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Mechanism of Biomagnification in Fish under Laboratory and Field Conditions

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While biomagnification of certain organic contaminants in food chains has been observed in field studies, the mechanism of the biomagnification process is still unresolved. Knowledge of the mechanism by which contaminants are absorbed and biomagnified in food chains is important in environmental risk assessment and studies of chemical bioavailability. In this study, we hypothesize a fugacity based model of the gastrointestinal absorption of contaminants. We test the model in a 73 day laboratory, gastrointestinal magnification study of 2,2′,4,4′,6,6′-hexachlorobiphenyl in adult rainbow trout (*Oncorhynchus mykiss*) and in a field study of the gastrointestinal magnification of PCB congeners in rock bass (*Ambloplites rupestris*). Both studies show that the fugacity of the test chemicals in the gastrointestinal tract (GIT) can increase to levels up to 7−8-fold greater than those in the consumed food. The fugacity increase in the GIT is the result of a drop in the chyme's fugacity capacity for the test chemicals (i.e. approximately 4-fold in this study) and an increase in chemical concentration due to food absorption in the GIT (i.e. approximately 2-fold in this study). Food digestibility and absorption are found to be critical factors controlling biomagnification factors and dietary uptake efficiencies under laboratory and field conditions.

Introduction

The presence of most bioaccumulative substances such as polychlorinated biphenyls (PCBs), DDT, dioxins, and mercury in the tissues of humans and mammals is largely the result of dietary intake and biomagnification [Biomagnification: The process where the chemical concentration in an organism (on a lipid weight basis, i.e., g chemical/g lipid) achieves a level that exceeds that in the organism's diet due to dietary absorption.] (*1, 2*). In aquatic organisms, dietary uptake is also the main source of organic chemical bioaccumulation for chemicals with octanol−water partition coefficients (K_{OW}) greater than approximately 10^5−10^6 (*3*). For chemicals with a K_{OW} less than 10^5, chemical uptake from the water and subsequent bioconcentration [Bioconcentration: The process where the chemical concentration in an aquatic organism achieves a level that exceeds that in the water as a result of exposure of the organism to a chemical concentration in the water via the respiratory surface (e.g. gills and/or skin) only.] is usually the main route of uptake (*4*). In food chains, biomagnification at each trophic interaction can result in food-chain bioaccumulation, [Food-chain bioaccumulation is the process where chemical concentrations in organisms (on a lipid weight basis, i.e., g chemical/g lipid) increase with each step in the food chain.] causing concentrations of contaminants in organisms at the top of food chains to be many times greater than those in organisms at the bottom of food chains even when differences in lipid content among the organism are taken into account (*5*−*7*). The importance of dietary accumulation in determining the effective dose of many bioaccumulative substances in humans, mammals, and aquatic organisms is generally recognized. However, the physiological and biochemical mechanisms of the dietary uptake and biomagnification process of contaminants are largely unexplored. Also, there is a high uncertainty in key parameters (e.g. dietary uptake efficiency, biomagnification factors) used in exposure and risk assessments of bioaccumulative substances (*8*). It is the objective of this study to improve the understanding of the mechanism of biomagnification of hydrophobic organic chemicals and to reduce uncertainty in dietary uptake parameters by investigating some of the factors that control dietary uptake and biomagnification.

The first mechanistic explanation of the food-chain bioaccumulation process was given by Woodwell (*9*), who proposed that biomagnification was due to biomass-to-energy conversion. Unable to reproduce the observations of Woodwell, Hamelink (*10*) proposed that bioaccumulation in aquatic food chains is due to a physical-chemical partitioning (or bioconcentration) of the chemical between the water and the organism. Connolly and Pedersen (*5*) showed that in food chains, chemical distribution could not be explained by equilibrium partitioning theory and that chemicals in food chains are transported against the thermodynamic gradient, i.e., from a low fugacity in the prey to a high fugacity in the predator. Gobas et al. (*11*) reported laboratory observations in guppies and goldfish which showed that chemical fugacities can be elevated in the gastrointestinal tract (GIT). This process was referred to as gastrointestinal magnification and can explain why fugacities of certain hydrophobic compounds in predators exceed those in their prey. The purpose of this study is to determine fugacity changes of some hydrophobic organic contaminants in the GIT of fish and to investigate how they occur under controlled laboratory and field conditions. The findings are interpreted in terms of the biochemical mechanism of contaminant absorption and the implications for dietary exposure studies and food-chain bioaccumulation modeling are discussed. Although the study was conducted in fish, it is expected that the investigated dietary uptake processes apply to many organisms.

Dietary Bioaccumulation Model and Hypothesis. A conceptual diagram of a fugacity based model describing the dietary absorption and biomagnification of hydrophobic organic chemicals is presented in Figure 1. A more detailed mathematical description of the model can be found in ref *11*. The reason for expressing the model in terms of fugacities is that net passive (i.e. diffusive) transport of a chemical between different (and temporally changing) media (i.e. food, digested food in the GIT and organism) occurs in response to fugacity, not concentration, differences between the media. Fugacity is a thermodynamic quantity that can be viewed as

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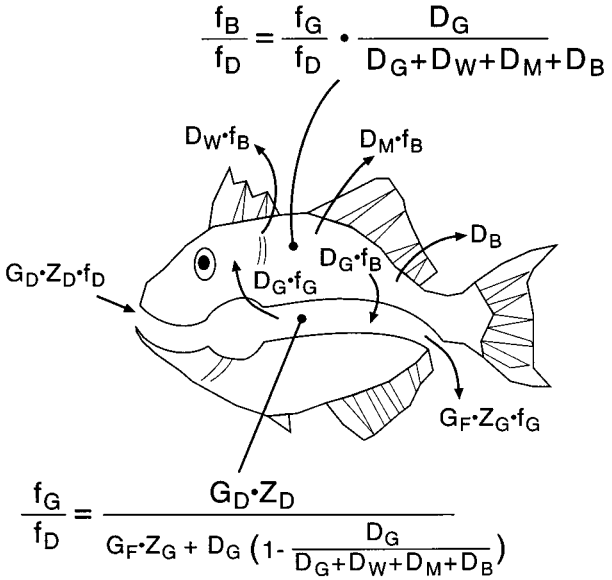

FIGURE 1. A conceptual diagram of a fugacity based model describing the dietary absorption and biomagnification of hydrophobic organic chemicals at steady-state. f_B, f_D, f_G, and f_W are the chemical fugacities (Pa) in respectively the organism (B), diet (D), GIT (G), and water (W); D_B, D_D, D_F, D_G, D_M, and D_W are the transport parameters (mol/Pa·d) of respectively growth dilution (B), chemical ingestion (D), chemical egestion by fecal excretion (F), chemical transfer between the GIT and the organism (G), metabolic transformation (M), and water-organism exchange via the gills (W); Z_B, Z_D, and Z_G are the fugacity capacities (mol/m^3·Pa) of respectively the organism (B), diet (D), and GIT contents (G); G_D and G_F are the rates (m^3/d) of food consumption and fecal egestion.

the "escaping tendency" of the chemical from its medium (*12*). It can be measured as the partial pressure that the chemical substance exerts and is hence expressed in units of pressure, i.e. Pascal (*12*). The chemical's concentration C in mol/m^3 and the fugacity f in the food in units of Pa are related as C equals $f \cdot Z$, where the fugacity capacity Z (in mol/m^3·Pa) reflects the ability of the matrix to "solubilize" or "store" the chemical.

In essence, the model expresses gastrointestinal uptake in simplified terms as a process where the GIT receives food at a rate G_D in m^3 food/day and emitting the gastrointestinal content at a rate G_F in m^3 feces/day. A chemical contaminant enters the GIT at a rate (in mol/day) of $G_D \cdot C_D$ or $G_D \cdot Z_D \cdot f_D$ and leaves the GIT at a rate of $G_F \cdot C_G$ or $G_F \cdot Z_G \cdot f_G$. Food absorption in the GIT will cause G_F to be smaller than G_D, and the change in food composition (especially the absorption of lipids in the food) will cause the Z_G to be smaller than Z_D. If chemical elimination via the gills, metabolic transformation, and growth are insignificant, then, at steady-state (such as may occur under field conditions), $G_D \cdot Z_D \cdot f_D$ can be expected to approach $G_F \cdot Z_G \cdot f_G$, and f_G will be greater than f_D. The increase of f_G over f_D is referred to as gastrointestinal magnification. This increase in chemical fugacity is also expected to occur under non-steady-state conditions (e.g. under laboratory conditions) if the contaminant absorption rate is smaller than the absorption rate of those food components, such as lipids, that provide the majority of the fugacity capacity of the food for the chemical contaminant. The importance of the increase in f_G over f_D is that simple passive diffusion tends to equalize f_B and f_G, causing the fugacity in the organism f_B to exceed the fugacity in the diet f_D (i.e. biomagnification). The latter will occur if the combined rate of chemical elimination via the respiratory surface area, metabolic transformation, growth dilution, and other processes are insignificant compared to the rate of fecal egestion (i.e. $D_W + D_M + D_B < D_G$ or following the equivalent kinetic approach $k_2 + k_M + k_G < k_E$ (*11*)). For most organic chemicals, the combined rate of chemical elimination via the respiratory surface area, metabolic transformation, growth dilution, and other processes can be expected to be significant and f_B will not be able to achieve f_G. If f_B is smaller than f_D, there is, by definition, no biomagnification. As a result of the high rate of chemical elimination via the respiratory surface area relative to the rate of fecal excretion, chemicals with a log $K_{OW} < 5$ generally do not show a tendency to biomagnify in aquatic food chains. Since chemical elimination rates via the respiratory surface to the water drop with increasing K_{OW}, chemicals with greater K_{OW} exhibit a significant biomagnification potential unless they are being metabolized at a significant rate. Because the essence of biomagnification model is the increase in the chemical fugacity in the GIT over that in the diet, this study investigates the hypothesis that, in the GIT, chemical fugacities are being elevated above the levels in the diet due to food absorption and digestion.

Experimental Section

Overview. The laboratory experiment involved the exposure of rainbow trout in a flow-through system to clean uncontaminated water and a diet consisting of fish chow, 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCBP), and 1% (w/w) chromic oxide. Throughout the exposure period, fish were sampled and their gastrointestinal tract content and tissues analyzed separately. The contents of stomach, interparietal fat, and four intestinal sections were analyzed for (i) concentration of HCBP, (ii) the fugacity of HCBP, (iii) lipid content, (iv) organic matter content, and (v) chromic oxide concentration with the main purpose to measure the change in HCBP fugacity that occurs upon ingestion and digestion of the food. Chromic oxide was included in the food to measure the food absorption efficiency in the fish. This method (*13*) was confirmed for measuring food absorption rates in fish (*14*) and has been used widely in rainbow trout and other species (*15, 16*). It is based on the virtual inabsorbability of chromic oxide from the diet. This provides a method to measure the degree of food absorption by the increase in concentration of chromic oxide in the intestinal tract as the food moves through the GIT, i.e. :M_G/M_D equals $C_{CO,D}/C_{CO,G}$,where M_D is the mass (g) of administered food to the fish, M_G is the mass (g) of the administered food remaining in the GIT at a particular location in the GIT, and $C_{CO,D}$ and $C_{CO,G}$ are the chromic oxide concentrations in respectively the administered food and the GIT at the location where the chromic oxide concentration is determined. The fraction of the administered food that is absorbed at any location in the GIT is $1 - (C_{CO,D}/C_{CO,G})$. The fraction of the administered mass of HCBP that remains at any location in the GIT can be determined as $C_G \cdot C_{CO,D}/C_D \cdot C_{CO,G}$, and the fraction of administered HCBP that has been absorbed is $1 - (C_G \cdot C_{CO,D}/C_D \cdot C_{CO,G})$.

To confirm the occurrence of (laboratory observed) fugacity changes in the GIT of fish under field conditions, lipid based concentrations of several PCB congeners in the food and intestinal content of rock bass were measured. The difference in the lipid based concentrations between the diet and the GIT contents is an adequate surrogate for the difference in fugacities between the diet and the GIT, because concentrations are proportional to fugacities as long as they refer to the same medium (i.e. lipids) with the same fugacity capacity Z (i.e. $C = f \cdot Z$). Direct measurements of the fugacity, as performed in the laboratory experiment, could not be made because the PCB congener fugacities were below the detection limit of the fugacity measurement method.

Statistics. Standard errors are reported in brackets. Changes in fugacities and concentrations in the GIT over time were tested for their statistical significance using analyses of co-variance. Statistically significant differences

between concentrations, fugacities, lipid contents in different gastrointestinal sections, and food were determined by student-t-tests. A Mann−Whitney U test (M−W) (*17*) was employed to determine differences in lipid content between crayfish and the intestinal content of rock bass. Multivariate Analyses of Variance (MANOVA) were performed to determine differences in PCB congener concentrations in crayfish and the intestinal content of the rock bass.

Diet Preparation. Ten grams of chromium(II) oxide (Sigma Chemical Co.) was blended with 1 kg of ground Silver Cup trout chow until a powder was formed. Nine hundred milligrams of 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCBP) was dissolved in 100 mL of petroleum ether and then mixed with water into the trout chow for a period of 2 h after which the solvent was evaporated. The mixture was then extruded through a solvent rinsed meat grinder having a die with 2 mm diameter holes. The resulting pellets were then dried in a fume hood overnight. The food for the untreated fish was prepared in a similar manner, but no HCBP was added. The high concentration of HCBP was required to make intestinal fugacity measurements. During the 73 day exposure period, internal concentrations in the fish were far below steady-state levels and no observable signs of fish toxicity (i.e. mortality, lack of activity or feeding) were observed.

Laboratory Exposure. Before the exposure period, adult rainbow trout (*Oncorhynchus mykiss*) were acclimated for 31 days while feeding on Silver Cup trout chow at a daily rate of 2 g food/ fish. Forty rainbow trout (weight: 365 (±66) g; lipid content: 6.6 (±0.72)% were then placed in a 240 L/h flow-through system consisting of a 3000 L bottom-draining tank containing uncontaminated water at 7 °C. The tank was divided into two connected compartments, separated by a screen through which water but no significant amounts of food pellets could pass. Each compartment contained 20 fish. Fish in only one compartment received the HCBP contaminated food. The purpose of including fish that were not exposed to dietary HCBP in the same flow-through tank with the HCBP exposed fish was to determine the extent of HCBP bioconcentration that may occur during dietary uptake of the HCBP exposed fish through dissolution of HCBP from food or feces into the water or through gill elimination by the fish. Bioconcentration of HCBP, if significant, interferes with the correct measurement of the dietary uptake efficiency in fish and could affect the HCBP fugacity in the GIT. In addition, the fish receiving food that did not contain HCBP provided a control population for observations of any possible toxic effects due to HCBP ingestion. No fish mortality, reduced activity, or lack of feeding was observed throughout the exposure experiment. The fish were fed 2.0 g food per fish per day. The initial daily feeding rate of 0.55 (±0.12)% of the fish's body weight was chosen to minimize the amount of nonabsorbed food. The feeding rate conformed with typical maintenance values of 0.5−10% (*18*), especially if it is taken into account that the lipid content of the administered food (18%) was greater than that (i.e. 8−11%) in the foods (*18*) used in typical fish maintenance programs. Fish collected over the 73 day exposure period exhibited a mean weight of 439 (±64) g for HCBP exposed fish and 409 (±86) for unexposed fish, indicating that fish grew throughout the exposure period and that the feeding rate as a percentage of body weight declined from 0.55 to approximately 0.46% per day. Two fish from each compartment were sampled on days 0 (to check for HCBP concentration in fish prior to the experiment), 1, 3, 6, 10, 18, 29, 43, and 73. The fish were killed and the GIT removed and divided into the stomach, interparietal fat, and four intestinal sections (referred to as the anterior, anterio central, posterio central and posterior sections), each approximately 5 cm in length.

Fugacity Analysis. Of each intestinal fraction, 0.5 mL was transferred into a 2 mL glass vial. For food, 0.5 g was transferred into a 2 mL glass vial. Two droplets of a 1 g/L mercuric chloride solution were added to each vial to prevent microbial growth. The sample was then distributed to cover the glass wall of the vial to increase surface area. Atmospheric air was replaced by nitrogen, and the vial was capped and airtight sealed. Samples were equilibrated at room temperature for 30 days, after which 80 μL of nitrogen gas was analyzed by gas chromatography (GC). The measured gaseous concentrations of HCBP were related to the HCBP fugacities through the Ideal Gas Law, i.e. fugacity (Pa) equals the product of concentration (mol/m^3), temperature (K), and the gas constant. The limit of quantification was 0.1 pg of HCBP per 80 μL injection, corresponding to a fugacity of 8.6×10^{-6} Pa. After headspace analysis, the vial contents were divided into two equal fractions for HCBP and chromic oxide analysis.

HCBP Concentration Analysis of Chyme (Gut Content). Each sample was homogenized in a mortar with 5 g of granular anhydrous sodium sulfate. The homogenized sample was transferred to a column containing, from bottom-to-top, glass wool, 1 g of granular sodium sulfate, 12 g of acidified-60 mesh silica gel, and 18 g of sodium sulfate. The column was eluted with 250 mL of petroleum ether over a 6 h period with recoveries greater than 94%, determined by elution of spiked samples. The eluent was then diluted and analyzed by GC. The limit of quantification was 0.1 μg/g.

HCBP and Lipid Content Analysis in Tissues. Weight and tissue analysis was conducted on the liver, interparietal fat, and the remainder of the fish (i.e. carcass). The GIT was inverted and washed with 75 mL of distilled water before being included as part of the carcass. References to the concentration and lipid content in the "whole fish tissue" refer to all tissues (i.e. liver + interparietal fat + carcass) and were derived as the sum of the HCBP or lipid masses in each part of the fish divided by the total weight of the fish tissues. The carcass was homogenized in a meat grinder and then mixed by hand. The liver was cut in small pieces and then homogenized with mortar and pestle. Interparietal fat was homogenized with mortal and pestle. Of the homogenates, 10 g (for carcass), 2 g (for liver), and 1 g (for interparietal fat) were used for HCBP analysis. HCBP analysis involved grinding each of the tissue samples in 20 g of anhydrous sodium sulfate. The mixture was then placed in 0.02 m × 1 m column containing 5 g of anhydrous sodium sulfate. This column was equilibrated overnight with petroleum ether and was eluted the next day with 250 mL of petroleum ether. The eluent was divided in two fractions, i.e. 10 mL for HCBP analysis and 240 mL for lipid content analysis. The 10-mL fraction of the eluent was transferred to a column containing, from bottom-to-top, glass wool, 1 g of granular sodium sulfate, 12 g of acidified-60 mesh silica gel, and 18 g of sodium sulfate. The column was eluted with 250 mL of petroleum ether over a 6 h period with recoveries greater than 95%, determined by elution of spiked samples. The eluent was then diluted and analyzed by GC. The limits of quantification were 0.063, 0.31, and 0.63 μg/g for fish carcass, liver, and interparietal fat, respectively. The 240-mL fraction was evaporated to dryness using a Rotavap and then placed in an oven overnight at 35 °C. The amount of lipid was then determined gravimetrically. The lipid content is expressed on a wet basis.

Gas Chromatography. GC analysis was conducted on a HP 5890 series II equipped with an on-column injection port, electron capture detector and a 5 m × 0.53 mm × 2.65 μm (film thickness) HP-1 (Methyl Silicone Gum) Instrument Column. Carrier gas was helium at a flow rate of 20 cm/s (at 35 °C). The temperature program was 35−270 °C at 20 °C/min. External standards were used for sample quantification. The limit of quantification of HCBP concentrations was 0.1 pg in a 1 μL injection.

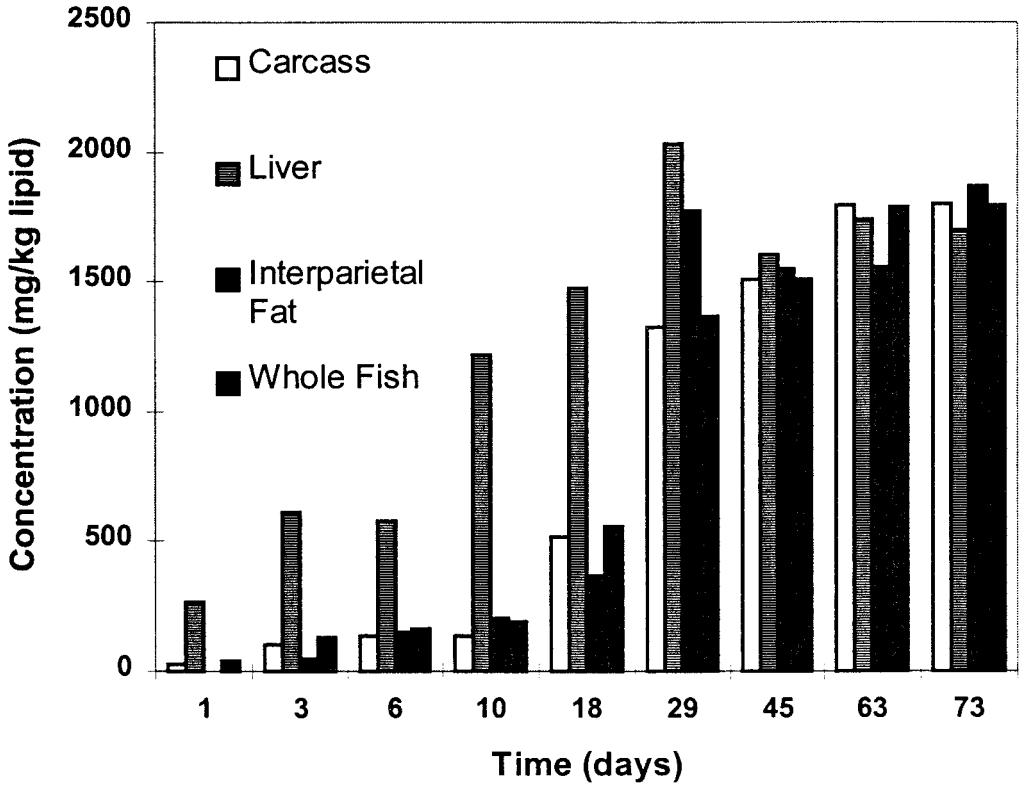

FIGURE 2. Mean observed lipid based concentrations (mg/kg lipid) of HCBP in the carcass, liver, interparietal fat, and whole fish, excluding the contents of GIT, throughout the exposure period.

Lipid Content Analysis of Food and Chyme. Two grams of gastrointestinal content or food was ground with sodium sulfate crystals and then transferred to a 0.02 m × 1 m column containing 18 g of sodium sulfate. The column was eluted with 150 mL of petroleum ether. The eluent was evaporated to dryness using a Rotavap and then placed in an oven overnight at 35 °C. The amount of lipid was then determined gravimetrically. The lipid content is expressed on dry weight basis for food and intestinal contents.

Organic Matter Analysis. In ceramic crucibles, 2 g of air-dried dietary sample was heated in a muffle furnace at 550 °C for 12 h, then cooled, and weighed. It was assumed that inorganic matter levels in the samples were insignificant.

Chromic Oxide Analysis. The sample was air-dried for 2 days, then weighed, and ashed at 450 °C in a muffle furnace. After cooling, the sample was (i) added to 2 mL of a digestion mixture (i.e. 10 g of sodium molybdate dihydrate dissolved in 500 mL of a 150:150:200 mixture of distilled water, concentrated sulfuric acid, and 70% perchloric acid following (*19*)), (ii) made up to 5 mL in distilled water, and (iii) analyzed for chromic acid at 440 nm with a Perkin-Elmer UV/vis absorbance spectrometer.

Field Collections. Seven rock bass (*Ambloplites rupestris*, weight: 77.8 (±14.0) g, lipid content: 0.21(±0.05)%) and 12 crayfish (*Orconectes propinquus*, weight: 7.8 (±0.71) g, lipid content: 1.1 (±0.26)%) were collected by gill net (rock bass) and trap (crayfish) from the Detroit River at (42°20′30″, 82°55′45″) in March 1992. Gastrointestinal tracts of fish were excised, and stomach contents were examined to determine prey consumption. Intestinal contents of fish were removed in entirety ($n = 7$), frozen at −20 °C, and then individually analyzed for PCB congeners 52, 87, 101, 153, 138, and 180 and lipid content according to methods, reported in ref *20*. Fish tissues, from which GIT contents were removed, were ground, stored at −20 °C, and analyzed for PCB congeners and lipid content following (*20*).

Results

Laboratory Experiments. Throughout the exposure period, concentrations of HCBP in the fish carcass, liver, and interparietal fat of the diet-exposed fish increased over time (Figure 2). Concentrations of HCBP in fish carcass, liver, and interparietal fat of the nondiet-exposed fish were right at or below the limits of quantification and showed no relationship with time. This indicates that exposure of the fish to aqueous HCBP was insignificant compared to the dietary exposure. During the uptake period, the HCBP concentration in the liver increased over time. However, the ratio of the lipid based HCBP concentrations (g HCBP/g lipid) in the liver (C_{liver}) and the fish carcass ($C_{carcass}$), i.e. $C_{liver}/C_{carcass}$, declined exponentially from a maximum value of 8.8 (after 1 day) to a value of 1.0 (i.e. internal equilibrium) after approximately 45 days. The whole fish concentration increased over time (Figure 2). Fitting the whole fish concentration (C_F) data to an Euler type numerical integration of the differential equation (*21*)

$$dC_F/dt = E_D \cdot F_D \cdot C_D - k_O \cdot C_F \quad (1)$$

where the fish weight normalized feeding rate F_D was 0.005 kg food/kg fish/d, the dietary concentration C_D was 920 mg/kg, k_O was the rate constant for chemical depuration by all routes, and t is time (days), indicated that the dietary absorption efficiency E_D of HCBP was approximately 70% and k_O was 0.018 d^{-1}.

In all fish, the anterior portion of the intestines contained a small amount of liquid that was insufficient for further analysis. The other sections of the intestines were filled with digested food in all cases. HCBP concentrations and fugacities as well as chromic oxide concentrations, lipid, and organic matter content in each section of the gastrointestinal tract did not change in a statistically significant fashion over the duration of the exposure period. Hence, for each gastrointestinal segment, measurements of each quantity taken

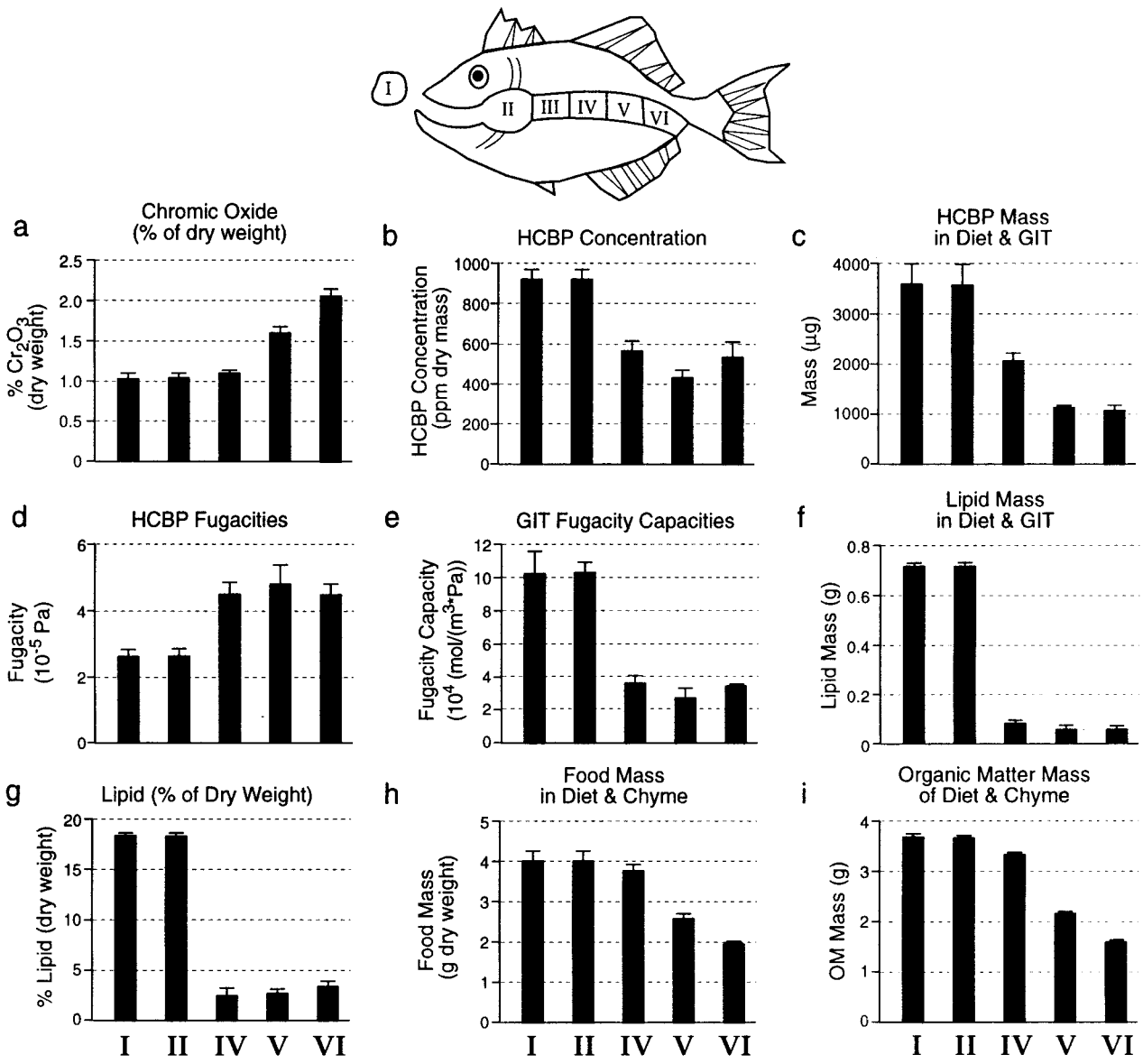

FIGURE 3. Observed mean concentrations of chromic oxide (a) and HCBP (b); the mass of HCBP (c), lipid (f), food (h), and organic matter (i); the fugacity (d) and fugacity capacity (e) of HCBP and the lipid content (g) with their standard errors in the diet and chyme in various sections of the gastrointestinal tract of adult rainbow trout throughout the exposure period.

over the duration of the entire experiment were pooled and their mean and statistical distribution determined. The mean concentration of chromic oxide in the stomach was not statistically different ($P < 0.05$) from that in the administered food. It increased 2-fold, representing a statistically significant change ($P < 0.05$), upon progression through the intestines of the fish (Figure 3). This indicates a net food absorption efficiency of 50%. The mean HCBP concentration in the stomach was not significantly different ($P < 0.05$) from that in the diet and dropped approximately 2-fold when progressing through the gastrointestinal tract (Figure 3). The reduction in HCBP concentration occurred predominantly between the stomach and the anterio central section of the intestines. Of the total mass of ingested HCBP, 29% was observed in the posterior section of the gut (Figure 3). This indicates an HCBP gastrointestinal absorption efficiency of approximately (i.e. some further HCBP absorption may occur prior to fecal egestion) 71%, which agrees well with the HCBP dietary absorption efficiency of 70% determined from HCBP whole fish tissue concentrations. Although the HCBP concentration dropped approximately 2-fold during progression through the GIT, the HCBP fugacity increased approximately 2-fold (Figure 3), representing a statistically significant increase ($P < 0.05$) over the fugacity in the food and stomach. The increase in fugacity was achieved between the stomach and the anterio central section of the intestinal tract. There were no statistically significant differences between the observed fugacities in the various section of the intestines. Since the concentration in the chyme equals the product of the HCBP fugacity in the chyme and the fugacity capacity of the chyme for HCBP, the increase in the HCBP fugacities was due to a 3.9-fold drop in the fugacity capacity of the gastrointestinal content upon progression through the GIT (Figure 3). Due to the high affinity of HCBP for lipids, it is possible that the drop in the fugacity capacity of the chyme matches the drop in lipid content due to lipid absorption. However, the 6.6-fold reduction in the lipid content of the chyme was greater than that of the fugacity capacity, i.e. 3.9-fold (Figure 3). The majority of the lipid absorption occurred between the stomach and the anterio central section of the intestinal tract. The total lipid absorption efficiency in the fish was 92%. The 3.9-fold reduction in the fugacity capacity of the gastrointestinal contents did not match the reduction in the organic matter content of the chyme, which

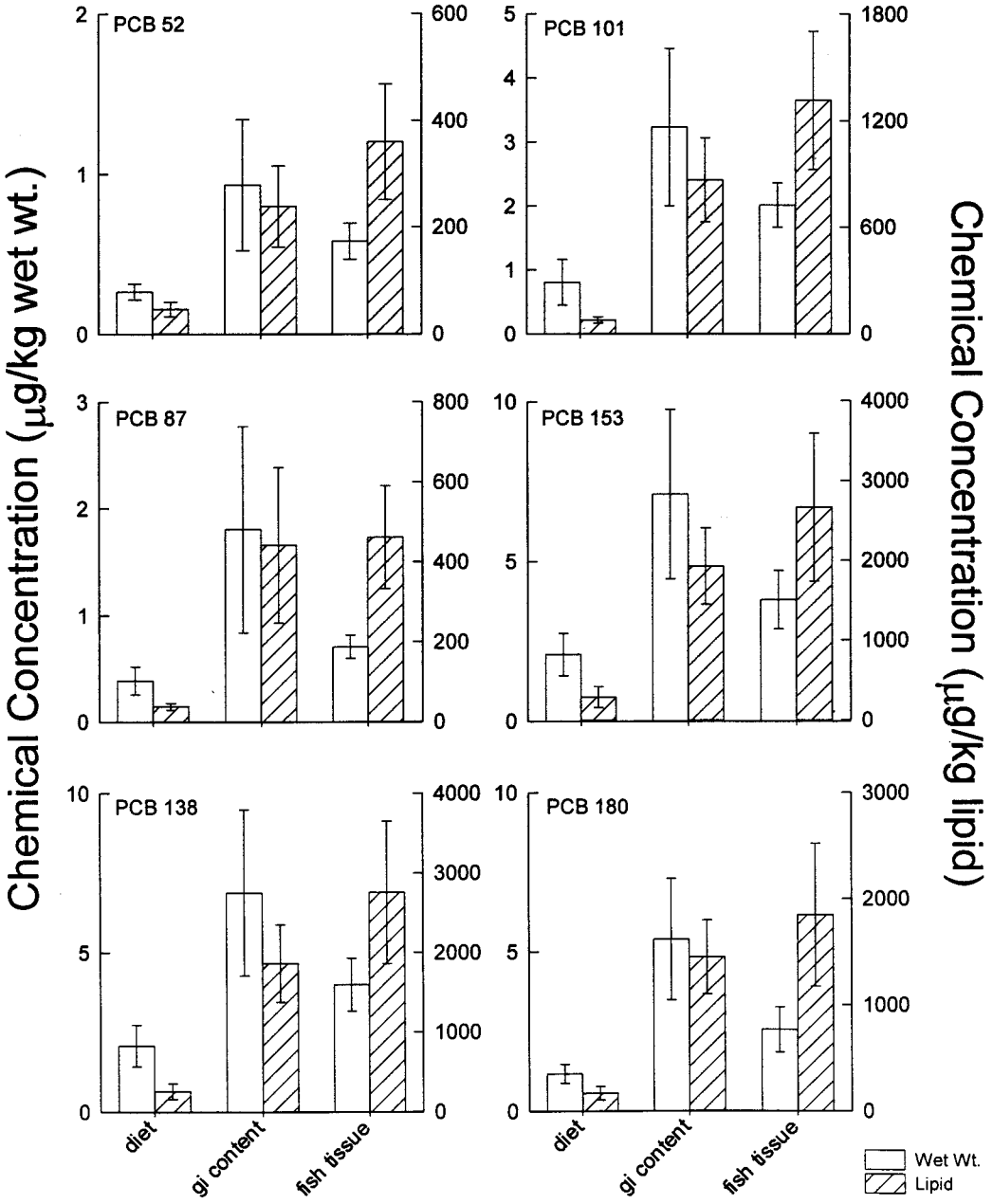

FIGURE 4. Concentrations (and standard errors) of several PCB congeners on a wet weight basis (left axis) and a lipid weight basis (right axis) in the diet (i.e. crayfish), intestines, and body tissues of rock bass collected from the Detroit River.

fell from 95% in the stomach to 83% in the posterior section of the gut, corresponding to an organic matter uptake efficiency of 57%.

Field Experiments. Stomach contents of rock bass exclusively contained crayfish carapaces and/or appendages, indicating that only crayfish had been consumed. The lipid content of the intestinal content in rock bass, i.e. 0.33 (±0.04)%, was 3.3-fold and significantly lower (Mann−Whitney U test = 68.5, $p = 0.025$) than the lipid content of the consumed crayfish, i.e. 1.1 (±0.26)%. This indicates that the rock bass absorb lipids from their diet. Figure 4 illustrates that lipid normalized concentrations of all PCB congeners (μg/kg lipid) in the intestinal content were 6−8-fold greater than those in the diet of the fish. This increase in concentration is statistically significant ($P < 0.05$) for all congeners as determined by a MANOVA. Assuming that the fugacity capacity of dietary and intestinal lipids are approximately the same, an increase of the lipid normalized concentrations is equivalent to a proportional increase in the fugacity of the PCB congeners in the GIT. The lipid based concentrations of PCBs in the fish were comparable to lipid based concentrations in the intestinal content, indicating that fish and intestinal content were close to a chemical equilibrium. Wet weight based PCB concentrations in the intestinal content were, with the exception of PCB 87, significantly ($P < 0.05$) greater than those in the diet. This indicates that under field conditions, dietary concentrations can increase in the GIT to levels greater than those in the diet.

Discussion

Dietary Uptake Mechanism. The observed increase in HCBP fugacity in the GIT in the laboratory and the increase in lipid based concentration upon food digestion in the field illustrate that gastrointestinal digestion is able to elevate the activity or thermodynamic potential of hydrophobic organic chemicals such as PCBs. This agrees with earlier laboratory (*11*) and field observations (*5, 22*), from which an increase in chemical fugacity due to digestion was inferred. The increase

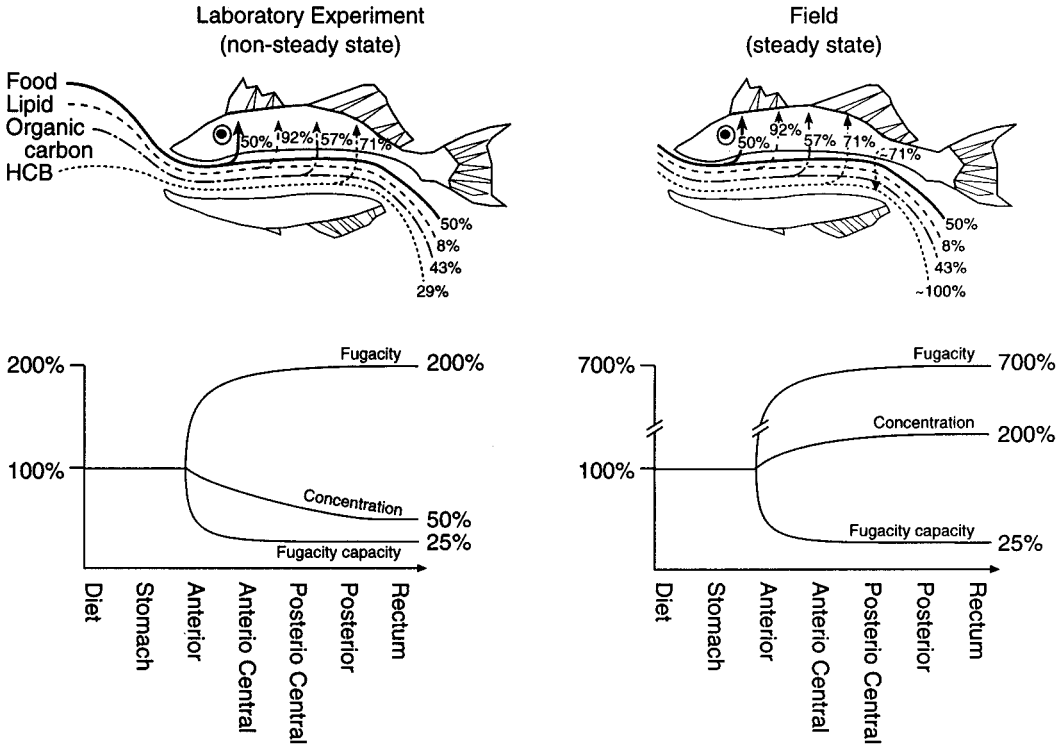

FIGURE 5. Schematic diagram of the lipid absorption efficiency, the food absorption efficiency, the HCBP absorption efficiency and the organic matter absorption efficiency, and resulting HCBP concentrations, fugacities, and chyme fugacity capacities in the gastrointestinal tract of the fish under non-steady-state conditions in this laboratory study and under steady-state conditions in the field study.

in HCBP fugacity is largely achieved between the stomach and the anterio central section of the GIT. It originates because the absorption efficiency of lipids (92%) is greater than the absorption efficiency of HCBP (71%). As a result, the lipid normalized concentration of HCBP in the GIT increases over that in the diet. The increase in the lipid-normalized concentration in the GIT corresponds to an increase in fugacity of HCBP in the GIT because the amount of lipid in the gastrointestinal content is a dominant factor controlling the chyme's fugacity capacity for HCBP. The food absorption efficiency (50%) is less than the HCBP absorption efficiency (71%), indicating that the HCBP concentration (in units of g HCBP/g chyme) will fall in the digestive tract. However, it should be stressed that the HCBP absorption efficiency in the laboratory experiment was measured under non-steady-state conditions where the HCBP concentration in the fish was essentially zero at the start of the experiment and far below its steady-state levels throughout the experiment (Figure 3). Under field conditions, concentrations in fish are much greater relative to the concentrations in the fish's diet, compared to the laboratory conditions in this experiment. Under field conditions, the net absorption efficiency of HCBP will be much smaller than the 71%, because, in contrast to our laboratory experiment, environmental fish tissues contain a considerable concentration of HCBP. Hence, there will be a considerable fish-to-GIT flux in addition to the GIT-to-fish flux, which reduces the net dietary absorption efficiency to values that only represent chemical losses through the gills and skin and through growth dilution. At or near steady-state, the concentration of HCBP can be expected to increase in the GIT, rather than drop as observed in our laboratory experiment, because the food absorption efficiency is then greater than the net HCBP absorption efficiency. Figure 4 confirms the occurrence of the increase in the wet weight based concentration of several PCB congeners in the field collected rock bass. The low net dietary uptake of HCBP near or at steady-state will also cause the fugacity in the intestinal tract to increase to values that are greater, i.e., approximately 7 to 8 times (i.e. 2×3.9) the fugacity in the food, than those observed in the laboratory experiment. An increase of this magnitude in the lipid based concentration of the intestinal content over that in the diet was observed in the rock bass (Figure 4). Figure 5 illustrates the interplay between absorption efficiencies of HCBP, food, and lipid and resulting changes in concentrations and fugacities of HCBP in the GIT under non-steady-state laboratory conditions and under steady-state field conditions.

Dietary Bioaccumulation Model. The successful application of the model for estimating biomagnification in biota relies on the assessment of (i) the change in fugacity capacity that occurs between the ingested and the digested food, i.e. Z_D/Z_G, where Z_D is the fugacity capacity of the diet and Z_G is the fugacity capacity of the chyme, and (ii) the degree of food absorption, which can be expressed as the ratio of the dietary ingestion (G_D) and fecal egestion rate (G_F), i.e. G_D/G_F. In the laboratory experiment, Z_D/Z_G appeared to be approximately 4. It is overestimated by the change in lipid content that occurs upon food digestion, i.e. $L_D/L_G = 6.6$ in our experiment. The discrepancy between Z_D/Z_G and L_D/L_F indicates that components other than lipids can contribute significantly to the fugacity capacity of the food and the intestinal content. The reduction in organic matter content (i.e. 1.14-fold) that occurs in the GIT appears to be a poor predictor of the gastrointestinal magnification factor. This is likely the result of the fact that both digestible and nondigestible matter in the experimental food predominantly consist of organic matter. Based on the observed changes in food composition, it is possible to estimate the extent to which lipids and organic matter other than lipids (i.e. carbohydrates, proteins, fibers and other) contribute to the fugacity capacity of the diet and intestinal content. To do this, it needs to be assumed that (i) food absorption and digestion do not affect the fugacity capacity of the nonlipid organic matter fractions of the diet and chyme for HCBP and (ii) chromic oxide and other inorganic substances do not contribute significantly to the fugacity capacity of the diet

and intestinal content. Under those assumptions, the measured fugacity capacities of the contents of the stomach (Z_S) and the second posterior intestinal fraction (Z_{PI}) can be represented as the sum of the product of the fugacity capacity of the lipid (Z_L) and the observed lipid content in the stomach (L_S) and second posterior intestinal fraction (L_{PI}), respectively, and the product of the fugacity capacity of the nonlipid organic matter (Z_{OM}) and the observed nonlipid organic matter content of the stomach (OM_S) and the second posterior intestinal fraction (OM_{PI}), respectively:

$$Z_S = (L_S \cdot Z_L) + (OM_S \cdot Z_{OM}) = 0.18 \cdot Z_L + 0.77 \cdot Z_{OM} = 10^5 \text{ mol/m}^3\text{·Pa} \quad (2)$$

$$Z_{PI} = (L_{PI} \cdot Z_L) + (OM_{PI} \cdot Z_{OM}) = 0.025 \cdot Z_L + 0.81 \cdot Z_{OM} = 2.9 \times 10^4 \text{ mol/m}^3\text{·Pa} \quad (3)$$

Solving these equations indicates that Z_L and Z_{OM} are approximately 4.8×10^5 and 1.6×10^4 mol/m^3·Pa, respectively. This suggests that the fugacity capacity of the lipids is approximately 30 times greater than that of the nonlipid organic matter. The dietary lipids provide approximately 88% of the total fugacity capacity of the diet in the stomach, but only 49% of the total fugacity capacity in egestable fecal matter. Based on these findings, the following tentative model can be proposed for estimating the contribution of food digestion to the gastrointestinal magnification factor

$$Z_D/Z_G = (L_D + 0.035 \cdot OM_D)/(L_G + 0.035 \cdot OM_G) \quad (4)$$

where L_D and L_G are the lipid contents of the diet and the chyme, and OM_D and OM_G are the nonlipid organic matter contents of the diet and the chyme, respectively.

Another aspect of our dietary bioaccumulation model is that the fish is viewed as a single homogeneous compartment. The observation that lipid based concentrations of HCBP in the liver were greater than those in the rest of the fish during the first 45 days of the experiment but then reached levels similar to those in the rest of the fish after 45 days, indicates that the assumption of homogeneity is reasonable for organisms in the field that (due to their longer exposure) may be close to steady-state levels. However, the assumption is not correct during short time exposures applied in the laboratory. The observation indicates that chemicals absorbed from the diet are being preferentially passed to the liver. However, it also demonstrates, as has been observed in many field studies, that internal distribution of unmetabolizable chemicals can result in homogeneous lipid based concentrations (and hence approximately equal fugacities) given sufficient time. The apparent time-lag in the distribution of HCBP in the fish provides an interesting method to assess whether fish under field conditions have recently consumed a "hot" meal, with higher than average contaminant levels. A significant elevation of the lipid based concentrations in the liver over that in the rest of the organism indicates the recent ingestion of a "hot" meal by the organism.

Dietary Uptake Experiments. The observation that the chemical fugacity in the GIT increases as a result of food digestion and absorption illustrates that the medium in which the chemical is exposed in dietary uptake studies can have an important effect on the rate of uptake in the test organism and the ultimate fugacity (and concentration) that the chemical will reach in the organism. For example, the fugacity of a chemical in the diet (f_D) of 1 Pa may be raised to 2 Pa in the GIT (f_G) if the diet is "poorly" digestible, whereas it may achieve a f_G of 8 Pa, if the diet is more "easily" digestible. Given the same gut wall permeation rate, the initial absorption rate would be four times greater for the more "easily" digestible food and the steady-state concentration in the organism would also be four times greater. The role of the dietary matrix on chemical uptake kinetics and biomagnification may explain the large degree of variability that has been reported in measurements of the dietary uptake efficiency of bioaccumulative substances (*8*). Dietary uptake efficiencies have been measured using a variety of chemical administration matrices, including oils (i.e. "easily digestible" substrates), gelatin, food pellets, and sand (i.e. a "poorly digestible" substrate). This and previous studies (*23*) suggest that dietary uptake rates can differ substantially depending on the matrix in which the chemical substance is administered.

Food-Chain Bioaccumulation. The results of this study indicate that concentrations of chemical substances increase with trophic levels not because of the transformation of (contaminant containing) biomass into energy (*9*) but because food digestion in the GIT raises the chemical's fugacity. This fugacity increase enables simple passive diffusion across the intestinal wall to cause a concentration in the predator that exceeds that in its prey. This study suggests that in food chains, lipid rich prey-items will not only result in a larger exposure of the predator to bioaccumulative substances but also result in larger gastrointestinal magnification factors which can lead to higher bioaccumulation factors in the predators. Organisms consuming a diet that is lipid-poor but rich in nonlipid-organic-matter (e.g. diets with high fiber contents) are expected to experience smaller gastrointestinal magnification factors. Due to the importance of dietary composition and digestibility of the dietary matrix, the application of universal biomagnification factors or food-chain multipliers for particular organisms can be associated with a substantial error.

Acknowledgments

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