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# Gastrointestinal Magnification: The Mechanism of Biomagnification and Food Chain Accumulation of Organic Chemicals

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Dietary bioaccumulation experiments with chlorobenzenes, PCBs, and mirex in guppies and goldfish are presented. The results demonstrate that, in the gastrointestinal tract of fish, the fugacity of very hydrophobic, nonmetabolizable chemicals (log $K_{OW}$ > 6) is elevated above the fugacity in the consumed food as a result of food digestion and absorption. Observed fugacities in fecal matter were up to 4.6-fold greater than the fugacity in the administered food. Fecal to food fugacity ratio ranged between 0.07 and 4.6 in guppies and between 0.014 and 4.5 in goldfish and increased with increasing $K_{OW}$. Food digestion in the gastrointestinal tract was found to increase the chemical fugacity in the food 5-fold by altering the fugacity capacity of the food. An additional 2–3-fold increase in the chemical concentration and fugacity in the gastrointestinal tract is due to food absorption from the gastrointestinal tract. The findings support the "digestion" hypothesis as being the driving force of the biomagnification and food chain accumulation of hydrophobic organic chemicals. The study further illustrates the application of static head-space analysis to measure chemical fugacities in food and fecal samples.

## *Introduction*

It is well-documented that persistent hydrophobic organic chemicals such as polychlorinated biphenyls (PCBs) and DDT can biomagnify, causing chemical concentrations to increase with every step in ecological food chains (*1*, *2*). Traditionally, this phenomenon is explained by the loss of biomass in the food chain due to respiration and excretion as biomass is transferred from one link in the food chain to another (*1*). However, recently, thermodynamic studies of PCBs and other organochlorines in aquatic food chains have shown that fugacities of very persistent, hydrophobic organic chemicals in organisms increase with every step in the food chain and that the fugacity in organisms of higher trophic levels exceed that in the water in which the organisms reside (*3*, *4*). Fugacity is equivalent to chemical activity or chemical potential, and a difference in fugacity provides a driving force for net passive chemical transport from high to low fugacity (*5*). Thus, in food chains, PCBs and other persistent hydrophobic organic chemicals appear to move against the thermodynamic gradient, i.e., from a low fugacity (in the prey) to a high fugacity (in the predator), which can only occur if organisms contain an active uptake transport mechanism. However, there is no evidence that an active uptake mechanism exists for simple hydrophobic organic chemicals in fish or other organisms. In fact, hydrophobic chemicals readily pass biological membranes by passive diffusion due to their lipophilicity (*6*), gill uptake of hydrophobic organic chemicals in fish is well recognized as being driven by a lipid–water equilibrium partitioning process where the chemical fugacity in the fish attempts to achieve (but not exceed) the fugacity in the water (*7*), and gastrointestinal absorption of PCBs in goldfish appears to be due to passive diffusion (*8*). But if there is indeed no active transport, then how do hydrophobic organic chemicals biomagnify in food chains?

To explain the phenomenon of biomagnification and food chain accumulation of persistent hydrophobic organic chemicals, it has been hypothesized that food digestion and absorption in the gastrointestinal tract (GIT) can raise the fugacity of persistent hydrophobic organic substances in the GIT above that of the consumed food (*3*, *9*). Food digestion in the GIT is believed to alter the composition of the food, causing the fugacity capacity of the food in the GIT to fall below that of the consumed food, hence raising the chemical fugacity in the GIT above that of the food (*9*). Food absorption is expected to "magnify" the chemical concentration in the food, hence raising the chemical fugacity in the GIT over that in the food (*9*). Food digestion and absorption combined thus raise the chemical fugacity in the GIT above that of the food, and simple passive diffusion of the chemical from the GIT into the fish can then explain why hydrophobic organic chemicals can achieve fugacities in the organism that exceed those in the food that these organisms consume. To test this hypothesis, experiments have been performed that indicate that passive diffusion is the main transport mechanism for gastrointestinal absorption in fish (*8*). The results provide indirect evidence for the proposed biomagnification mechanism, but they do not demonstrate the increase in chemical fugacity in the GIT that is the essence of the proposed mechanism.

In this study, we present direct measurements of the change in the fugacity of various persistent hydrophobic organic substances in the GIT of two fish species. The results demonstrate that the fugacity of some nonmetabolizable hydrophobic organic chemicals in the food increases while the food is being digested in the gastrointestinal tract. These findings provide, in our opinion, conclusive evidence supporting the digestion hypothesis for the mechanism of biomagnification and food chain accumulation of hydrophobic organic chemicals.

## *Theoretical Section*

Bioconcentration is the process where biological organisms absorb chemical substances from the water, resulting in chemical concentrations in the organisms that exceed those in the water. For organic substances, the driving force of the bioconcentration process is an equilibrium partitioning process of the chemical between the

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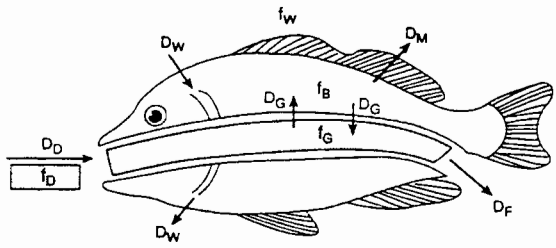


**Figure 1.** Conceptual diagram of the kinetics of chemical uptake and elimination in fish using fugacity-based terminology. The transport parameter $D_W$ represents chemical exchange via the gills, $D_G$ represents chemical exchange across the GIT, $D_D$ represents chemical intake through food intake, $D_F$ is chemical elimination through the egestion of fecal matter, and $D_M$ is metabolic transformation. $f_D$ is the chemical fugacity in the diet, $f_G$ is the fugacity in the GIT, and $f_B$ is the fugacity in the organism.

organism and the ambient water. This partitioning process attempts to achieve a chemical equilibrium where the chemical fugacities in the organism ($f_B$) and water ($f_W$) are equal (*10*). A "chemical equilibrium" is defined here as being different from a "steady-state" in which chemical fluxes into and out of a compartment are equal. A chemical equilibrium can only be reached if (i) the chemical substance is not metabolized by the organism or degraded (*11*), (ii) the chemical is not eliminated to a significant extent through fecal egestion (*12*), (iii) sufficient time is allowed to reach equilibrium (*13*), and (iv) the growth of the organism is insignificant (*14*). If any of these conditions do not apply (e.g., the chemical is metabolized), then a chemical equilibrium cannot be reached, and the fugacity of the chemical in the organism $f_B$ will be less than that in the water $f_W$. If the bioconcentration theory is applied to describe the distribution of a persistent, nonmetabolizable chemical (e.g., PCBs) in a food web, then chemical fugacities in all organisms of the food web are similar and equal or less than the chemical fugacity in the water. However, a fugacity-based analysis of the actual distribution of PCBs and some other high $K_{OW}$ organochlorines ($\log K_{OW} > 5.5$) in aquatic food chains shows that chemical fugacities in organisms are exceeding those in the water ($f_B > f_W$) and increase with the trophic status of the organism (*3, 4*). This increase in chemical fugacity within the food chain cannot be explained by bioconcentratic and is believed to be due to biomagnification.

Biomagnification or dietary accumulation can be viewed as a two-step process (*8*). First, the chemical enters the GIT in association with food. Secondly, the chemical is absorbed by the organism from the GIT (Figure 1). If, as previous work suggests, passive diffusion from high to low fugacity is indeed the only significant mode of gastrointestinal transport, then the chemical entering the GIT at a fugacity in the food of, for example, 1 Pa and remaining in the GIT at a fugacity of 1 Pa would result in a chemical fugacity in the organism of no more than 1 Pa. Biomagnification and food chain accumulation thus could not occur unless there is an active uptake mechanism. However, if the chemical fugacity in the food is elevated from 1 to 5 Pa in the GIT, then passive diffusion could result in a chemical fugacity in the organism of up to 5 Pa and biomagnification could occur. To date such an increase of the chemical fugacity in the GIT has not been observed, but it has been suggested that food digestion could lead to an elevation of the fugacity in the GIT (*3, 8, 9*).

A mechanism for the magnification of the chemical fugacity in the GIT was formalized and documented in earlier work (*8, 9*) and is illustrated in Figure 2. In essence, the GIT is viewed as a continuously stirred compartment, which receives a constant flow ($m^3$/day) of food ($G_D$) and emits a constant flow of fecal matter ($G_F$). The difference between $G_D$ and $G_F$ is the flow of food that is actually absorbed by the organism ($G_A$). The volume of the GIT contents is thus viewed as being constant, representing a "average" condition over an extended period of time. It is assumed that the GIT contents are well-mixed and that fecal matter and GIT contents have the same composition and hence the same fugacity capacity ($Z_G$). The mechanism by which biomagnification is hypothesized to occur can then be illustrated by the following example (Figure 2), starting with a fish containing a chemical fugacity that

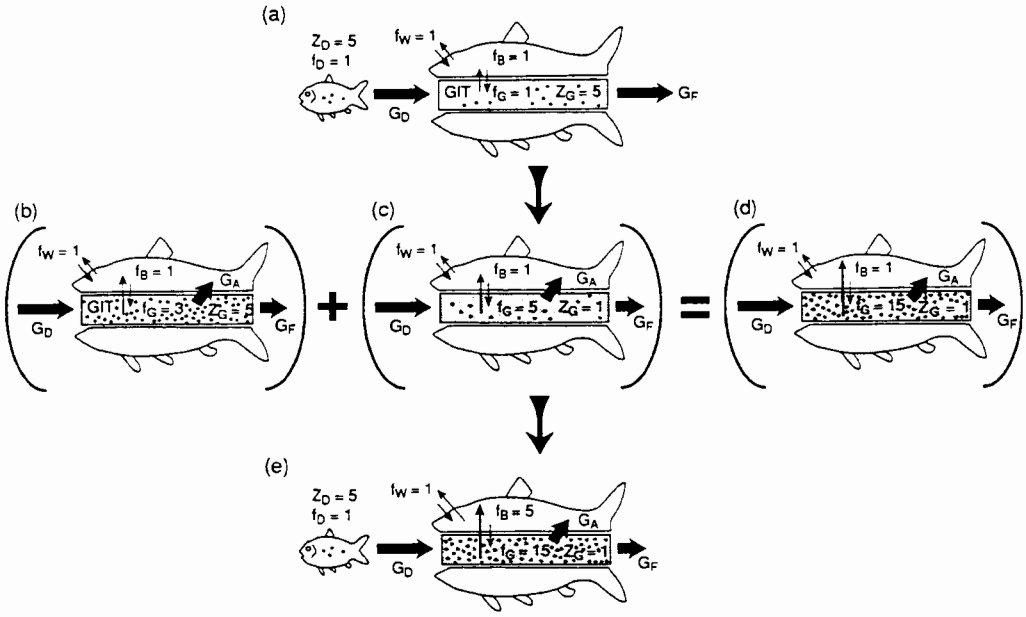


**Figure 2.** Conceptual diagram of the proposed mechanism of organic chemical biomagnification in fish, illustrating the increase of the chemical fugacity in the GIT due to food digestion and absorption. See text for explanation. Dots in GIT represent chemical concentration, gray shading represents change in fugacity capacity of the GIT contents.

**Table I. Summary of Equations Formalizing the Mechanism of Organic Chemical Magnification in Gastrointestinal Tract and Biomagnification in Fish**

mass balance equation in gastrointestinal tract:

$$N_G = V_G\, dC_G/dt = (V_G Z_G\, df_G)/dt = D_D f_D + D_G f_B - (D_F + D_G) f_G \quad (1)$$

mass balance equation in fish:

$$N_B = d(V_B C_B)/dt = d(V_B Z_B f_B)/dt = D_W f_W + D_G f_G - (D_G + D_W + D_M) f_B \quad (2)$$

assume steady state in GIT ($N_G = 0$):

$$f_G = (D_D f_D + D_G f_B)/(D_G + D_F) \quad (3)$$

substitution of eq 3 into eq 2:

$$N_B = D_W f_W + D_D D_G f_D/(D_F + D_G) - D_F D_G f_B/(D_F + D_G) - (D_W + D_M) f_B \quad (4)$$

where

chemical intake from water (mol/day): $N_W = D_W f_W = k_1 V_B C_W$ (5)

chemical intake from diet (mol/day): $N_D = D_D D_G f_D/(D_F + D_G) = G_D E C_D$ (6)

dietary uptake efficiency: $E = D_G/(D_F + D_G)$ (7)

chemical elimination to the water (mol/day): $D_W f_B = k_2 V_B C_B$ (8)

chemical elimination in feces (mol/day): $N_F = D_F D_G f_B/(D_F + D_G) = G_F E K_{GB} C_B$ (9)

chemical elimination through metabolic transformation (mol/day): $N_M = D_M f_B = k_M V_B C_B$ (10)

steady-state fugacity ratios:

fugacity-based GIT magnification factor ($f_W = 0$): $f_G/f_D = D_D/\{D_F + D_G(1 - D_G/(D_G + D_W + D_M))\}$ (11)

fugacity-based biomagnification factor ($f_W = 0$): $f_B/f_D = (f_G/f_D) D_G/(D_G + D_W + D_M)$ (12)

fugacity-based bioconcentration factor ($f_D = 0$): $f_B/f_W = D_W/(D_W + D_G + D_M)$ (13)

supporting equations:

concentration = fugacity × fugacity capacity

Glossary

| | |
|---|---|
| $C_B$, $C_D$, $C_G$, $C_W$ | chemical concentration (mol/m$^3$) in, respectively, organism, diet, GIT, and water |
| $f_B$, $f_D$, $f_G$, $f_W$ | chemical fugacity (Pa) in, respectively, organism, diet, GIT, and water |
| $N_B$, $N_G$ | chemical net flux (mol/day) into, respectively, the organism and the GIT |
| $N_D$, $N_W$, $N_M$ | chemical flux (mol/day) from, respectively, diet-to-organism and water-to-organism and the metabolic transformation flux |
| $V_B$, $V_G$ | volume (m$^3$) of organism and GIT |
| $D_D$, $D_F$, $D_G$, $D_M$, $D_W$ | transport parameter (mol/Pa·day) of, respectively, chemical intake through food consumption; chemical egestion by fecal excretion; chemical transfer across the gut between the GIT and the organism; metabolic transformation; and water-organism exchange through the gills |
| $Z_B$, $Z_D$, $Z_G$ | fugacity capacity (mol/m$^3$·Pa) of, respectively, organism, diet, and GIT contents |
| $G_D$, $G_F$, $G_A$ | rates (m$^3$/day) of, respectively, food intake, fecal egestion, and food absorption from the GIT |
| $E$ | dietary absorption efficiency (no units) |
| $k_1$, $k_2$, and $k_M$ | rate constants (day$^{-1}$) of, respectively, gill uptake, gill elimination, and metabolic transformation |
| $K_{GB}$ | chemical partition coefficient between GIT contents and organism (no units) |
| $\phi_D$ | rate of food intake by fish (in units of kg of food/day) |
| $\rho_D$ | density of food (kg/L) |

is equal to that in the water and the diet of the fish, i.e. $f_B = f_W = f_D = 1$ (Figure 2a). If the ingested food would pass through the GIT without being absorbed or digested, then the chemical fugacity in the GIT ($f_G$) would be that of the diet ($f_D$) and there would be no net uptake of the chemical from the GIT into the fish because $f_G$ would equal $f_B$, whereas a difference between $f_G$ and $f_B$ is required for net passive chemical transport across the GIT wall (Figure 2a). If food absorption occurs (Figure 2b), then GIT content is removed from the GIT, but initially no chemical is absorbed from the GIT since $f_G$ equals $f_B$. As food absorption from the GIT ($G_A$) is associated with an inflow of contaminated food ($G_D$) that replaces the absorbed food (hence $V_G$ is constant), the chemical mass (in moles) in the GIT essentially increases while the volume of the GIT remains the same. The result is an increase of the chemical concentration as well as the fugacity ($f_G$) in the GIT, which now increases above $f_D$ and $f_B$ of 1 Pa (Figure 2b). In addition, food absorption from and digestion in the GIT alter the composition of the consumed food in the GIT (Figure 2c). For example, in the GIT, lipids are first hydrolyzed (digestion), and the reaction products are then absorbed, causing the ability of the GIT contents to dissolve hydrophobic organic substances to fall below that of the consumed diet. Food absorption and digestion thus reduce the fugacity capacity of the GIT for the chemical ($Z_G$) below that of the consumed diet ($Z_D$), hence raising $f_G$ above $f_D$ and $f_B$ as the fugacity is inversely proportional to the fugacity capacity (Figure 2c). Food absorption and digestion combined (Figure 2d) thus raise the chemical fugacity in the GIT ($f_G$) above that of the food ($f_D$) and provide the driving force for net passive uptake of chemical from the GIT into the organism that is able to elevate $f_B$ over $f_D$ (i.e., biomagnification). The extent to which the fugacity in the organism ($f_B$) is raised above that of the food ($f_D$) and water ($f_W$) as a result of

the net uptake of the chemical from the GIT is dependent on the rate of chemical elimination from the organism through gill, skin, urine excretion, and metabolic transformation as well as the rate of growth. If, as illustrated in Figure 2e, the combined rate of chemical transformation, gill elimination, and growth dilution is small compared to the rate of chemical elimination through fecal egestion, then the high chemical fugacity in the organism can be maintained and biomagnification has occurred. However, if the rate of metabolic transformation and elimination is large compared to the rate of fecal elimination, then a chemical fugacity in the organism that is larger than that in the water cannot be maintained, the chemical fugacity in the organism will adopt a value that is equal or less than the fugacity in the water, and there is no biomagnification. For this reason, many hydrophobic organic chemicals that are metabolized or that have a $K_{OW}$ less than approximately $10^6$ do not biomagnify in aquatic food chains. However, for very hydrophobic, nonmetabolizable organic chemicals ($K_{OW}$ exceeds approximately $10^6$), such as PCBs, DDT, mirex, and toxaphene, the rate of chemical elimination via the gills, skin, urine, or metabolic transformation is too small to reduce a high fugacity in the organism, and these chemicals thus tend to biomagnify in food chains.

The mathematical model representing the mechanism described in Figure 2 is summarized in Table I. The essence of the hypothesized mechanism is formalized in eq 11, which represents the increase of the chemical fugacity in the GIT above that of the food. It is this hypothesis that is being tested in the experiments outlined below by measuring the fugacities of several hydrophobic organic chemicals in the food and feces of two fish species.

## *Experimental Section*

**Materials.** 1,2,4,5-Tetra-, penta-, and hexachlorobenzene (purity >99%) were obtained from Aldrich. 2,2′,5,5′-Tetra- and 2,2′,4,4′,6,6′-hexachlorobiphenyl and mirex were obtained from Analabs. Analytical grade *n*-hexane and petroleum ether were obtained from BDH Inc. (Vancouver, Canada). Florisil 60/100-μm mesh and silica gel 100/200-μm mesh were obtained from Supelco Canada Ltd. Anhydrous sodium sulfate, from J. T. Baker Chemical Co., was heated to 650 °C overnight and stored at 130 °C before use.

**Food Preparation.** The lipids of 143 g of dried fish food (Tetramin) were extracted in 750 mL of petroleum ether. A total of 106 g of Tetramin was then added to this lipid extract, together with 22 mg of tetrachlorobenzene, 23 mg of pentachlorobenzene, 31 mg of hexachlorobenzene, 128 mg of 2,2′,5,5′-tetrachlorobiphenyl, 460 mg of 2,2′,4,4′,6,6′-hexachlorobiphenyl, and 20 mg of mirex. This mixture was slowly stirred for 24 h at 45 °C to ensure complete mixing. The petroleum ether was then removed, while being stirred continuously, by slow evaporation over a period of 4 h. The newly prepared fish food was then analyzed for chemical concentrations, and the lipid content was measured to be 16.4% according to methods described earlier (*8*). This food was administered to the fish during the first 14 days of the experiment. In a similar manner, a series of foods were prepared with lower chemical concentrations. These foods were used to test the relationship between the chemical concentration and fugacity in the food and to measure the response of the fecal fugacity to changes in the chemical concentration in the food.

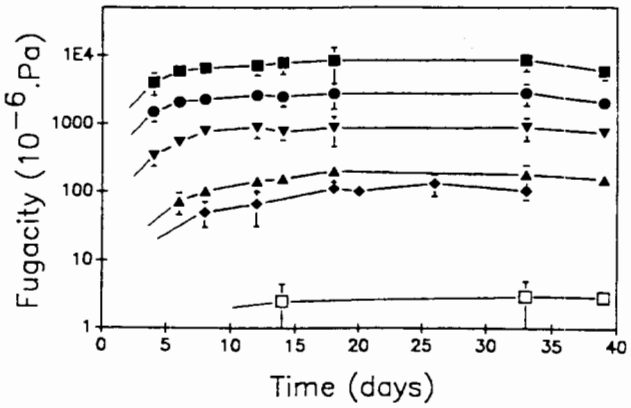


**Figure 3.** Observed fugacities (in units of $10^{-6}$ Pa) with their standard deviations of tetrachlorobenzene (■), pentachlorobenzene (●), hexachlorobenzene (▼), tetrachlorobiphenyl (♦), hexachlorobiphenyl (▲) and mirex (□) in fish food as a function of the time (in days) before head-space analysis.

**Experiments.** One hundred male guppies (*Poecilia reticulata*), with an average weight of 0.132 (±0.052) g and a lipid content 1.0 (± 0.3) %, and 48 goldfish (*Carassius auratus*), with an average weight of 0.71 (±0.23) g and a lipid content of 1.6 (±0.5) %, were exposed in individual 55-L glass fish tanks to contaminated fish food for 36 days. Throughout the experiment, the water was kept at (21 ± 2 °C), and the water was continuously aerated ($O_2$ concentration was 8.0 ppm), carbon/ammonia filtered at a rate of 3 L/min, and carbon-filtered at a rate of 15 L/min. The guppies and goldfish were fed daily at a rate of 0.015 and 0.010 g of food/g of fish, respectively. To test the response of the fecal fugacity to the chemical concentration in the food, food with "high" chemical concentrations was administered during the first 14 days of the experiment, and food with "low" chemical concentrations was administered throughout the remaining 22 days of the experiment. After 1, 5, 8, 14, 16, 20, 26, and 35 days, 50 guppies and 10 goldfish were taken from the exposure tanks, 2 h after feeding, and transferred into individual 1.0-L glass beakers containing an aluminum bottom screen, for a period of 20 h to collect feces. The fish were then returned to the exposure tanks before the next feeding period, and the feces were collected from the bottom of the glass beakers below the screen. The feces, in wet form (i.e., the feces were not dried to conserve the fecal phase characteristics), were immediately transferred in a 1.5-mL glass vial together with five glass beads. Nitrogen was then added to the vials (to replace air), and the vials were airtight sealed and kept at room temperature for a period of 21 days, after which time head-space analysis was performed. Each vial was shaken daily for approximately 30 s to homogenize the gas phase in the vials and to increase feces-to-air surface area by creating a "film" of feces on the inner wall of the vial. After head-space analysis, feces were analyzed for chemical concentrations. Samples of three guppies and two goldfish were taken from the exposure tanks after 2, 6, 14, 16, 20, 26, 34, and 36 days of exposure. The fish were killed in liquid nitrogen and frozen until analysis.

**Chemical Analysis in Fish and Food.** Fish were thawed, measured, and weighed independently. No statistically significant changes ($P < 0.05$) in fish weight were observed throughout the duration of the experiment, suggesting that there was no significant growth of the fish. Fish stomach and gut, including remaining contaminated

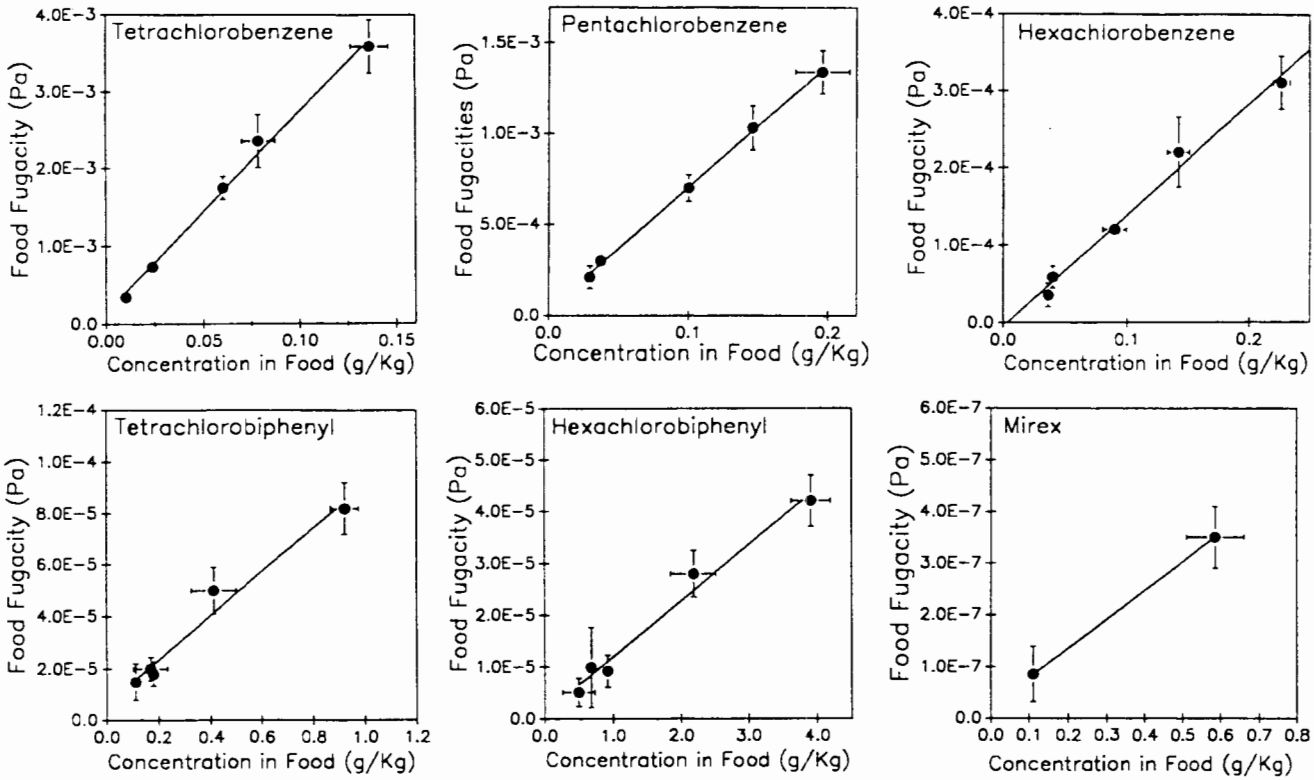


**Figure 4.** Relationship between the concentration (in g/kg) and fugacity (in Pa) of the test chemicals in fish food. Error bars represent standard deviations.

food in the GIT, were then removed. The fish were then ground to a paste in a mortar with 20 mL of anhydrous sodium sulfate. This paste was added to a 0.025 × 0.60 m glass column, containing 10 mL of anhydrous sodium sulfate, 10 mL of acidified silica [40% (w/w) sulfuric acid], 10 mL of silica gel, and glass beads. This column was eluted with 250 mL of hexane over an 8-h period. The extract was concentrated in 1 mL of isooctane and diluted in hexane for GC analysis. Food and fecal analysis were done in a similar manner. The recovery of the entire fish and food extraction procedure ranged between 87 and 100% depending on the chemical substance.

Gas chromatographic analysis was performed on a Hewlett-Packard HP5890, equipped with a 30-m DB-5 capillary column (J&W Scientific), a $^{63}$Ni electron capture detector, an on-column injector, and an integrator. Detector temperature was 300 °C, and column temperature was programmed from 30 to 290 °C. The carrier gas was ultra-high-pure grade helium. The makeup gas was ultra-high-pure grade 5% methane–95% argon. Head-space analysis was performed by injecting 100 μL of nitrogen from food and fecal sample vials with a gas-tight Hamilton syringe. Standards were prepared from the pure chemicals. Standard deviations are reported in parentheses.

## *Results and Discussion*

**Fugacity Measurements.** Figure 3 illustrates that gaseous concentrations above the fish food reached a chemical equilibrium in approximately 12–20 days, demonstrating that head-space analysis can be used to make direct measurements of the fugacity of hydrophobic organic substances in fish food. The chemical fugacity in the gas-phase $f_N$ is directly related to the measured gaseous concentration in the air $C_N$ as $f_N$ equals $C_N/Z_N$, where $Z_N$, following the ideal gas law, is 0.00041 mol/m$^3$·Pa at the experimental temperature of 21 °C (*5*). Since at chemical equilibrium the chemical fugacity in the food $f_D$ equals the chemical fugacity $f_N$ in the gas phase above the food, chemical concentrations measured in the gas phase ($C_N$) are directly related to the chemical fugacity in the food $f_D$. In a similar manner, the chemical fugacity in the feces $f_G$ can be found from $C_N$ measured in the head space above fecal samples. Chemical losses from the food and feces to the head space ranged from approximately 0.005% (tetrachlorobenzene) to $2 \times 10^{-7}$% (mirex) for the food samples and from 0.12% to $6.7 \times 10^{-6}$% for fecal samples and were considered to be insignificant. Figure 4 illustrates that the measured chemical fugacities in the food are directly proportional to the chemical concentration in the food $C_D$. The slope of the fugacity concentration plot is the reciprocal of the fugacity capacity of the food, i.e., $1/Z_D$, which is listed in Table II. As fish food was administered in the water, the effect of water content in the food on the chemical fugacity of the food was investigated. Adding water up to 100% of the weight of the dried fish food, which saturates the fish food, was found not to have a statistically significant effect ($P < 0.05$) on the chemical fugacity in the food, which can be explained by the very low fugacity capacity of the water relative to that of the food. This illustrates that the administration of the food to the water did not affect the chemical fugacity in the food. Chemical losses from the feces to the water during the 20-h feces collection have the potential to significantly reduce the fugacities of the chlorobenzenes and tetrachlorobiphenyl in the feces if the rate of feces to water transfer is large. Losses of hexachlorobiphenyl and mirex from the feces to the water are probably insignificant as estimates based on complete equilibration of the water and feces suggest chemical losses of less than 1%.

**Table II. Observed Fugacity-Based Gastrointestinal Magnification Factors $f_G/f_D$ after 14 Days of Exposure and Fugacity Capacities (mol/m³·Pa) of Diet $Z_D$ and Fecal Matter $Z_G$ for Several Hydrophobic Organic Chemicals**

| chemical | log $K_{OW}$ | $f_G/f_D$[a] | $f_G/f_D$[b] | $Z_D$ | $Z_G$[a] | $Z_G$[b] |
|---|---|---|---|---|---|---|
| tetrachlorobenzene | 4.50 | 0.070 | 0.014 | 248 | 50 | 45 |
| pentachlorobenzene | 5.03 | 0.32 | 0.13 | 833 | 167 | 167 |
| hexachlorobenzene | 5.47 | 0.64 | 0.36 | 3430 | 755 | 720 |
| tetrachlorobiphenyl | 6.10 | 2.3 | 1.2 | $5.46 \times 10^4$ | 9280 | $1.26 \times 10^4$ |
| hexachlorobiphenyl | 7.20 | 3.2 | 2.4 | $3.50 \times 10^5$ | $7.0 \times 10^4$ | $7.0 \times 10^4$ |
| mirex | 7.50 | 4.6 | 4.5 | $4.57 \times 10^6$ | $1.1 \times 10^6$ | $8.7 \times 10^5$ |

[a] Data for guppies. [b] Data for goldfish.

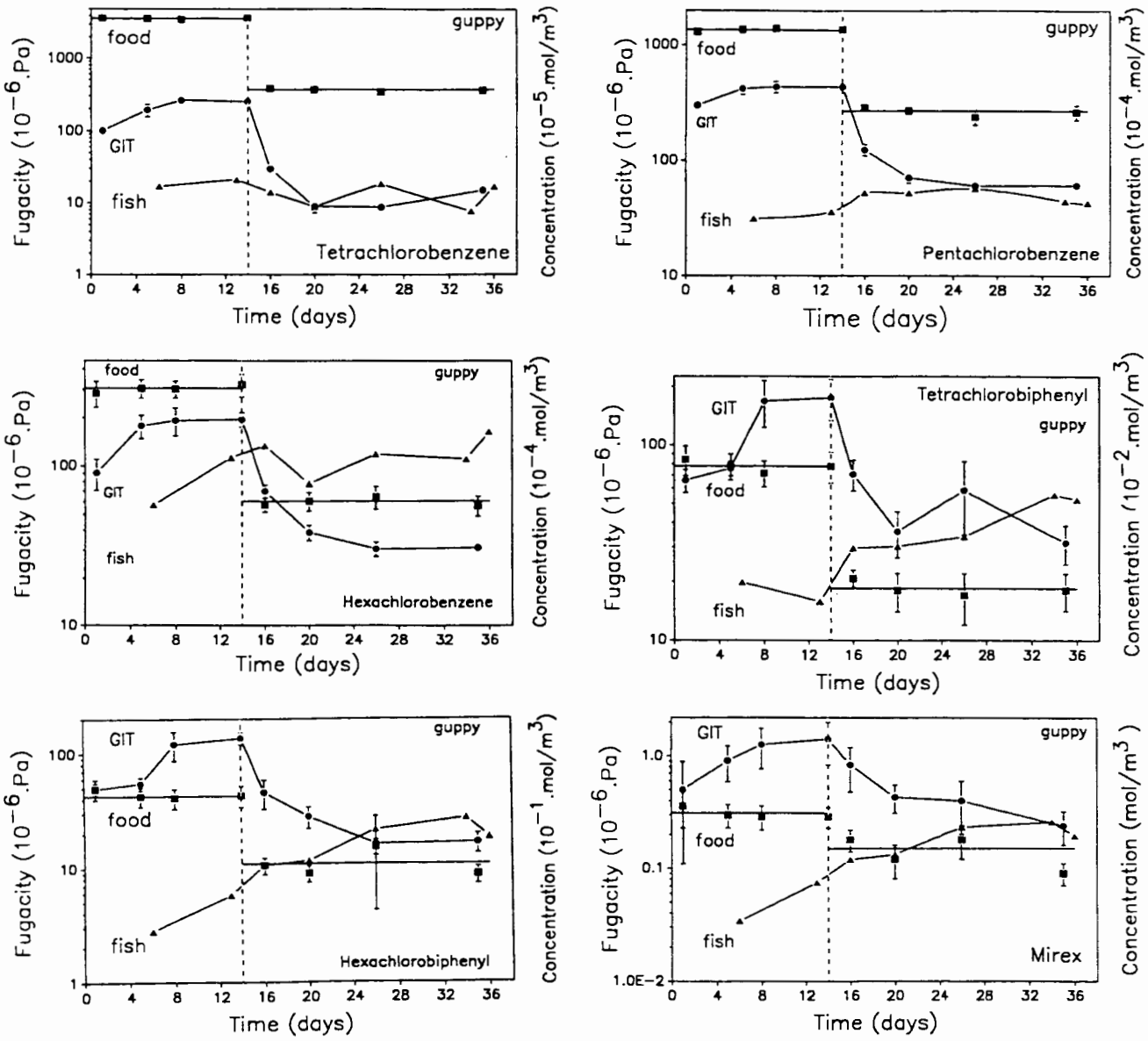


**Figure 5.** Observed fugacities (in $10^{-6}$ Pa) of the test chemicals in fish food (■) and fecal matter (●) of guppies and observed concentrations (in mol/m³) in guppies (▲) as a function of exposure time (in days). Error bars represent standard deviations.

**Gastrointestinal Magnification.** Figures 5 and 6 show that during the first 14 days of the experiment, the fugacity of tetrachlorobenzene in the feces of guppies and goldfish reached a steady-state value, which is respectively 16 and 66 times lower than the fugacity in the administered food, i.e., $f_G/f_D$ was 0.070 for guppies and 0.014 for goldfish. This shows that in the GIT the fugacity of tetrachlorobenzene reduces substantially under the experimental conditions where the chemical concentration in the water was essentially zero. However, with increasing $K_{OW}$ of the chemical, the reduction of the chemical fugacity in the GIT becomes smaller. For example, after 14 days of dietary uptake, the fugacity of hexachlorobenzene (log $K_{OW}$ = 5.47) in the feces of guppies and goldfish was respectively 1.6 and 2.8 times lower than that in the administered food. For tetra- and hexachlorobiphenyl and mirex, which have even higher $K_{OW}$ (Table II), the chemical fugacity in the feces exceeded that in the food by up to 4–5 times. This

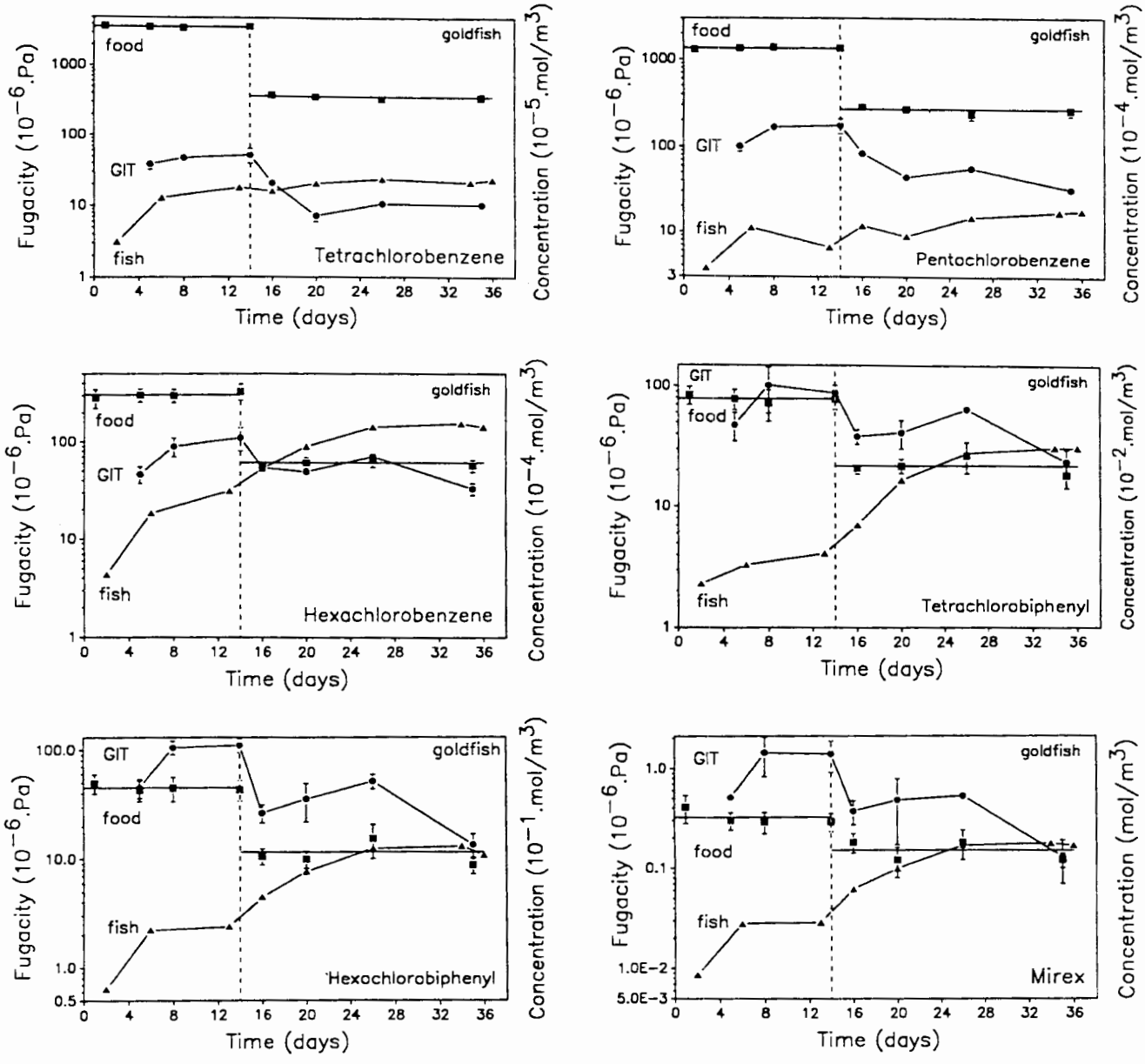


**Figure 6.** Observed fugacities (in $10^{-6}$ Pa) of the test chemicals in fish food (■) and fecal matter (●) of goldfish and observed concentrations (in mol/m$^3$) in goldfish (▲) as a function of exposure time (in days). Error bars represent standard deviations.

proves that the absorption and digestion of food in the GIT can result in a magnification of the fugacity of very hydrophobic and nonmetabolizable chemicals in the GIT over that in the food that is administered. The observed fugacity-based gastrointestinal magnification factors after the first 14 days of exposure are listed in Table II, and their relationship with $K_{OW}$ is illustrated in Figure 7. As can be observed from Figures 5 and 6, fugacities of the higher $K_{OW}$ chemicals in the GIT were not at steady state, indicating that steady-state gastrointestinal magnification factors may actually be somewhat higher than those presented in Table II and Figure 7. During the following 22 days of exposure to the low fugacity food, the fugacities of the test chemicals in the feces dropped to respond to the lower fugacity in the food, suggesting that the fugacity in the feces is proportional to the fugacity in the food.

One of the factors causing the increase of the chemical fugacity in the GIT is the reduction of the fugacity capacity of the food in the GIT. Measurements of the chemical fugacity $f_G$ and concentration $C_G$ in the feces show that

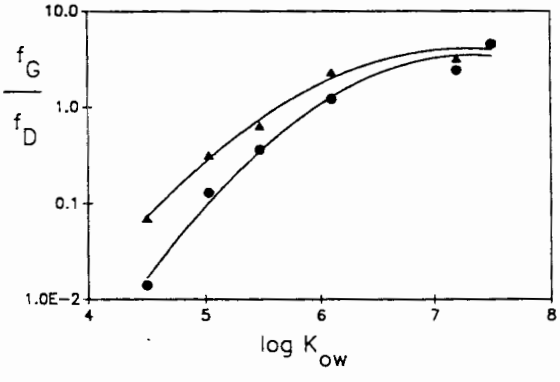


**Figure 7.** Gastrointestinal magnification factors $f_G/f_D$ of the test chemicals in guppies (▲) and goldfish (●).

the fugacity capacity of the feces $Z_G$ or $C_G/f_G$ for the test chemicals in this study is approximately 5 times smaller than that of the food that was administered (Table II). The reduction of the fugacity capacity of the food upon

**Table III. Transport Parameters (in units of μmol/day·Pa) for Food Ingestion $D_D$, Gastrointestinal Uptake $D_G$, Fecal Egestion $D_F$, and Dietary Absorption Efficiencies $E$ (%) in Guppies and Goldfish for Several Hydrophobic Organic Chemicals**

| chemical | guppies | | | | goldfish | | | |
|---|---|---|---|---|---|---|---|---|
| | $D_D$[a] | $D_G$[b] | $D_F$[c] | $E$[d] | $D_D$[e] | $D_G$[b] | $D_F$[f] | $E$[d] |
| tetrachlorobenzene | 0.38 | | 0.030 | | 1.4 | 0.32 | 0.11 | 74 |
| pentachlorobenzene | 1.3 | 0.12 | 0.10 | 55 | 4.6 | 0.38 | 0.36 | 51 |
| hexachlorobenzene | 5.2 | 0.62 | 0.47 | 57 | 18.7 | 4.2 | 1.7 | 71 |
| tetrachlorobiphenyl | 83 | 18 | 5.8 | 75 | 299 | 47 | 21 | 69 |
| hexachlorobiphenyl | 532 | 79 | 43 | 65 | 1 910 | 336 | 150 | 69 |
| mirex | 6960 | 840 | 668 | 56 | 25 000 | 2477 | 2400 | 51 |

[a] For guppies: $D_D$ is $(\phi_D/\rho_D) \times 10^{-3} Z_D$ where $\phi_D$ is $1.98 \times 10^{-6}$ kg of food/day, $\rho_D$ is 1.3 kg of food/L, and $Z_D$ is from Table II. [b] $D_G$ was derived by fitting observed concentrations in fish and fugacities in feces over the 36-day exposure period to eq 2 according to the Biofit procedure (*17*). Under the experimental conditions, eq 2 is equivalent to $dC_B/dt = k_u Z_G f_G - k_e C_B$, where $k_u$ and $k_e$ are the constants that are fitted. $D_G$ then follows as $k_u V_B Z_G$. [c] For guppies: $D_F$ is $0.4.(\phi_D/\rho_D) \times 10^{-3} Z_G$, where $Z_G$ is from Table II. [d] $E$ is derived from $D_G$ and $D_F$ according to eq 7. [e] For goldfish: $D_D$ is $(\phi_D/\rho_D) \times 10^{-3} Z_D$ where $\phi_D$ is $7.1 \times 10^{-6}$ kg of food/day, $\rho_D$ is 1.3 kg of food/L, and $Z_D$ is from Table II. [f] For goldfish: $D_F$ is $0.4.(\phi_D/\rho_D) \times 10^{-3} Z_G$, where $Z_G$ is from Table II.

ingestion is largely due to the absorption of lipids and possibly other organic matter from the food in the GIT during digestion. The lipid content of egested fecal matter of goldfish was found to be less than 3.5% and more than 4.6-fold lower than the lipid content of the administered food. This reduction of the fugacity capacity of the food in the GIT tends to raise the fugacity $f_G$ of the chemical in the GIT as $f_G$ is inversely proportional to $Z_G$.

A second factor causing the increase of the chemical fugacity in the GIT is the absorption of consumed food from the GIT, resulting in a fecal excretion rate $G_F$ that was measured to be 40 (±10) and 40 (±8)% of the corresponding dietary intake rate $G_D$ in guppies and goldfish, respectively. As explained earlier, food absorption tends to magnify the chemical concentration the GIT as the food and the chemical are absorbed independently. If the food is absorbed but the chemical is not absorbed or chemical absorption is at a lower rate, then food absorption will result in a greater mass of chemical in the GIT and, hence, a higher concentration and fugacity.

Since the reduction of the fugacity capacity of the food in the GIT and food absorption apply to all test chemicals equally, similar gastrointestinal magnification factors should have been observed for all chemicals. Figure 7 illustrates that this is not the case and that the gastrointestinal magnification factor increases with increasing $K_{OW}$. The main reason for the increase in $f_G/f_D$ is that, during the exposure period, fish were exposed to water with a virtually zero chemical concentration and fugacity in the water ($f_W = 0$). Thus, the chemical absorbed by the fish was continuously being eliminated to the water. However, it is well-recognized that the rate of chemical elimination to the water drops with increasing $K_{OW}$. Because of the slower fish-to-water elimination rates of the higher $K_{OW}$ chemicals, chemicals with a high $K_{OW}$ tend to build up a higher fugacity in the organism than chemicals of low $K_{OW}$, causing a larger flux of chemical from the organism back into the GIT and, hence, a larger fugacity in the GIT. This is further illustrated by eq 11, which shows that if $(D_W + D_M)$ becomes very small compared to $D_G$ (i.e., fish-to-water elimination is insignificant compared to chemical elimination in fecal matter), $f_G/f_D$ approaches $D_D/D_F$ or $G_D Z_D/G_F Z_G$, which is the actual "fugacity pump" that drives the chemical fugacity in the GIT above that of the food. In this ratio, $Z_D/Z_G$ reflects the change in fugacity capacity in the GIT due to digestion (and is approximately 5 in our experiments), and $G_D/G_F$ represents the increase in fugacity due to food absorption (and is approximately 2.5 in our experiments). The maximum possible gastrointestinal magnification factor in guppies and goldfish is thus expected to be approximately 12–13.

Further insights into the dynamics of chemical absorption from the GIT can be obtained by deriving the transport parameters for dietary intake ($D_D$), chemical permeation across the GIT ($D_G$), and fecal excretion rate ($D_F$) from the experimental data (Table III). Table III shows that in goldfish there is a slight tendency for $D_G$ to drop relative to $D_F$ as $K_{OW}$ increases, indicating that the rate of chemical transfer across the GIT drops relative to the rate of chemical egestion in fecal matter as $K_{OW}$ increases. These findings indicate a small decline of the dietary uptake efficiency with increasing $K_{OW}$ (Table III), especially for very high $K_{OW}$ chemicals, which agrees with results from other studies (*9*, *15*). Similar findings could not be confirmed for guppies, although the dietary uptake efficiency of the highest $K_{OW}$ chemicals also appeared to fall with increasing $K_{OW}$.

**Biomagnification and Food Chain Accumulation.** Gastrointestinal magnification can only result in biomagnification in the fish (i.e., $f_B > f_D$) if gill elimination and metabolic transformation in the fish are small compared to gastrointestinal uptake (i.e., $D_W + D_M < D_G$), causing the chemical fugacity in the fish to approach that in the GIT. The fugacity-based biomagnification factor in the fish $f_B/f_D$ is thus a function of (i) the extent of food digestion $Z_D/Z_G$, (ii) the extent of food absorption $G_D/G_F$, and (iii) the rate of gill elimination and metabolic transformation relative to the rate of gastrointestinal uptake ($D_W + D_M/D_G$). Growth of the organism can also have a significant effect on the extent of biomagnification in the organism as growth tends to reduce the chemical fugacity in the organism in a fashion similar to chemical elimination or metabolic transformation.

Since the digestive processes in fish, mammals, and birds share many common features, it is expected that the model and mechanism of biomagnification described in this study also apply in other organisms. However, since our results indicate that biomagnification is directly related to certain physiological characteristics of the digestive system of the organism, it can be expected that biomagnification factors vary among different organisms. Organisms with an

efficient digestive system may exhibit a very high $Z_D/Z_G$ and $G_D/G_F$ ratio and, thus, biomagnify nonmetabolizable substances of high $K_{OW}$ to a greater extent than organisms with a less efficient digestive system. The properties of the chemical substance are also important. Polar substances with low $K_{OW}$ are often rapidly eliminated by organisms (through the gills in fish, in urine in mammals and birds), and biomagnification cannot occur despite an effective digestive system because of a high elimination rate. Also, chemicals that are metabolized by organisms will show no or low biomagnification and food chain accumulation potential.

## *Conclusion*

The experimental findings discussed above provide conclusive evidence in support of the hypothesis that the biomagnification of hydrophobic organic chemicals in food chains is the result of food digestion and food absorption in the GIT. Food digestion and absorption can act as a fugacity pump by raising the fugacity or activity of the chemical in the GIT above that of the food that is consumed through altering the phase characteristics and, hence, the fugacity capacity of the food and through increasing the chemical concentration in the GIT. This fugacity pump applies each time that one organism is consumed by another organism causing the fugacity and activity of slowly eliminating chemical substances to increase with each step in the food chain, thus providing the driving force for food chain accumulation. For modeling the food chain accumulation of hydrophobic organic chemicals, it is important to include the biomagnification mechanism. Incorporation of the outlined biomagnification mechanism in a food chain model provided excellent agreement between predicted and observed concentrations of PCBs and some other hydrophobic organic chemicals in various organisms in Lake Ontario (*16*).

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