

DIFFERENTIAL TOXICITIES OF TWO LOTS OF AROCLOR 1254

Deborah E. Burgin, Janet J. DilibertoH, Prasad R. KodavantiI, Linda S. BirnbaumH

UNC Curriculum in Toxicology/US Environmental Protection Agency, MD 74, 86 T.W.

Alexander Dr., Research Triangle Park, NC 27711, USA

H Experimental Toxicology Division, USEPA, NHEERL, RTP, NC 27711, USA

I Neurotoxicology Division, USEPA, NHEERL, RTP, NC 27711, USA

Introduction

Aroclors are complex commercial mixtures of polychlorinated biphenyl (PCBs). Aroclor 1254 is defined by the weight percentage of chlorine; but the PCB congener composition varies from lot to lot. In previously reported toxicity studies using Aroclor 1254 there has been a discrepancy in the results. Two lots that have been used in toxicity studies and are representative of commercial Aroclor mixtures, #124-191 and #6024, were analyzed for their congener composition. A relative potency ranking scheme was created for risk assessment of complex mixtures of dioxin-like PCBs. (1). The TEF (toxic equivalency factor) assigns relative potency value to polyhalogenated aromatics based on mechanism of action via the Ah receptor. 2,3,7,8 tetrachlordibenzo-*p*-dioxin (TCDD) is the most toxic of the structurally related polyhalogenated aromatic hydrocarbons and thus has a TEF = 1. Lot #6024 has approximately ten times the dioxin toxic equivalents (TEQ) of Lot #124-191 (see Table 1). The purpose of this study was to determine if the difference in the TEQ for the two lots explained the different responses seen on a weight basis. Male Long Evans rats (70 days old) were treated with graded doses of 0 to 1000 mg/kg po for each lot (See Table 2). Hepatic EROD, MROD, PROD and serum T3/T4 concentrations were determined. Dose levels were chosen based on the paper by Nishida *et al* (2) where they exposed rats to Aroclor 1254 and looked for behavioral and motor activity effects. Calculated TEQs were based on factors from the WHO Consensus TEFs for Mammals, Fish, and Birds (3), and the congener concentrations present in the two lots were determined by ECD and GC/MS (see Table 1).

Materials and Methods

Chemicals: The two Aroclor lots were obtained from AccuStandard (New Haven, CT).

Animal Treatment Male Long Evans rats (Charles River Laboratories, Inc., Raleigh, NC), 70 days old were maintained on a 12-hr light/dark cycle at 21.0±2°C with 55±5% relative humidity and housed individually with free access to food and water. Dose groups (n=5; 300-400 grams) were treated via oral gavage in corn oil (See Table 2). Three days after treatment, animals were killed by CO₂ asphyxiation. Blood was collected for total T3/T4 analysis; livers were removed, weighed and analyzed for enzymatic activity.

Cytochrome P450 activity: Ethoxyresorufin O-deethylase (EROD), marker for CYP1A1, methoxyresorufin O-deethylase (MROD), marker for CYP1A2, and pentoxyresorufin O-deethylase (PROD), marker for CYP2B1 activities were measured in hepatic microsomes (4).

Circulating thyroid hormones: Total T4, and T3 were determined with the standard radioimmunoassay kits (Diagnostic Products Corp.) based on the competitive protein binding technique. Blood was collected and allowed to clot on ice for 1 h. Blood samples were centrifuged at 750 g for 15 min to separate serum. Serum samples were stored at -80°C until radioimmunoassay (5).

Protein concentration assay: The Micro BioRad Protein Assay was used according to package directions and with BSA as standard.

Results and Discussion

On a weight basis, Lot #6024 led to greater responses for EROD (Fig. 1A), MROD (Fig. 2A), and PROD (Fig. 3A), total T4 (Fig. 4A) and total T3 (data not shown). The differences in TEQ explained the values for EROD (Fig. 1B) and MROD (Fig. 2B) but did not account for the lower response of Lot #124-191 for PROD (Fig. 3B) or T4 (Fig. 4B). The PROD effects were not due to dioxin-like congeners and thus could not be predicted by the TEQ. The T4 results may be due to multiple mechanisms. There were no consistent dose-response findings for T3. These results illustrate that the contrast in the TEQ explains the variances in the dioxin-like effects, but the nondioxin-like congeners cause other responses that are not associated with the Ah receptor. Thus, overall toxicity cannot be entirely predicted based on the TEQ values, and in the future it is vital that the lot number and congener composition are reported in studies conducted with Aroclor 1254.

Implications

It is important for researchers to be aware that differences exist from lot to lot of Aroclor in terms of enzyme inducibility and toxicity. Regulators need to be aware of the differences that exist and that lab results may not predict environmental impact when the congener composition does not coincide.

This paper does not necessarily represent EPA policy. DEB supported by NIEHS T32-ES07126 and EPA CT902908.

References

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Table 1: Congener Analysis and TEQ Determination

Derivation of TEQ values: $TEQ = \sum ([Congener \times TEF_{i,j}])^{(6)}$

Congeners	Lot # 124-191 mg/g	TEF (3)	TEQ ug/g	Lot #6024 mg/g	TEF (3)	TEQ ug/g
<i>Mon-ortho</i>						
105	51	0.0001	5.1	130	0.0001	13
118	127	0.0001	12.7	124	0.0001	12.4
123	0.6	0.0001	0.06	2.1	0.0001	0.21
131/114/122	0.5	0.0005	0.25	0.8	0.0005	0.4
156	4.8	0.0005	2.4	51	0.0005	25.5
157	0.36	0.0005	0.18	26.3	0.0005	13.15
<i>Coplanar</i>						
77	<.01	0.0001	<.001	27.2	0.0001	2.72
81	<.011	0.0001	<.0011	0.28	0.0001	0.028
126	0.167	0.1	16.7	3.24	0.1	324
169	<.013	0.01	<.13	0.022	0.01	0.22
<i>Furans</i>						
4 Cl	0.001678	0.1	0.1678	0.001693	0.1	0.1693
5 Cl	0.002933	0.1	0.2933	0.014151	0.1	1.4151
6 Cl	0.004744	0.01	0.04744	0.017191	0.01	0.17191
7 Cl	0.001649	0.01	0.01649	0.004724	0.01	0.04724
8 Cl	0.00356	0.001	0.00356	0.000946	0.001	0.000946
Grand Total			38.05069			393.4325

Table 2 – Dose Schedule for Two Lots of Aroclor based on Weight and TEQ values.

Dose mg/kg Basis		Dose ugTEQ/kg Basis	
Lot 124-191	Lot 6024	Lot 124-191	Lot 6024
0	0	0.000	0.000
Not dosed	1	0.033	0.033
Not dosed	3	0.099	0.099
10	10	0.330	0.330
30	30	0.991	0.991
100	100	3.305	3.305
300	300	Not dosed	10.478
1000	1000	Not dosed	34.927

Figure 1: Comparison of EROD enzymatic activity based on A) weight and B) TEQ

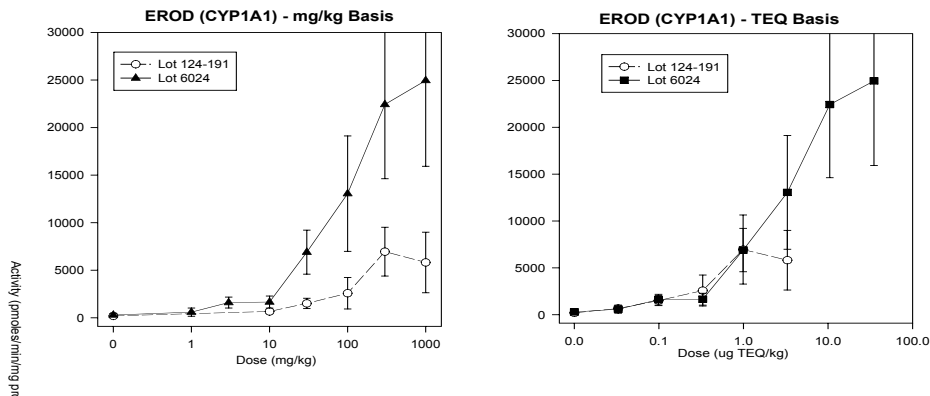


Figure 2: Comparison of MROD enzymatic activity based on A) weight and B) TEQ

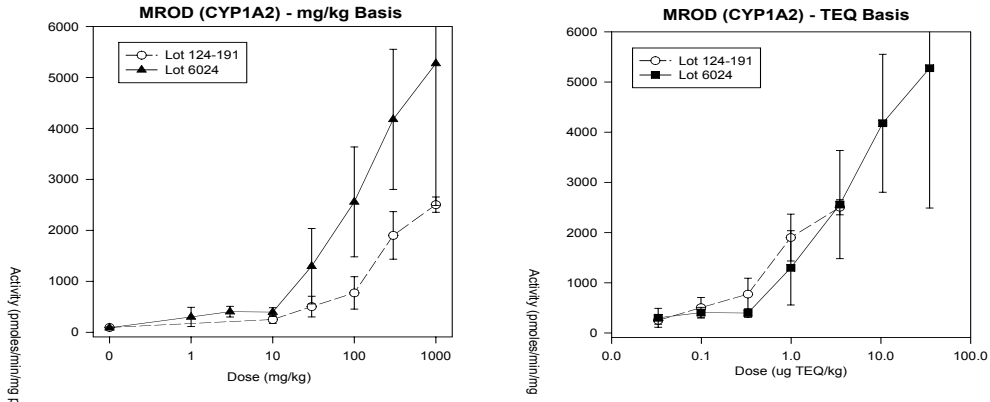


Figure 3: Comparison of PROD enzymatic activity based on A) weight and B) TEQ

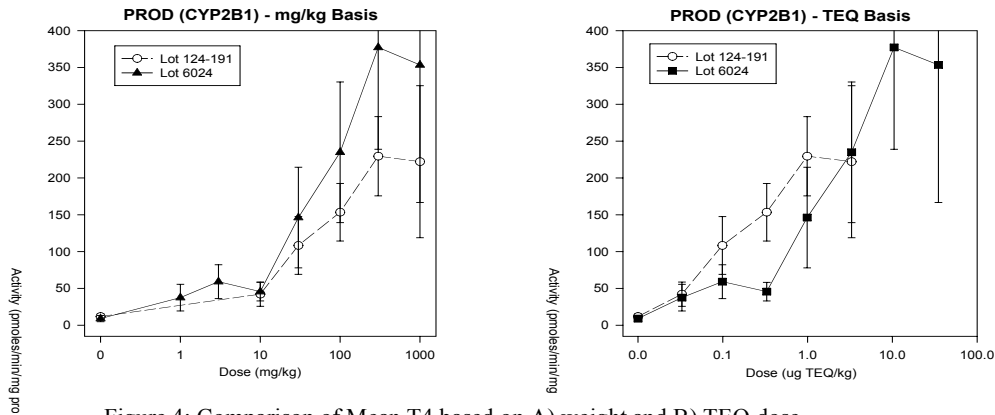


Figure 4: Comparison of Mean T4 based on A) weight and B) TEQ dose

