Comparison of functional endpoints of PCB exposure: Dose-response analysis with Aroclor 1260

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Introduction

Polychlorinated biphenyls (PCBs) elicit multiple toxic effects, including hepatic enzyme induction, alteration of the endocrine system, and suppression of the immune system, especially at high exposure levels.[1-4] Information about the relative sensitivity of various endpoints such as endocrine disruption or immune suppression is useful for predicting the type of toxicity that might be expected at different exposure levels. Comparative information about the relative sensitivity of different responses can be gleaned from published studies, but these studies often involve single PCB congeners and differ with respect to the species tested, dosages used, and duration and route of exposure.

The present study was undertaken to determine the relative sensitivity of functional responses to PCB exposure over a wide range of dosages within the same experimental design. Aroclor 1260, a technical PCB mixture, was selected as a surrogate environmental mixture on the basis that the congener composition of Aroclor 1260 is somewhat similar to that of environmental samples, which typically consist of more highly chlorinated congeners with a relatively high concentration of non-coplanar di-*ortho* -substituted PCBs. Responses including anti-SRBC titer, thyroid hormone levels, hepatic microsomal CYP1A and CYP2B enzyme induction and testosterone hydroxylase activities were measured in adult male rats following a ten-day exposure to Aroclor 1260.

Materials and Methods

Chemicals: Aroclor 1260 (Monsanto Chemical Co., St. Louis, MO) was a gift from Dr. S.H. Safe (Texas A& M University, College Station, TX). The Aroclor 1260 sample was from the same batch as that used and analyzed previously.[5]

Animal treatment: Nine-week old male Long Evans rats were treated with Aroclor 1260 by oral gavage for 10 consecutive days at dosages ranging from 0.025 to 156 mg/kg/day and killed two days after the last treatment. On day 4 of treatment, all rats were injected with sheep red blood cells ($2x10^8$ SRBCs), for determination of humoral immunity. Blood samples were collected on day 10 of treatment for anti-SRBC IgM determination, and at the time of termination for hormone assays. Liver, thymus, testes, seminal vesicle, and ventral prostate were dissected, blotted dry and weighed. Liver samples were used for preparation of hepatic microsomes.

Assays: Anti-SRBC IgM titer was measured as described.[6] Serum testosterone and thyroxine concentrations were determined using RIA kits. Hepatic microsomal testosterone hydroxylase activity was measured as described previously.[7] Quantitative immunoblot analysis for hepatic microsomal CYP1A1, CYP1A2, CYP2B1, CYP2B2, and CYP2C11 protein levels was performed as described previously.[5] No observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), and ED_{50} values were calculated for each endpoint measured.

Statistical analysis: One-way analysis of variance (ANOVA) with Student-Newman-Keuls multiple comparisons post test was performed using Instat software Ver. 3.0 (GraphPad Software, Inc., San Diego, CA). A p value less than 0.05 was considered to be statistically significant. For data that conformed to a sigmoidal dose-response relationship, curve fitting equations supplied by SigmaPlot Ver. 8.0 (SPSS Sciences, Inc., Chicago, IL) were used to calculate ED_{50} values.

Results and Discussion

Treatment with Aroclor 1260 had a minimal effect on body weight, but resulted in increased liver weight at dosages greater than 1.25 mg/kg/day. There was no change in thymus weight or on testis, seminal vesicle, and ventral prostate weights. Aroclor 1260 also produced marked dose-dependent reductions in anti-SRBC IgM and serum thyroxine levels beginning at dosages of 1.25 and 3.125 mg/kg/day, respectively. There was a large dose-dependent induction of hepatic CYP2B protein levels and a corresponding alteration in CYP-mediated testosterone metabolism following treatment with Aroclor 1260. Testosterone 2β - and 16β -hydroxylase activities were increased, while testosterone 2α -hydroxylase activity was decreased, at dosages of 1.25-3.125 mg/kg/day and greater. Similarly, CYP2B1 and CYP2B2 enzyme levels were induced, whereas CYP2C11 enzyme was suppressed, at dosages greater than 1.25 mg/kg/day. Hepatic CYP1A1 and CYP1A2 protein expression was also induced following Aroclor 1260 exposure but to a much lesser extent than CYP2B enzymes.

To determine the relative sensitivity of immune, endocrine, and hepatic responses following Aroclor 1260 exposure, ED_{50} values, NOAEL levels, and LOAEL levels were calculated and compared (see Table 1). The results indicate that induction of hepatic testosterone 16β–hydroxylase activity and suppression of anti-SRBC IgM titer with ED_{50} values of 1 to 1.5 mg/kg/day are the most sensitive responses to Aroclor 1260 exposure, followed closely by induction of testosterone 2β–hydroxylase activity, induction of CYP2B2 and CYP2B1 protein levels, and suppression of serum T4 levels, in that order. In contrast, body weight was the least sensitive response to Aroclor 1260 exposure.

Table 1. No Observable Adverse Effect Levels, Lowest Observable Adverse Effect Levels and ED_{50} Values of Responses Measured in Rats Following Treatment with Aroclor 1260

Response	ED_{50} (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)
Hepatic testosterone 16β -hydroxylase activity	1.2-1.3	0.625	1.25
Serum anti-SRBC IgM titer	1.4-1.5	1.25	3.125
Hepatic testosterone 2β -hydroxylase activity	2.2-3.1	1.25	3.125
Hepatic CYP2B2 content	3.1-4.0	1.25	3.125
Serum T4 concentration	3.5-4.7	3.125	6.25
Hepatic CYP2B1 content	5.4-6.3	1.25	3.125
Total hepatic CYP content	6.3	0.625	1.25
Liver weight	6.4-15.6	1.25	3.125
Hepatic CYP1A2 content	6.3-21.3	15.625	31.25
Hepatic testosterone 2α -hydroxylase activity	7.7-10.4	1.25	3.125
Hepatic testosterone 7α -hydroxylase activity	7.7-15.6	3.125	6.25
Body weight	n.a.	78.125	156.25

Note: ED_{50} , medial effective dose, denotes the estimated exposure level at which the response is half of the observed maximal response. A range of ED_{50} values, which were determined using different curve fitting models, is presented. NOAEL, no observable adverse effect level is defined as the highest exposure level at which the response between the treated and control group is not significantly different. LOAEL, lowest observable adverse effect level is defined as the lowest exposure level at which the response between the treated and control group is significantly different.

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