Development and Application of a Simplified *in vivo* Test for Estimating Biotransformation Rate Constants of Organic Chemicals in Fish

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Introduction

Many chemicals manufactured for industrial and domestic use are hydrophobic [1]. Hydrophobic organic chemicals (HOCs) are of concern due to their ability to achieve high concentrations potentially causing biological effects in organisms. Therefore, such chemicals require evaluation to identify any potential adverse effects they may incur on humans and the environment [2]. Bioaccumulation metrics, such as bioconcentration factors (BCFs), are often used in regulatory evaluations to assess a chemical's potential for bioaccumulation in organisms [3,4]. Protocols exist to measure BCFs (e.g. OECD 305). However, these experiments have long completion times and require large amounts of material and animals [2]. Therefore, streamlined methodologies for acquiring BCF data are needed. Passive dosing (PD) systems are now being used frequently for aquatic *in vitro* bioaccumulation tests. PD systems involve the partitioning of HOCs from a loaded reservoir (e.g. polymer) into the test medium (e.g. water) [5,1]. PD is considered a practical alternative for dosing HOCs in experimental systems as it overcomes many of the limitations identified in other dosing designs used for measuring BCFs [1]. However, PD has not previously been used for *in vivo* bioaccumulation tests. In this study, an experimental approach for a streamlined aqueous *in vivo* bioaccumulation test using a PD system was developed and applied to measure depuration rate constants (k_T) and biotransformation rate constants (k_{MET}) that can be used in whole organism bioaccumulation models to estimate the BCF. This study demonstrates that BCF can be measured directly using a passive-dosing design. The method incorporates the use of non-biotransformed reference chemicals for calculating biotransformation rates. The kinetics of test chemicals in rainbow trout are compared to those of reference chemicals to derive k_{MET} [4]. The study further aims to generate high quality data on biotransformation rates of test chemicals that will be used to test the ability of quantitative structure–activity relationship (QSAR) models and in vitro-in vivo (IVIV) extrapolation methods to estimate biotransformation rates and corresponding bioconcentration factors of HOCs in fish [6,7,8].

Materials and methods

Partitioning experiment

A 52 L glass aquarium containing 40 L of clean filtered de-chlorinated water was set up with a filter containing the passive dosing system. The passive-dosing system consisted of 100 g of poly(ethylene co-vinyl acetate) (EVA) beads dosed with the test and reference chemicals contained in a mesh bag. The filter containing the passive dosing system was run for 14 days and water was sampled throughout. For analysis, the analytes concentrations were obtained using solvent-solvent extractions followed by gas-chromatography mass spectrometry (GCMS) analysis using an Agilent 6890 gas chromatograph (GC) attached to an Agilent 5973N mass spectrometer (MS) (Agilent technologies, Santa Clara , United States).

Fish experiment

Rainbow trout, approximately 10g in weight, were purchased from a local trout hatchery. Three 52 L glass aquariums with filters (flow rate) were used for the tests: One for control, one for the test chemical and one for a

mixture of the test and reference chemicals. The aquaria were held in a cold room at Simon Fraser University with a 12 h light/12 h dark photoperiod and 30 fish were transferred from acclimatization tanks to each aquarium for the exposure phase. Fish were exposed to levels of the test chemical concentrations (~ 2µg/L) for 7 days. During the exposure no fish were sampled. Following the exposure, fish were transferred to three separate flow-through tanks supplied with de-chlorinated water to begin a 14-day depuration phase. The fish were sampled in triplicate (n=3) from each aquarium based on a schedule determined using a bioaccumulation model for each test chemical. Analytes were extracted from fish tissue using a Quick, Easy, Cheap, Effective, Rugged and Safe (QuECHERS) technique and analyzed using GCMS (Agilent technologies, Santa Clara, United States).

Results and discussion

Partitioning experiment

Water concentrations measured throughout the partitioning experiment revealed that the chemicals reached equilibrium between the EVA and the water after 2 days of running the filter (Figure 1). This confirmed the first component of the research to identify whether partitioning would be adequate to obtain quantifiable concentrations in the water. EVA-water partition coefficients (K_{ew}) were calculated from the partitioning experiment by measuring the chemical concentrations in the EVA and the water once the water reached equilibrium. The measured log K_{ew} and log K_{ow} values obtained from the literature as shown in Figure 2 indicate that there is a relationship between the two measures [9,10]. This relationship will be further investigated.

Fish experiment

No fish mortalities were observed throughout the experiments in either the exposure or control groups. Behavior and appearance of fish in exposure and control groups were similar. The analysis is still in progress and therefore results are limited to the partitioning study. Fish samples taken during the depuration phase will be used to determine the biotransformation rate constant (k_M), the growth rate constant (k_G), the elimination rate constant (k_E) and the wet weight, lipid normalized and growth corrected BCF of the test chemicals. The chemical concentrations in the fish are expected to decrease throughout the 14-day depuration phase. There should be no difference in depuration rates between fish exposed to one test chemical and fish exposed to a mixture of the test and reference chemicals.

Implications

This study will provide a method for calculating hydrophobic organic chemical biotransformation rate constants in fish and potentially contribute to efforts for testing hydrophobic organic chemicals more efficiently for their biotransformative and bioaccumulative behaviors. This research tests the feasibility of a stream-lined test design for measuring bioaccumulation metrics in fish to reduce measurements without compromising accuracy. Data acquired from this research will then be compared to *in vitro* results from previous research to determine the accuracy of in vitro studies [11]. Finally, the proposed research will yield insight into whether the presence of multiple chemicals has an effect on the biotransformation rate of individual substances [12].

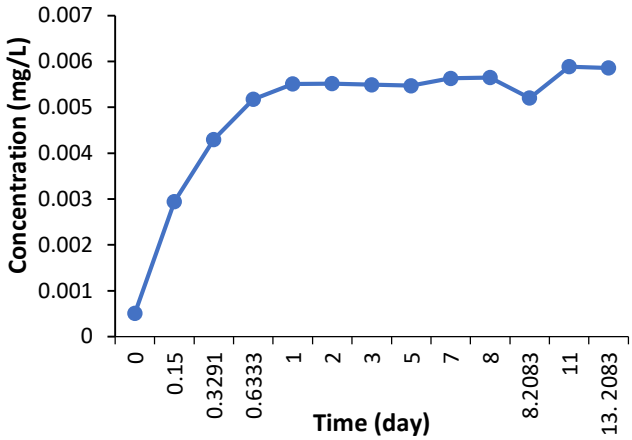

Figure 1. The concentration of methoxychlor in water throughout the partitioning experiment (0.005-0.006 mg/L after day 1).

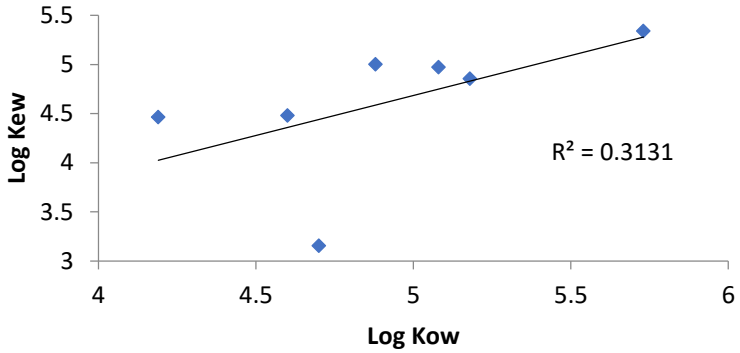

Figure 2. The relationship between log K_{ow} (octanol-water partition coefficient) and log K_{ew} (EVA-water partition coefficient). Log K_{ow} values are obtained from the literature [9,10].

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