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Calibration of the Freshwater Mussel, *Elliptio complanata*, for Quantitative Biomonitoring of Hexachlorobenzene and Octachlorostyrene in Aquatic Systems

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The fresh water mussel Elliptio complanata has been frequently used to monitor organic contaminant exposure in the Great Lakes (Curry 1977/78; Kauss and Hamdy 1985; Pugsley et al. 1985; Muncaster 1987). However, to translate observed body burdens in the mussel to chemical concentrations in the water, the relationship between chemical concentrations in the water and the mussel should be established. This relationship is controlled by the uptake and elimination kinetics of chemicals in the mussel. This study reports a bioconcentration experiment in Elliptio complanata for hexachlorobenzene and octachlorostyrene. It demonstrates (i) the derivation of uptake and elimination rate constants from the experimental data, (ii) the use of kinetic rate constants in establishing chemical specific relationships for chemical concentrations in the mussel and the water and (iii) the role of these relationships in the planning and interpretation of biomonitoring studies.

MATERIALS AND METHODS

Hexachlorobenzene (HCB) was purchased from the Aldrich Chemical Co., Milwaukee WI and octachlorostyrene (OCS) was obtained from Foxboro/Analabs, North Haven CT. Glass distilled acetonitrile and petroleum ether were from BDH Inc., Toronto Ont. Pesticide grade n-hexane was from Caledon Laboratories Ltd., Georgetown Ont. Florisil (60-100 mesh) was from Supelco Canada Ltd., Oakville Ont. Anhydrous sodium sulphate from J.T. Baker Chemical Co., Phillipsburg NJ was heated to 650°C overnight and stored at 130°C before use.

Mussels (Elliptio complanata) were from Balsam Lake, Ontario (44°23'N,78°50'W), and held in 700-L tanks until used experimentally. Windsor tap water (hardness=104 ug/L, pH=7.4, dissolved O_2=10 ug/L) was continuously filtered,

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aerated, and held at 10°C. The 69 mussels used in the experiment averaged 6.02 (± 0.30) g in weight and 67.4 (± 1.0) mm in length. Analysis of collected mussels before the start of the experiment showed no detectable levels of HCB and OCS.

The uptake phase of the experiment was performed in a continuous flow apparatus similar to that used by Bruggeman et al. (1984), Opperhuizen et al. (1985) and Gobas et al. (1989). It consisted of a 100-L glass tank filled with dechlorinated Windsor tap water at 20°C. To contaminate the water, an Asti teflon pump (Cole-Parmer Instrument Co., Chicago IL) circulated water through a generator column and a glass fish tank at 60 L/hr. All fittings and tubing were of teflon and glass. The generator column was prepared by dissolving 150 mg of HCB and OCS in hexane. The hexane was evaporated from the solution onto glass wool, resulting in "coating" the glass wool with HCB and OCS. The contaminated glass wool was placed at the bottom of a 1-L column and covered with hexane rinsed glass wool. The water was equilibrated for 3 wk before mussels were added. Three mussels and two water samples were taken daily for 11 days. All mussels were shucked and weighed when sampled, then frozen immediately.

The elimination phase was performed in a 50-L glass tank. The water was filtered continuously by an Eheim activated carbon power filter at a rate of 270 L/hr. Mussels were sampled in triplicate for 11 days in the same manner as previously described.

Individual mussels were homogenized for approximately 1 min in a mixture of 120 mL of acetonitrile and 40 mL distilled, deionized water using a Brinkman Polytron (Sybron Canada Ltd., Rexdale Ont.). The mixture was filtered by suction through a Whatmann no. 1 glass filter and the liquid phase collected for liquid-liquid extraction in petroleum ether. The filtered aqueous phase was extracted 3 times in a total of 300 mL of petroleum ether. The organic phase was separated and passed through a column containing 30 g sodium sulphate (for drying), then concentrated to a 5 mL volume in a Kuderna-Danish (K-D) evaporator. This extract was passed through a 20-g florisil column and eluted with 200 mL petroleum ether to remove lipids. This extract was concentrated and analyzed by gas chromatography.

Water samples of 500 mL were collected daily during the uptake phase and were extracted 3 times in a total of 300 mL petroleum ether. The extract was dried by passing it through a sodium sulphate column, then concentrated on a K-D evaporator and analyzed by gas chromatography.

Gas chromatographic analysis was performed on a Hewlett-Packard 5790A (Hewlett-Packard Canada Ltd., Mississauga Ont.) equipped with a DB-5 capillary column (J&W Scientific, Folsom CA), a ^{63}Ni electron capture detector, a Hewlett-Packard auto-injector and a Hewlett-Packard 3390A integrator. Injector temperature was 250°C, detector temperature was 300°C, and column temperature was programmed from 50 to 250°C. Carrier gas was ultra high purity helium at 1.5 mL/min. Make-up gas was 5% methane-95% argon at 60 mL/min, and the injection mode was splitless. Injection volume was 1 uL.

Average lipid content of the mussels was determined by homogenizing 10 clams in a blender in a solution of 60% chloroform and 40% methanol. The mixture was filtered by suction through a Whatmann no. 1 glass filter and the solvent was collected for evaporation after which the lipids were determined by weight.

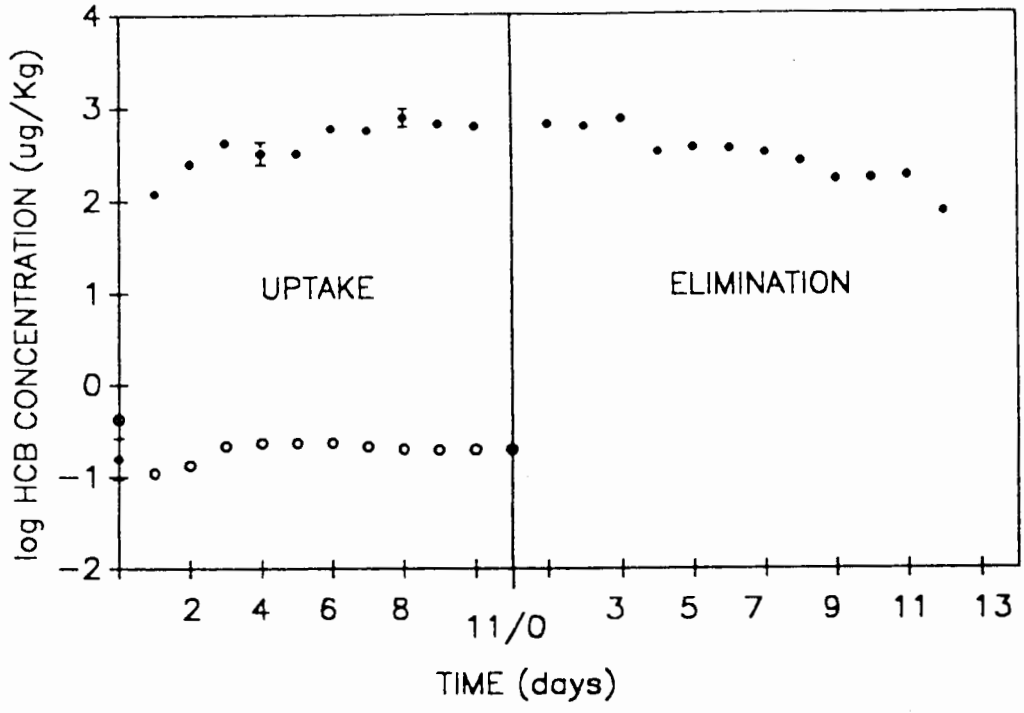
RESULTS AND DISCUSSION

Figure 1 illustrates the observed concentrations of HCB and OCS in the water and in the mussel as a function of the exposure time. It shows that the initial concentrations of HCB (0.43 ug/L) and OCS (0.16 ug/L) in the water are below the reported aqueous solubilities of 4.69 ug/L for HCB (Miller et al. 1985) and 2.5 ug/L (Bjerk et al. 1980) for OCS. This indicates that HCB and OCS were truly dissolved. After the mussels were added, the HCB and OCS concentrations in the water dropped rapidly due to the high initial uptake rates in the mussels. HCB and OCS concentrations in the water restored after 3 to 4 days to approximately half the initial water concentration and remained approximately constant throughout the remainder of the uptake period. At the end of the exposure period a steady-state appears to have been reached for HCB but not for OCS. During the depuration phase, where the mussels were exposed to uncontaminated water, concentrations of HCB and OCS in the mussel declined logarithmically over time. This suggests that the kinetics of these chemicals can be well described by a simple mussel-water two-compartment model with first order rate constants and thus by the following differential equation.

$$dC_M/dt = k_1.C_W - k_2.C_M \qquad (1)$$

where C_M is the chemical concentration in the mussel, C_W is the concentration in the water, t is time and k_1 and k_2 are the rate constants for chemical uptake from the water and elimination to the water. If the C_W is constant, equation 1 can be integrated to give:

$$C_M = C_W.(k_1/k_2).(1-\exp(-k_2.t)) \qquad (2)$$



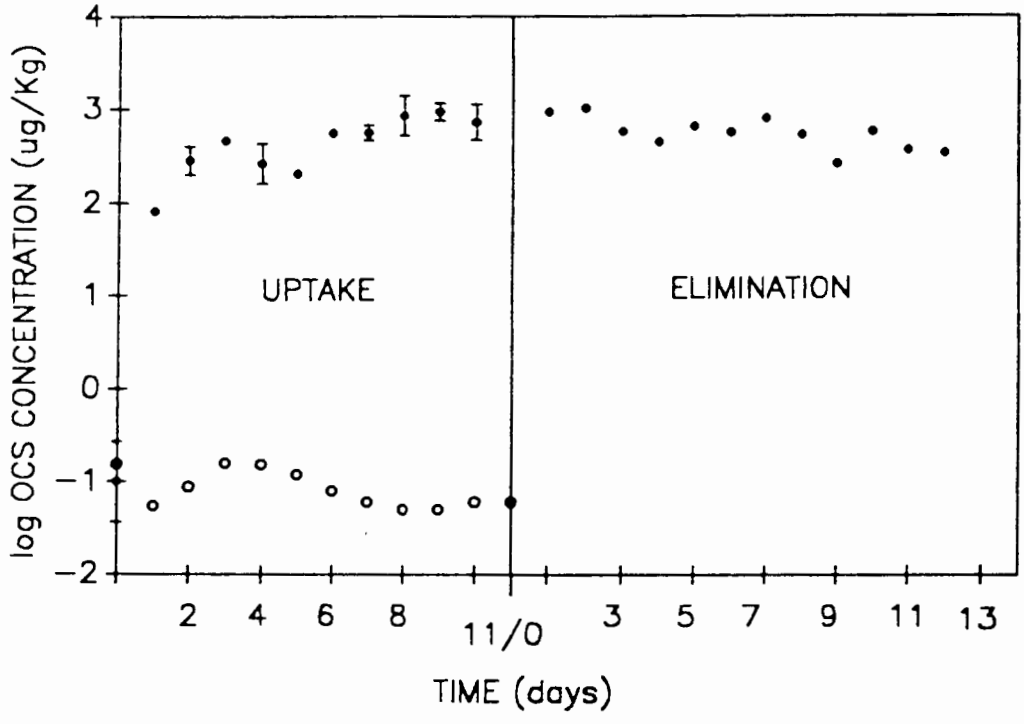


Figure 1. Logarithms of chemical concentrations in the mussel in ug/Kg (closed circles) and in the water in ug/L (open circles). Error bars on mussel tissue concentrations are 95% confidence intervals. When not shown, confidence intervals were too small to be depicted.

At steady-state (i.e., infinite exposure time or $dC_M/dt=0$) the mussel/water concentration ratio (C_M/C_W) reaches a constant value, i.e., the bioconcentration factor (BCF) k_1/k_2. If lipids are the primary storage site for organic chemicals in the mussel, the BCF may reflect the chemical's lipid-water partition coefficient K_L through the mussel's lipid content L_M, i.e., K_L equals $(k_1/k_2.L_F)$ or $(C_M/C_W.L_F)$.

The elimination rate constant k_2 can be determined from the slope of the log C_M versus time plot as $2.303.d(\log C_M)/dt$. This results in values of 0.178 (± 0.036) d^{-1} for HCB and 0.070 (± 0.045) d^{-1} for OCS, where the values within brackets are 95% confidence intervals. This corresponds to half-life times of respectively 3.9 and 9.9 days and demonstrates that chemicals with higher K_{OW} (i.e. log K_{OW} of HCB and OCS are respectively 5.45 and 6.29) have higher half-life times in the mussel. This corresponds to observations by Pruel et al. (1986) in the blue mussel Mytilus edulis and Gobas et al. (1989) in fish. It follows that to reach a steady-state for OCS, mussels should be deployed for a longer period of time than for HCB.

Since the HCB and OCS concentrations in the water were not constant during the uptake phase and the uptake phase was too short for OCS to reach a steady-state, k_1 and BCF can not be simply deduced from the data using equation 2 or by data fitting computer programs such as BIOFAC (Blau and Algin 1978). To derive the correct values for k_1 and BCF under these conditions, Gobas et al. (1989) suggested a numerical integration procedure. This procedure involves the calculation of increments in mussel concentrations dC_M over time intervals dt from equation 1, i.e.:

$$dC_M = (k_1.C_W - k_2.C_M).dt \qquad (3)$$

where dt was chosen to be 0.04 d, k_2 was 0.178 d^{-1} for HCB and 0.07 d^{-1} for OCS and C_M is the sum of all previous dC_M, i.e., ΣdC_M. The chemical concentration in the water C_W at every exposure time t, i.e., Σdt, was estimated by fitting the observed water concentrations to a series of linear functions, which each connect the observed water concentration data at two consecutive exposure times. The uptake rate constant k_1 was then selected to produce the best agreement between calculated and observed concentrations in the mussel. The best fit of the observed data was the one with the k_1 value, for which the sum of the squared differences between calculated and observed concentrations in the mussel was the smallest. The uptake rate constants were determined to be 650 d^{-1} for HCB and 1010 d^{-1} for OCS.

The BCFs of HCB and OCS can now be calculated as k_1/k_2 and

are respectively 3,650 and 14,500. The k_1 and BCFs calculated following this procedure are not affected by the duration of the uptake period or by variations in the concentration in the water. These BCFs can also be expressed on a lipid weight basis, as the ratio of the chemical concentrations in the lipid tissue of the mussel and the water, i.e., K_L, by dividing the BCFs by the weight-based lipid fraction of the mussels, i.e., 0.0084. This results in log K_L values of 5.64 for HCB and 6.24 for OCS, which correspond excellently with the reported log K_{OW} values of 5.45 for HCB and 6.29 for OCS. This suggests that the 1-octanol-water partition coefficient is an excellent predictor of the bioconcentration factor of these chemicals in Elliptio complanata.

Studies by Roesijadi et al. (1978a and 1978b) and by Pruel et al. (1986) in Mytilus edulis suggest that water is the predominant source for PAH and PCB bioaccumulation and that uptake of chemical from particulate matter is a relatively insignificant route of exposure. This is further supported by field studies, in which Elliptio complanata was deployed at particulate rich and poor sites (Muncaster 1987). If the water is indeed the major source for uptake and bioaccumulation of HCB and OCS, Elliptio complanata can be used to monitor the concentrations of these chemicals in the water. Measured steady-state concentration in the deployed mussel can simply be related to the chemical concentrations in the water through the BCF or K_{OW}, i.e., C_W equals $C_M/(L_M.K_L)$ or $C_M/(L_M.K_{OW})$.

The length of time, for which the mussels should be deployed to reach 95% of steady-state, is $-\ln 0.05/k_2$ or $3.0/k_2$. For HCB this means that the mussels should be deployed for 17 days and for OCS 43 days. Deployment times of 21 days (Kauss and Hamdy 1985) and 40 days (Pugsley et al. 1985, Muncaster 1987) may therefore be sufficient for monitoring of HCB, but they are too short for OCS.

It can be concluded that when organisms are to be used as a tool to measure chemical concentrations in water, they need to be "calibrated". This can be achieved by determining the uptake and elimination kinetics of the chemicals of interest in a laboratory experiment, but preferably in the field. As demonstrated for HCB and OCS in the mussel, this results in practical relationships between chemical concentrations in the organism and the water and an appropriate duration of deployment. These relationships can be improved when experimental data regarding the role of physical and physiological factors such as temperature and oxygen levels become available.

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