DETERMINATION OF THE DIETARY ASSIMILATION EFFICIENCY OF HEXACHLOROBENZENE INTO THE CHANNEL CATFISH (*Ictalurus punctatus*)

K.B. Woodburn[1], M. Goodrich[2], D.L. Rick[1], T.A. Marino[1], E.L. McClymont[1], and J.M. Barber[3]

[1] The Dow Chemical Company, Midland Michigan
[2] URS Corporation, Nashville, Tennessee
[3] The Dow Chemical Company, Freeport, Texas

Introduction

The evidence on how efficiently fish species assimilate or transfer lipophilic persistent organic pollutants (POPs) from contaminated food into their tissue has often been contradictory or absent in the scientific literature. From 1940 to the early 1970s, hexachlorobenzene (HCB) was used as a seed dressing for several crops to prevent fungal disease. The principal current sources of HCB to the environment are as a by-product of industrial production, application of HCB-contaminated pesticides, incineration of wastes, and long-range transport of HCB[1]. Clark and Mackay[2] found that HCB accumulated rapidly to a steady-state condition in freshwater guppies (*Poecilia reticulata*) with body burdens achieving 10% of the HCB concentrations present in food. These data contrast with HCB in oil-dosed food fed to a cold-water Salmonidae species, the rainbow trout, where an absorption efficiency of 80 to 90% was measured and an elimination half-life of 7 months was later reported[3]. The contradictory evidence for food chain transfer of HCB may lead to highly variable estimates of how such a lipophilic chemical accumulates within an ecosystem food web. In addition, no studies have been conducted on HCB assimilation efficiency with benthic-feeding fish species, such as the channel catfish. Current environmental risk assessment (ERA) models often assume a worst-case assimilation efficiency value of 90% for HCB in development of predicted residue values for protection of wildlife organisms.

Objective

The objective of this study was to measure the assimilation efficiency of HCB from dietary exposure into a bottom-dwelling fish species, the channel catfish. The common default assumption for HCB assimilation efficiency in fish species is typically a value of 90%. Bottom-dwelling fish, such as catfish, represent a critical exposure pathway for the receptors of concern in a sediment-based wildlife exposure food web model and none of the reported assimilation efficiencies for HCB are specific to benthic-feeding fish. Finally, an empirical measurement of HCB assimilation efficiency in the catfish will allow for calibration of the modeling schemes used in ERAs, which will result in more accurate estimates of accumulation of HCB in an ecological food web.

Assimilation Efficiency Model

The following schematic diagram (Figure 1) identifies the important exposure pathways and pharmacokinetic parameters that relate exposure to uptake, clearance, and potential accumulation of environmental chemicals from water and food.

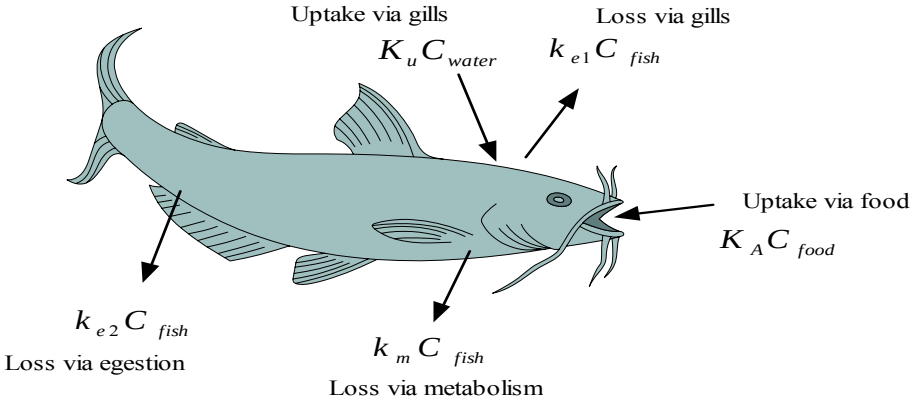

Figure 1. Critical uptake and clearance routes for chemicals in fish.

Aquatic organisms in contact with hydrophobic chemicals accumulate the chemical via water uptake across the gills and from their diet. Clearance, on the other hand, is a function of metabolism and excretion (egestion) and/or loss via elimination through the gills. The accumulation of very hydrophobic compounds (log K_{ow} >5) into fish is often dominated by dietary input[4]. In addition, work on HCB found that ingestion of contaminated sediments is a greater contributor to body burden HCB levels of a bottom-dwelling fish species (gizzard shad) than water uptake/ventilation[5]. We can model this food/chemical assimilation process with a simple 'two-box' system:

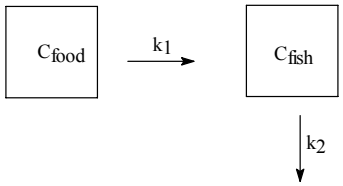

Figure 2. Two-box model describing dietary uptake and clearance of chemicals in fish.

The differential equation describing the change in chemical concentration in fish with respect to time is:

$$dC_{fish}/dt = k_1C_{food} - k_2C_{fish} \quad (1)$$

where C_{fish} and C_{food} are the specific chemical concentrations (ng-chemical/g-wet weight basis) in fish tissue and foodstuffs, respectively, and k_1 (g-food adsorbed/g-fish/day) and k_2 (day^{-1}) are the uptake and clearance rate constants, respectively.

Solving analytically,

$$C_{fish}(t) = k_1/k_2 * C_{food}(1 - \exp^{-k_2 t}) \quad (2)$$

The biomagnification factor or BMF expresses chemical accumulation via the diet at steady state:

$$BMF = C_{fish}/C_{food} = k_1/k_2 \quad (3)$$

The intestinal absorption efficiency (α) for a chemical administered through the food vector moving across the intestinal epithelium into fish tissue can be estimated from knowledge of k_1 (the uptake rate) and the food feeding rate ('f'):

$$k_1 = f*\alpha \quad (4)$$

where 'f' is the feeding rate (g-food consumed/g-fish/day) and α is the assimilation efficiency (g-food adsorbed/g-food consumed).

Measurement of ^{14}C activity in both food (C_{food}) and fish (C_{fish}) residues, respectively, from the two-box system allowed use of Equation (1) to solve for rate constants k_1 and k_2. Knowledge of k_1 and k_2 and the feeding rate 'f' allows determination of an experimental value for the assimilation efficiency (α) for HCB into channel catfish.

Materials and Methods

The test material was uniformly labeled ^{14}C-HCB from New England Nuclear, with a nominal specific activity of 30.725 mCi/mmole (239 disintegrations per minute [DPM] per ng) and a radiochemical purity of 98.9%. The ^{14}C-HCB was dissolved in acetone and a 100-mL aliquot mixed with 1000 g of catfish food ("Silver Cup" catfish food manufactured by Nelson & Sons, Inc. of Murray, UT) to achieve a nominal dose level of 340 ng ^{14}C-HCB/g of food; the mean measured concentration was of 327 ng ^{14}C-HCB/g. A negative control of unspiked feed was also set concurrently with the test treatment.

Channel catfish (*Ictalurus punctatus*) weighing approximately 2 to 5 g each and approximately 5-10 cm in total length were used in the study. Limited evidence suggests that metabolic systems are essentially unchanged with fish size, with the principal differences being a larger gill surface area-to-mass ratio and higher lipid content for smaller fish[6].

One set of fish samples was dissected to remove the undigested ^{14}C-labeled food material from the catfish digestive system. In the second set of samples taken on the same sampling day, fish were left intact and all ^{14}C activity was measured, including undigested ^{14}C-labeled food material in the gut contents of the fish.

The study design was based on a fish bioconcentration study guideline of the U.S. EPA. The test design included:

- 90 fish/treatment, with 4 fish dissected and analyzed per sampling date. Aquaria of 135 L volume.
- Fish were individually measured for total ^{14}C activity (per wet weight of fish).
- Fish were sampled and solvent extracted for HPLC radioassay of fish ^{14}C activity on exposure days 14, 21, and 28.
- Continuous flow-through exposure at 10 turnovers/day.
- Fish were fed at a nominal rate of 2% per day (measured rate of 2.1% per day). Uneaten food was removed after 1 hour.
- Water temperature: 22 +/- 1 ^{o}C (thermostated)
- 28-day exposure to treated food, followed by 14-day clearance period (untreated food).
- Fish sampled for total ^{14}C activity on exposure days 0, 3, 5, 7, 10, 14, 21 and 28, and clearance days 0, 3, 7, 10, and 14.

Results and Discussion

Measured ^{14}C activity was detected in fish from initial day 3 of exposure through clearance day 14 (cumulative day 42). As shown in Figure 3, the experimental residue data were fit to the first-order two-box feeding model. Mean values of 0.0135 g-food absorbed/g-fish/day and 0.037 day^{-1}, respectively, were calculated for the uptake (k_1) and clearance (k_2) rate constants for catfish with the undigested ^{14}C-spiked food particles removed from their intestines.

Knowledge of the mean feeding rate of 2.1% per day allows determination of the assimilation efficiency value (α) for HCB in catfish of 64%. The steady-state biomagnification factor or BMF for ^{14}C-HCB in whole fish was 0.4, which indicates that catfish exposed to dietary HCB will accumulate 40% of an exposed dose under steady state conditions. Application of the first-order feeding model to the elimination rate constant (k_2) produces an elimination half-life of 19 days for ^{14}C-HCB in channel catfish. The elimination half-life for total ^{14}C activity in channel catfish indicates that total body burden of HCB will decline following removal of the contaminant source.

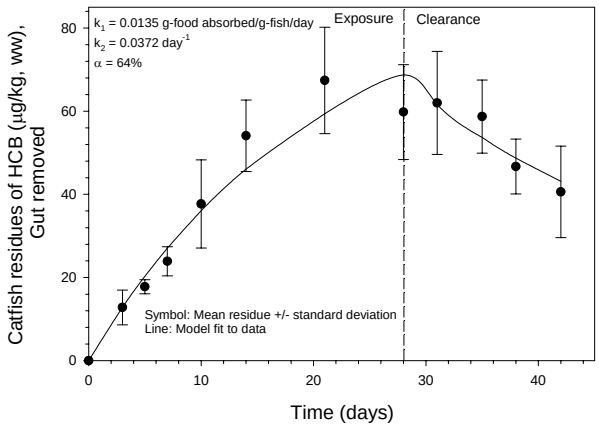

Figure 3. Application of a two-box kinetic feeding model (line) to mean measured residue concentrations (symbols) of ^{14}C-HCB in channel catfish.

HPLC radioassay of extracted ^{14}C activity from whole catfish found essentially no measurable metabolism of ^{14}C-HCB activity in catfish tissue following 28 days of dietary exposure.

Whereas the assimilation efficiency (α) from the ERA model-assumes a value of 90% for HCB, the experimentally measured value is 64% in channel catfish. The measured assimilation efficiency reduces the uncertainty inherent in the ERA model assumption (90%) and results in an estimated increase in

allowable HCB sediment/food residues for protection of wildlife by approximately 1.5- to 2.0-fold. In this assessment, catfish food was used as a surrogate material for sedimentary materials containing HCB. It is a worst-case scenario, as the assimilation of HCB from sediment material would likely have a lower efficiency than from fish food for bottom-dwelling fish species.

As a conservative estimate of transfer efficiency, the measured food assimilation efficiency value of 64% in the benthic feeding catfish may be used in any risk assessment process for establishing remediation limits for HCB in sediment. The use of this measured transfer efficiency may have significant implications for any corrective actions proposed for HCB-contaminated sediment. Additionally, the measured assimilation of HCB in catfish allowed for a point calibration of the proposed octanol-water partitioning regressions for assimilation coefficients of POPs suggested by numerous authors[3,7,8,9]. The final regression considered to present the best fit is that of Fisk *et al*.[8] and it has been re-calibrated to include the observed catfish assimilation for HCB of 64% (see Figure 4):

$$\alpha = 10^{-1.8 + (1.085 \times \log K_{ow}) - (0.08 \times \log K_{ow}^{2})} \quad [5]$$

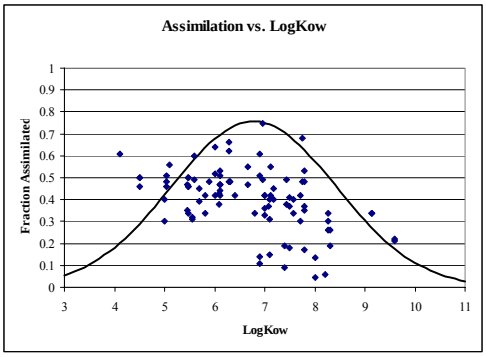

Figure 4. Assimilation efficiency as a function of log K_{ow}; data from Fisk et al.[8] but also including 64% value for HCB/catfish.

The resultant regression as calibrated using the catfish data is plotted here with the assimilation efficiencies reported in the literature. The plot shows that the use of a common default value of $\alpha = 90\%$ is unrealistic and that a calibrated regression based on the data present here remains conservative for the evaluation of food chain transfers of not only HCB but other persistent organic pollutants as well.

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