Detecting the Effects of Environmental Contaminants on Thyroid Hormones: A Preliminary Report on a Short-Term Dosing Model in the Rat

Kevin M. Crofton*, Elena S. Craft*, David J. Ross**and Michael J. DeVito**

*Neurotoxicology Division (MD-74B) and **Experimental Toxicology Division (MD35), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Abstract

Previous reports have suggested that developmental exposure to polychlorinated biphenyls (PCBs), dioxin, and other PHAHs (polyhalogenated aromatic hydrocarbons) decrease levels of the circulating thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). The purpose of this project is to develop and validate an animal model to test for additive or non-additive interactions of mixtures on thyroid hormone homeostasis. Initial efforts have focused on developing a short-term dosing protocol to characterize contaminant effects on serum thyroid hormones and CYP1A1 (EROD activity) as biomarkers for disruption of thyroid hormone homeostasis and Ah receptor activation, respectively. In this study, Long Evans rats (female, 27 days of age) were dosed with varying concentrations of PHAHs for four consecutive days. Dose response curves were generated for the following chemicals: PCB 118, PCB 77, PCB 153, PCB 126, PCB 162, TCDD, and 2,3,4,7,8-PeCDF. One day post-dosing, the animals were sacrificed and serum and livers were harvested. T_3 and T_4 were measured via radio-immunoassays. CYP1A1 activity was determined from liver microsomes. All PCB congeners, dioxins, and PeCDF were found to produce abundant decreases in T_4 and only slight sporadic decreases in T_3. PHAHs, with the exception of PCB153 and PCB 162, also caused increased EROD activity. Data collected to date suggest that this short-term dosing protocol is adequate for detecting PHAH-induced hypothyroxenemia and CYP1A1 induction. Future studies will involve dosing animals with combinations of these chemicals to determine the possibility of additive and/or non-additive effects on thyroid hormones.

Introduction

There is mounting evidence, from both animal models and epidemiological studies, that a number of diverse classes of xenobiotics act as thyrotoxicants (1-3). Thyrotoxicants, broadly defined, are chemicals that disrupt any aspect of thyroid hormone (TH) synthesis, release, transport, metabolism, and/or activation of nuclear receptors. There are several ways that environmental xenobiotics can alter circulating thyroid hormone concentrations. First, the chemicals can alter the synthesis and/or release of thyroid hormones from the thyroid gland (4). Second, these chemicals may displace T4 from serum binding proteins such as transthyretin (TTR) (5,6). A third pathway through which PHAHs can alter circulating T4 is by increasing

glucuronidation through induction of UDPGT isomers (7,8). It is possible that chemicals acting on different sites of thyroid hormone regulation will cause additive or synergistic interactions.

Understanding the effects of endocrine disrupting chemicals on thyroid hormone is important in determining the overall threat that these chemicals may pose to human health. Effects of low level exposure to endocrine disrupting chemicals are of particular concern because most exposures are to complex mixtures of these compounds. Before studying the adverse effects of these mixtures on thyroid hormones, however, it is necessary to determine dose-response functions for each individual chemical. Once this data is collected, then mixture studies devoted to examining the additive or non-additive interactions of these various compounds can be completed. The present study examined the effects of various PHAHs on serum TH concentrations in a short-term dosing protocol. In addition, CYP1A1 activity was determined in order to compare the relative potencies of Ah receptor activation with TH decreases. Data collected from the short-term dosing protocols will provide a basis for future studies examining the additive and/or non-additive effects of mixtures of thyroid disrupting chemicals.

Materials and Methods

Subjects: For each chemical tested, 24 day-old Long Evans female rats (Charles River Laboratories, NC) were received and given a four-day acclimation period. The animals were housed 2 per cage in standard plastic hanging cages (24x20x45 cm) in a AAALAC approved facility. Food (Purina Lab Chow) was available ad libitum and the photoperiod was 12L:12D (0600-1800). Temperature was maintained at 21.0°C ±2°C while the relative humidity was 50 ±10%. All animal care and research procedures were approved by the National Health Effects and Environmental Research Laboratory of the U.S. Environmental Protection Agency.

General Procedures: All PCB congeners (commercially available from AccuStandard, Inc.), dioxin, and PeCDF were dissolved in corn oil and administered in either 1.0 or 2.0 ml/kg by oral gavage. For each dose group, 6-8 animals were weighed daily and dosed for four consecutive days. The dose range for each chemical is listed in Table 1. Blood and livers were collected from animals killed 24 hours post-dosing. Samples were frozen at -80°C until assayed.

Table 1: Chemical names, common abbreviations, and administered dosages.

Chemical Name	Abbreviation	Dosages
2,3,7,8-tetrachlorodibenzo-p-dioxin	TCDD	0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 ug/kg
2,3,4,7,8-pentachlorodibenzofuran	4-PECDF	0.03, 0.09, 0.3, 0.9, 3, 9, 30, 90 ug/kg
3,4,3',4'-tetrachlorobiphenyl	PCB 77	0.1, 0.3, 1, 3, 10, 30 mg/kg
2,4,5,3',4'-pentachlorobiphenyl	PCB 118	0.01,0.03, 0.1, 0.3, 1, 3, 10 mg/kg
3,4,3',4',5'-pentachlorobiphenyl	PCB 126	0.03, 0.1, 0.3, 1, 3, 10, 30, 100 ug/kg
2,4,5,2',4',5'-hexachlorobiphenyl	PCB 153	0.9, 3, 9, 20, 30, 90 mg/kg
2,3,5,3',4',5'-hexachlorobiphenyl	PCB162	15, 60 mg/kg

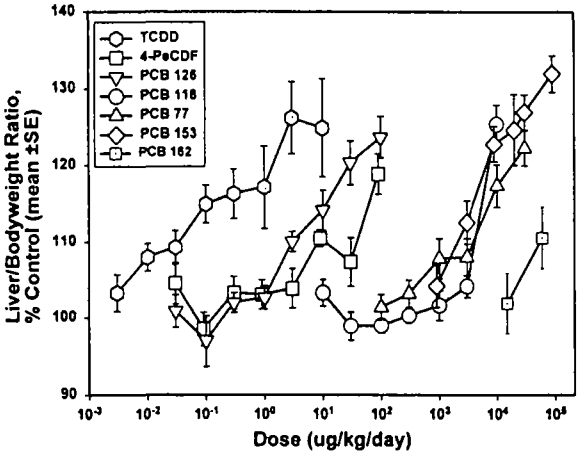

Figure 1. Increased liver-to-body weight ratios after short-term repeated exposure to PHAHs.

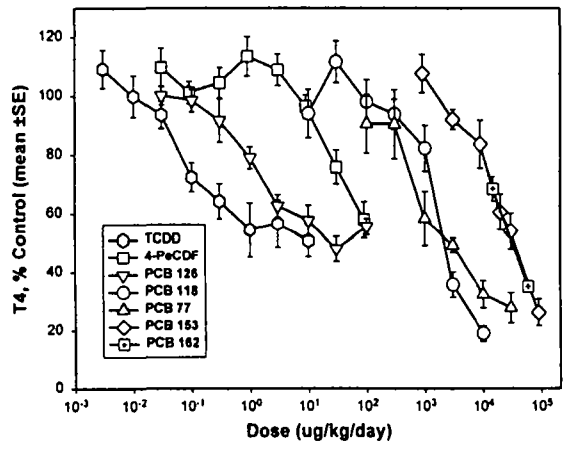

Figure 2. Concentration of total serum T4 decreased following four-day exposure to PHAHs.

Thyroid Hormones : T_3 and T_4 hormone concentrations were measured from serum using standard solid phase ^{125}I radioimmunoassay Coat-A-Count kits from Diagnostic Products Corporation. The assays were completed according to the methods of Goldey et al (9).

CYP1A1 Activity: The CYP1A1 assay was completed from microsomes prepared from liver samples collected during the experiment. EROD activity was determined according to Devito et al (10).

Results

Body and liver weight data demonstrated that PHAHs significantly increased liver weight gain in accordance with dose concentration. Body weight/liver weight ratios are shown in Figure 1. All PCBs, dioxin, and PeCDF were shown to significantly decrease circulating levels of T4 (see Figure 1). Dioxin and the dioxin-like chemicals (such as 4-PeCDF and PCB 126) were more potent, yet appeared to be less efficacious, compared to the other PCBs tested. Only slight and sporadic decreases were noted in serum T3 concentrations (data not shown). EROD activity (a biomarker for CYP1A1 induction also increased in relation to the dose concentration

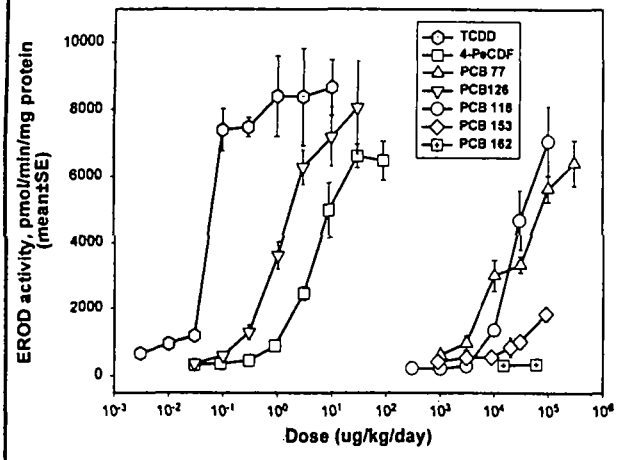

Figure 3. EROD activity, a marker for CYP1A1 induction, following short-term exposure to PHAHs.

received for all chemicals, except PCB 162 (Figure 3).

The induction following PCB153 is likey a result of cross-reactivity with CYP2B isoforms. The induction following PCB162 was extremely minimal, only about 50% over control levels, and was not statistically significant.

Discussion

Although there has been a limited number of chemicals studied, current data suggest that the short-term dose protocol used in this experiment may be an adequate model to detect and characterize environmental chemicals that adversely impact thyroid hormone homeostasis. Potencies for induction of EROD activity, as well as depression of T4 are consistent with other reports (Safe, 1994).

Future work on this model with involve: 1) expanding the investigation to include other classes of chemicals such as TH synthesis inhibitors (e.g., amitrole) or iodine uptake blockers (e.g., perchlorate); 2) characterizing the potency for induction of UDPGT activity; and 3) determining whether mixtures of environmental xenobiotics are capable of additive and/or non-additive interactions.

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