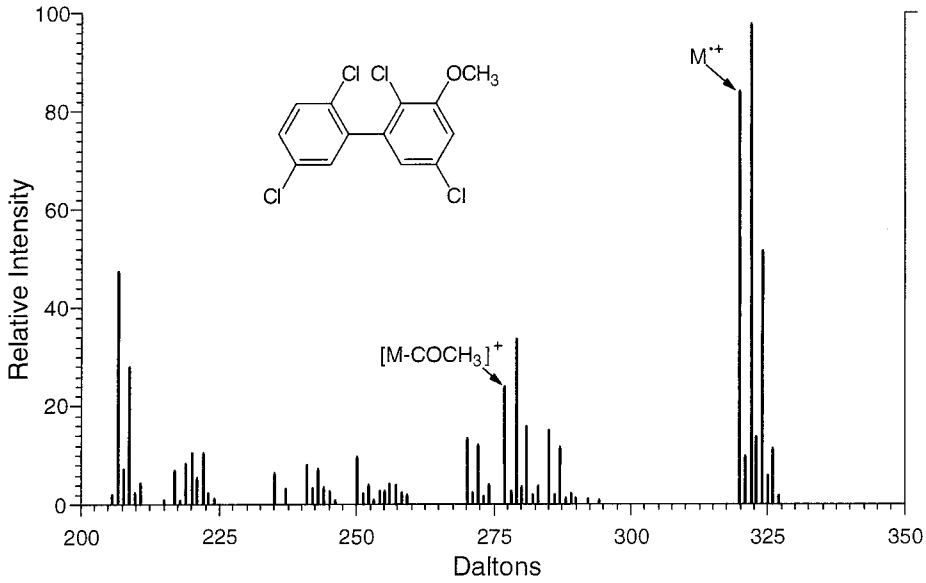

FIG. 11. *Electron ionization mass spectrum of 3-methoxy-TCB.*

A hydroxylated metabolite of TCB was produced by hepatic microsomes from rat and rainbow trout. Hydroxylation at position 3 rather than at position 4 is proposed because of the lack of a chlorine isotope pattern at *m/z* 305 ($[M\text{-}CH_3]^+$).

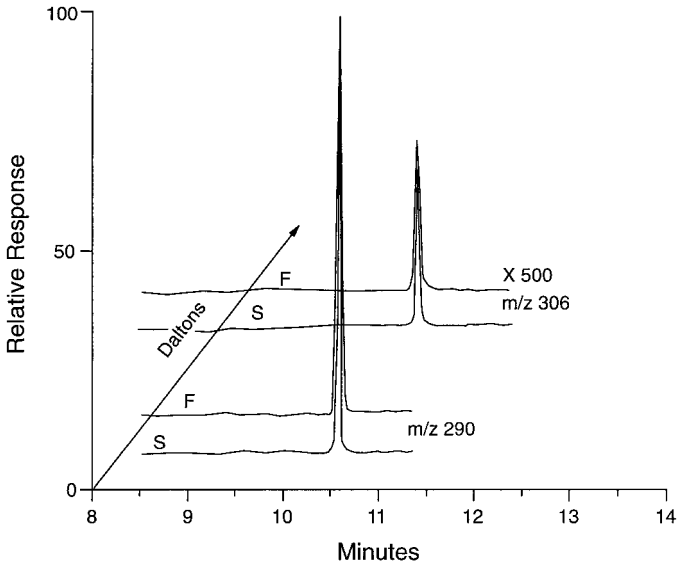

FIG. 12. *GC/MS identification of TCB (290) and 3-hydroxy-TCB (306) in feces (F) of rainbow trout compared with standard (S) material.*

Feces were obtained from the lower intestine of rainbow trout 24 h after feeding. The retention time of each analyte was the same for both samples.

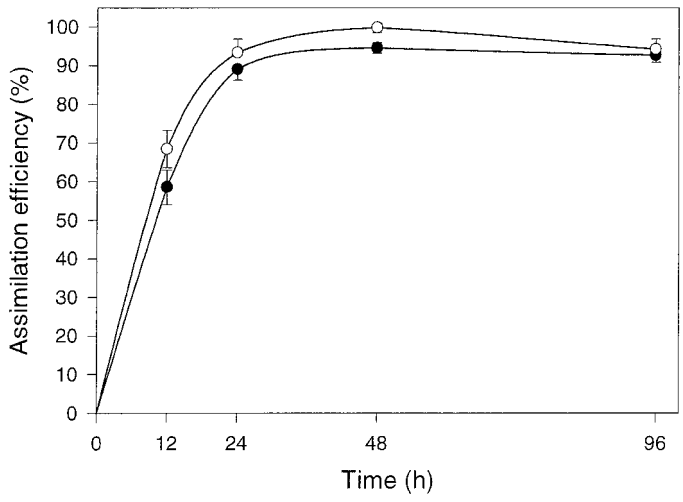

FIG. 13. *Net assimilation of TCB from the diet.*

Net assimilation efficiencies for high- (●) and low (○)-dose exposures were calculated as the ratio of total TCB mass in trout tissues to that which was fed. Each point represents the mean ± S.E., $N = 4$.

(Bruggeman et al., 1981). The goal of the present study was to characterize the dietary uptake kinetics of TCB by rainbow trout following consumption of contaminated prey. The highest dose used in this effort is comparable with total PCB concentrations in forage fishes from the lower Great Lakes (Oliver and Niimi, 1988), while the low dose is similar to summed levels of tetrachlorinated congeners. To our knowledge, this is the first dietary exposure with fish in which the chemical time course is described for discrete gut segments as well as in tissues.

It is well known that the rate and extent of digestion in fish varies with feeding frequency and meal size (Persson, 1986; Ruggerone, 1989; Rand et al., 1994). An effort was made, therefore, to conduct these investigations using environmentally realistic feeding parameters (4% of body weight once every 48 h). Few estimates of daily ration have been obtained for piscivorous salmonids. Juvenile coho salmon (*Oncorhynchus kisutch*) collected from a natural lake system consumed sockeye salmon fry at a rate of 2.1 to 4.4% body weight/day (Ruggerone, 1989). This value is similar to that calculated on an annual basis for adult Lake Ontario chinook salmon (*Oncorhynchus tshawytscha*, 2.2 to 3.1% body weight/day, with variation due to changes in the forage base; Rand et al., 1994).

The gastric evacuation of fathead minnows by trout was well described using an exponential model. This pattern is similar to that seen in previous feeding studies with fish using live prey items (Persson, 1986). TCB concentrations in the stomach contents also declined exponentially indicating that TCB mass was evacuated more rapidly than the mass of the meal. In contrast, the TCB time course in stomach tissue closely followed the kinetics observed in blood. Thus, despite the transient presence of extremely high TCB concentrations

in stomach contents, absorption of TCB by stomach tissues appeared to be minimal.

TCB concentrations in both chyme and upper intestinal tissues increased rapidly between 6 and 12 h, declining thereafter (Fig. 10). Viewed separately, these data suggested that uptake from the upper intestine was greatest at 12 h. However, when the TCB concentration in chyme at 12 h was multiplied by the amount of material present, the total mass of TCB was equal to only 2.2% of the ingested dose. Even after taking into account that portion of the dose that was contained in the pyloric ceca (probably less than 5%), and adding to this the one-third that was still in the stomach (45% of the ingested meal volume $\times$ 65% of the original concentration), it is clear that by 12 h nearly two-thirds of the ingested dose had passed to the upper intestine, and that most of this had already been absorbed.

In an effort to gain insight into the processes responsible for this uptake, data from both intestinal segments were expressed as tissue/blood and lumenal content/tissue TCB concentration ratios. Both ratios for the upper intestine were highest at 6 h and then declined to nearly constant values by 48 h (Figs. 5, 6, and 8). The most likely explanation for these observations is an early liquid phase release of lipid from the stomach to the upper intestine (Jobling, 1987), followed by coassimilation of lipid and TCB (Vetter et al., 1985; Van Veld, 1990). Observed changes in the lipid content of material from the stomach and upper intestine are consistent with this conclusion. The absence of an increase in the lipid content of chyme is especially noteworthy and suggests that dietary lipid is efficiently absorbed in the pyloric ceca and upper intestine. The lipid content of tissues from the upper intestine was not determined. It can be speculated, however, that the high tissue/blood TCB ratio at 6 h was accompanied by a transient increase in tissue lipid content, effectively creating an activity gradient for chemical diffusion during the period of lipid absorption. A similar proposal, termed the "fat flush hypothesis", was advanced to explain the dietary uptake of PCBs and other chlorinated organic compounds by humans (Schlummer et al., 1998).

It has been suggested that diffusion limitations in the GI tract of fish may retard dietary absorption of compounds with log K_{OW} values greater than 7 (Gobas et al., 1988; Opperhuizen and Sijm, 1990). Diffusion limitations external to the intestinal epithelium (for example, in the unstirred water layer adjacent to the brush-border membrane) would tend to reduce the tissue/blood concentration ratio and increase the lumenal contents/tissue ratio. Diffusion limitations within the tissue (for example, at the capillary endothelium) would tend to increase the tissue/blood concentration ratio. The data collected in this study were not sufficient to determine whether lumenal contents, tissues, and blood were at or close to chemical equilibrium. However, the observed rapid uptake of TCB from the upper intestine argues against any large diffusion limitations.

TCB concentration ratios in the lower intestine differed greatly from those of the upper intestine. The tissue/blood ratio remained unchanged throughout the study suggesting a near-equilibrium condition (Figs. 5 and 6). In contrast, the lumenal contents/tissue ratio increased sharply between 6 and 48 h and then returned to an apparent baseline value (Fig. 8). The origins of this pattern are not clear. One possible explanation is that lipid and TCB that were not absorbed in the upper intestine were subsequently concentrated in the lower intestine. This seems unlikely, however, since digestive events that tend to reduce meal volume occur primarily in the pyloric ceca and upper intestine. A second possibility is that lipid or some other material with affinity for TCB was shed into the lower intestine, perhaps due microbial activity or sloughing of the gastrointestinal epithelium. In this case, a transient increase in the lumenal contents/tissue ratio would result if TCB that had already been absorbed by fish were to partition back into feces. This suggestion is supported by earlier work on rats dosed orally with hexachlorobenzene (HCB) in corn oil (Rozman et al., 1985). A partial jejunectomy had no effect on fecal elimination of HCB by rats, while a colectomy markedly reduced the amount of HCB in feces. Based on these results it was concluded that processes occurring in the lower intestine control the fecal elimination of hydrophobic organic compounds, and that the major source of these compounds in feces is direct transfer from blood to lumenal contents of the large intestine. In the present study, however, the lipid content of feces at 24 h was only 50% higher than levels measured earlier or later. This transient increase in lipid content does not appear to be sufficient to explain observed changes in TCB distribution, regardless of its origins.

A third possibility is that radioactivity in feces was associated with a metabolite of TCB and not with the parent chemical. In previous studies with rainbow trout, Melancon and Lech (1976) isolated a metabolite of TCB from bile and tentatively identified it as 4-hydroxy-TCB. If this or another metabolic product of TCB became concentrated in gut contents, a description of chemical distribution based on total activity data could be misinterpreted. In the present study, experiments with rat and trout liver microsomes showed that both species are capable of hydroxylating TCB, and that in both cases the predominant product is 3-hydroxy-TCB. Similar in vitro results were reported in earlier studies with rats (Preston and Allen, 1980). It is unlikely, however, that metabolism had a discernible impact on the kinetics of TCB in vivo in trout. The only TCB metabolite detected in this study was a small amount of 3-hydroxy-TCB in feces, accounting for less than 1% of the total activity (Fig. 12). Bile samples collected at 24 h were too small to analyze by GC/MS. It is possible that some or all of the activity measured in bile was present as a metabolite of TCB. However, the total amount of TCB-derived activity contained in bile accounted for less than 0.1% of the ingested dose.

A fourth explanation for the observed distribution of radioactivity at 24 h is that kinetic limitations on TCB uptake resulted in a transient chemical disequilibrium between tissues and contents of the lower GI tract. As indicated previously, it is unlikely that there were substantial limitations on diffusive uptake of TCB. It is possible, however, that diffusion was sufficiently limited to prevent an equilibrium distribution of chemical.

The kinetics of TCB in muscle, kidney, liver, stomach, and lower intestine followed the chemical kinetics in blood. A slightly elevated liver/blood concentration ratio at 6 h may have been due to uptake of TCB and lipid via the hepatic portal vein, but this value did not differ statistically from ratios developed at later sampling times. Tissue/blood concentration ratios in other lean tissues remained constant throughout the exposure and probably represented near equilibrium conditions. In studies with yellow perch and rainbow trout, TCB was shown to distribute between skin, viscera, skeletal muscle, and "carcass" (whole fish minus the other tissues) in rough accordance with tissue lipid content (Guiney and Peterson, 1980). The results of the present study suggest that an equilibrium distribution among lean tissues, including blood, occurred very rapidly, perhaps within a few hours.

TCB concentrations in fat, in contrast to those in lean tissues, increased between 0 and 48 h. This resulted in a redistribution of chemical mass from lean tissues to fat and an increase in the fat/blood concentration ratio (Fig. 7). TCB concentrations in fat declined slightly between 48 and 96 h; however, the fat/blood concentration ratio continued to increase, suggesting that an equilibrium condition was never achieved. A redistribution of TCB from lean tissues to fat was reported previously in studies with rainbow trout (Guiney et al., 1977). This result is most likely due to differences among tissues in

TABLE 1

Dietary assimilation of TCB in feeding studies with fish

Species	Life Stage (Age/Wt)	Dosing Method	Assimilation	Estimation Method	Reference
			%		
Guppy	Juvenile (0–18 wk)	Spiked into prepared diet; 5 feedings/wk × 18 wk	15–17	Kinetic estimate of ε	Sijm et al., 1992
Guppy	Subadult (21–30 wk)	Spiked into prepared diet; 5 feeding/wk × 10 wk	27–32	Kinetic estimate of ε	Sijm et al., 1992
Guppy	Subadult (1 yr)	Spiked into prepared diet; fed daily for 70 days	51	Kinetic estimate of ε	Bruggeman et al., 1984
Northern pike	Juvenile (10–30 g)	Injected into trout prey; fed once	50[a]	Net, from whole-body analysis	Burreau et al., 1997
Goldfish	Subadult (1 yr)	Spiked into prepared diet; fed daily for 150 days	53	Kinetic estimate of ε	Bruggeman et al., 1981
Rainbow trout	Adult (900 g)	Oil-filled capsule; fed once	77	Extrapolation of depuration data	Niimi and Oliver, 1983
Rainbow trout	Subadult (80–100 g)	Starch-filled capsule; fed once	70–80[b]	Net, from whole-body analysis	Guiney and Peterson, 1980
Yellow perch	Subadult (70–120 g)	Starch-filled capsule; fed once	70–80[b]	Net, from whole-body analysis	Guiney and Peterson, 1980
Carp	Adult	Spiked into prepared diet; fed daily for 5 days	80–90[c]	Amount fed minus Σ feces	Tanabe et al., 1982

[a] Estimated from Fig. 2.
[b] Estimated from data provided in this article at 1 to 3 days after feeding.
[c] Range of values for all tetrachlorinated congeners.

blood perfusion rate and chemical capacity. Initially, chemical mass distributes primarily to well perfused tissues. Redistribution occurs because the rate of chemical transfer from lean tissues to fat, although limited by a low perfusion rate, exceeds the overall rate of elimination.

The last major finding of this study was that trout fed "naturally" contaminated prey at a realistic feeding rate absorbed virtually all of the TCB presented to them (Fig. 13). These assimilation estimates are best characterized as "net" values. In principle, true assimilation efficiency could be calculated from a quantitative collection of feces [(amount of TCB in diet − amount in feces)/amount in diet], but this was not attempted. The collection of gut contents at any point in time does not permit such a calculation, since chemical present in the stomach and upper intestinal contents at early time points may eventually be absorbed by the fish, while at later time points a summation of chemical in the gut will not account for compound that has already been eliminated.

For comparison, TCB assimilation efficiencies reported in other studies with fish are given in Table 1, along with the methods used for their estimation. Although highly variable, all of these values are lower than estimates obtained in the present study. Factors that may be responsible for differences between this and other studies include the dosing vehicle, fish species, and study design. The relative oral bioavailability of TCB spiked into a prepared diet, loaded into a gelatin capsule, or assimilated naturally by prey is poorly known, as are differences among species with respect to digestion and processing of dietary lipid. It has also been suggested that juvenile fish assimilate chemicals from the diet less efficiently than adults of the same species due to decreased digestive efficiency (Sijm et al., 1992). Study design is particularly important when comparing the results of short (one or a few feedings) and longer-term feeding studies. The results of the present study suggest that dietary assimilation efficiency can approach 100% when fish are exposed to a hydrophobic compound for the first time. Chemical accumulation with repeated dosing will reduce the diffusion gradients that favor dietary uptake, reducing assimilation efficiency.

The goal of the present study was to reproduce the conditions under which wild fish encounter hydrophobic dietary contaminants. The fact that dietary assimilation efficiencies may be extremely high for chemically "naive" fish has important implications for food web modeling efforts. Additional information on the gastrointestinal physiology of fish is needed to provide an improved basis for interpretation of these results. There is also a need to expand these observations to include compounds with higher log K_{OW} values and to characterize chemical uptake after repeated exposure to contaminated prey.

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