An Innovative Injury Quantification Approach for Organisms Exposed to AhR-Active Compounds

David F. Ludwig*, Timothy J. Iannuzzi*, Kurunthachalam Kannan**, John P. Giesy**, Stephen H. Safe***, Linda M. Schmeising****, Nicholas W. Gard*****, Michael L. Moore*****, and Kevin T. Connor*.

*Exponent, 8201 Corporate Drive, Suite 680, Landover, MD, 20785, USA
** Michigan State University, Dept. of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI, 48824-1222, USA
*** Texas A&M University, College Station, TX, 77843, USA
**** Exponent, 4940 Pearl East Circle, Suite 300, Boulder, CO, 80301, USA
***** Exponent, 15375 SE 30th Place, Suite 250, Bellevue, WA, 98007, USA

Introduction

Halogenated aromatic hydrocarbons (HAHs) that act by binding to the aryl hydrocarbon receptor (AhR) in vertebrates are reportedly additive in their toxicological effects on biological endpoints affecting reproduction in organisms including fish, birds, and mammals (1, 2, 3). These endpoints are particularly important for natural resource damage (NRD) injury assessments because they are potentially linked to impacts on populations of organisms.

In aquatic sediments, particularly in urban and/or industrial waterways, complex mixtures of pollutants are often present. A number of these chemicals may have AhR, and thus contribute to biological effects mediated by the AhR. It is often critical, for effective risk management decision making, to understand effects that may be specifically attributable to one or more component of the chemical mixture.

The objective of the investigation reported here is to quantify the incremental contribution of polychlorinated biphenyls (PCBs) to total AhR-mediated toxicity in a river system containing a large number of different pollutants. To accomplish this, we developed and implemented an approach that allows us to partition potential PCB effects from total AhR activity in a sample, and apportion the PCB contribution to total potential ecological injury.

Materials and Methods

The study site is a relatively large river in an agricultural, residential, and industrial watershed of the northern United States. The river bottom is generally depositional in nature. Sediments have accumulated a large number of organic chemicals and metals, including halogenated compounds, polyaromatic hydrocarbons, pesticides, and other compounds with toxic effects known or suspected to be mediated through the AhR. PCBs in the river are being investigated by government and industry on an ongoing basis.

Fish of several species, representing the most important trophic guilds (forage fish, large bottom feeders, water column feeders, carnivores) were collected over a three-year period throughout the

river and the large freshwater embayment into which the river flows. Fish tissues were extracted and analyzed for individual PCB congeners by gas chromatography (GC/MS and GC/ECD), and for total AhR with H4IIE rat hepatoma cell bioassays. Fish tissues were extracted using procedures such that the final extracts to be used for the bioassays would contain all PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), structurally related halogenated aromatic hydrocarbons (e.g., polybrominated biphenyls, chloronaphthalenes, diphenyl ethers), chlorinated pesticides (e.g. dieldrin, DDE, mirex) and polycyclic aromatic hydrocarbons (PAHs). Split samples were generated following homogenization of whole body samples, prior to methylene chloride extraction and cleanup with gel permeation chromatography. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) concentrations in each fish extract was determined by assessing ethoxyresorufin-O-deethylase (EROD) activity using the H4IIE cell bioassay. Stock cultures of H4IIE rat hepatoma cells purchased from the American Type Culture Collection (ATCC No. CRL 1548) were cultured using standard cell tissue culture procedures (5). EROD activity and protein concentration were determined in triplicate on a CytoFluor® 2350 plate reader. TCDD standard curves were generated for each sample batch. Samples were diluted in 400 mL DMSO to ensure the maximal activity of each extract would be observed. The final results were expressed as the mean (±1 standard deviation) TCDD equivalent concentration per unit mass of wet weight tissue. Approximately 112 individual fish were analyzed for both PCB congeners and total AhR.

PCB congener data were converted to potential congener-specific AhR effects concentrations by applying toxic equivalency factors (TEFs) established in other studies with HII4E bioassays (4). PCB contribution to total AhR-mediated activity in each sample was evaluated by comparing the sum of congener-specific TEFs and the site-specific H4IIE bioassay results.

Results and Discussion

Assessing incremental injuries to organisms from individual AhR active chemicals requires the evaluation of the total AhR activity, for all AhR active compounds present as well as the incremental activity from individual chemicals. A reasonable approach to this problem is to determine the total AhR activity using a bioassay, then to measure the concentration of specific chemicals for which the injury assessment is being performed. Using a toxicity equivalence scheme, measured concentrations of individual chemicals can be used to estimate their incremental contribution to the total AhR activity. Alternatively, an attempt to estimate total AhR activity could be made by individually measuring the concentrations of all Ah-active chemicals. There are two key limitations to this latter approach. First, many of the chemicals that exhibit AhR activity have not been identified, and the contributing chemicals vary between sites. Thus, the total analytically determined AhR activity may substantially underestimate the actual activity. Second, the analytical work associated with measuring multiple chemicals is often cost prohibitive. The holistic approach presented here suffers from neither of these limitations and possesses the considerable advantage of being applicable for determining the incremental contribution of any group of AhR active chemicals found in environmental media.

The results of bioassay investigations for fish from the study site suggest that the injuries that may be reasonably predicted on the basis of AhR activity are the result of a variety of compounds. Table 1 presents the results of the H4IIE bioassays for each of several species caught in the study

site. The TEQs measured in the bioassay ranged from 90 to 3,299 ppt. The mean TEQ for most species was near the high end of the range of bioassay TEQs reported in other studies of Great Lakes fish (6, 7). TEQs calculated on the basis of measured concentrations of individual PCB congeners and corresponding TEFs ranged from 1.5 to 1,600 ppt. The incremental contribution analysis suggests that PCBs contribute 7 to 23 percent of total AhR activity based on congener-specific TEF summation, but that the PCB contribution for individual species was as low as 1 percent and as high as 57 percent. The remaining activity is likely attributable to the large number of halogenated and other organic compounds present in environmental media to which fish are exposed.

Table 1. Toxicity equivalents (TEQs) in extracts of different fish species caught in the study site: Comparison of bioassay derived TEQs and TEQs calculated on the basis of analytical data.

Species	Bioassay TEQs			Calculated TEQs			
	Min.	Max.	Mean	Min.	Max.	Mean	$\mu_{Calc\text{-}TEQ}/\mu_{Bio\text{-}TEQ}$
Alewife (n=5)	147	309	228	1.6	205	53	23%
Carp (n=17)	90	780	339	1.5	251	34	10%
Gizzard Shad (n=8)	265	3299	1105	1.7	212	78	7%
Northern Pike (n=15)	125	2877	711	1.5	453	99	13%
Smallmouth Bass (n=13)	248	676	406	1.5	165	41	10%
Walleye (n=19)	134	837	386	1.5	1600	220	57%
White Sucker (n=3)	222	593	407	1.6	86	30	7.3%
Yellow Perch (n=18)	151	884	350	1.5	13	3.3	0.94 %

All values in ppt.

The ultimate application of these findings is for injury determination and environmental management decision making. Management decisions for PCBs must account for the fact that PCBs contribute a relatively low proportion of total sediment AhR activity. A substantial component of ecological risks associated with such activity is due to compounds other than PCBs. Sources of compounds include runoff from agricultural and urbanized areas, point source discharges of industrial chemicals, and aerial deposition of a broad range of natural and anthropogenic compounds. Environmental restoration potential is also constrained by the ability of the system to recover to levels represented by the effects of pollutants remaining after the PCB increment is accounted for. Thus, quantitative understanding of the PCB contribution to total sediment activity is important both for understanding the effects of pollutants in the system and for making decisions regarding remediation and restoration of sediments.

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