Differential Effects of Two Lots of Aroclor 1254 on Enzyme Induction, Thyroid Hormones, and Oxidative Stress

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Aroclor 1254 is a commercial mixture of polychlorinated biphenyls (PCBs), which is defined as being 54% chlorine by weight. However, the congener composition varies from lot to lot. Two lots which have been used in toxicity studies, 124-191 and 6024 (AccuStandard), were analyzed for their congener composition. Lot 6024 has approximately 10 times the dioxin toxic equivalents (TEQ) of lot 124-191. The purpose of this study was to determine if the difference in the TEQ of the two lots explains the different *in vivo* responses seen on a weight basis. Male Long-Evans rats (70 days old) were treated orally with a single dose of 0–1,000 mg/kg of each lot. Hepatic ethoxy-, methoxy-, and pentoxyresorufin *O*-deethylase (EROD, MROD, and PROD, respectively) activities as well as serum thyroxine (T_4) concentrations and measures of oxidative stress were determined 4 days after treatment. Results, on a weight basis, indicate that lot 6024 led to a greater induction of EROD, MROD, and PROD but not total T_4 reduction. The differences in TEQ between the lots explained the differential induction of EROD and MROD but did not account for the induction of PROD nor decreases in T_4. PROD induction is not due to dioxin-like congeners, whereas the decrease in serum T_4 levels may involve multiple mechanisms. Effects on the antioxidants ascorbic acid and uric acid were seen only at the highest mass dose for both lots and were not explained by the difference in TEQ. These results illustrate that the differences in the TEQ explain the differences in the strict dioxin-like effects (EROD, MROD induction), but the non–dioxin-like congeners cause other effects that are not associated with the aryl hydrocarbon receptor (e.g., PROD). In addition, supra-additive effects also occur in the mixture (T_4, oxidative stress). Thus, current results demonstrate that overall toxicity cannot be predicted on the basis of the TEQ values. It is also critical that the lot number is reported in studies conducted with Aroclor 1254 because the congener composition and therefore the effects observed can be very different. *Key words*: Aroclor 1254, dioxin, hepatic enzymes, oxidative stress, polychlorinated biphenyls, thryroid hormones, toxic equivalents. *Environ Health Perspect* 109:1163–1168 (2001). [Online 5 November 2001]

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Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products before their production was banned in the United States in the 1970s. More than 1 million tons of PCBs have been produced (*1*) and more than than 70% of the PCBs made are still in use (*2*). The chemical properties that made PCBs desirable in industrial applications (inflammability, chemical stability, and miscibility with organic compounds, or lipophilicity) are the same properties that have contributed to their environmental problems. Once in the environment, stable PCBs degrade slowly and undergo cycling and transport and are thus ubiquitous environmental contaminants. They are frequently found as complex mixtures of congeners in soil and dust and on surfaces in homes and factories. Of the 209 possible PCB congeners, only around 130 are detectable in commercial mixtures, and far fewer are found in the environment (*3–6*).

PCB mixtures induce a variety of biochemical and toxic responses in humans and animals (*4*). Many of these effects resemble those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons that act through the aryl hydrocarbon receptor (AhR) signal transduction pathway (*7*). Dioxin-like PCBs principally include the coplanar PCBs such as IUPAC 77, 81, 126, 169. Dioxin-like effects include weight loss, thymic atrophy, enzyme induction, immunotoxicity, teratogenicity, dermatologic effects, carcinogenicity, and endocrine disruption (*4*). The mono-*ortho* coplanar PCBs such as 105, 114, 118, 123, 156, 157, 167, 189 have both dioxin-like effects via the AhR and other mechanisms of action, such as a phenobarbital-like spectrum of enzyme induction. *Ortho*-substituted PCBs, those that do not bind to the AhR, elicit a different pattern of toxicity. For example, *ortho*-substituted nonplanar PCB congeners elicit enzyme induction, neurotoxicity, carcinogenicity, and endocrine disruption (*7–9*). Exposure to complex mixtures of polychlorinated dibenzofurans (PCDFs) and PCBs have been linked to developmental and cognitive dysfunctions seen in children born to mothers who consumed PCDF- and PCB-contaminated rice oil in Japan (Yusho) and Taiwan (Yu-Cheng) (*10*). Reduced levels of thyroid hormones are detected after developmental exposure to TCDD, non-*ortho*, mono- and di-*ortho* chlorinated PCB congeners as well as after exposure to technical mixtures of PCBs (*9,11*).

Oxidative stress involves a depletion of the protective antioxidant defenses of the body. Oxidative stress from TCDD exposure causes increased production of reactive oxygen species, enhanced lipid peroxidation, decreased glutathione content, decreased hepatic membrane fluidity, and DNA damage (*12,13*). Persistent organohalogen compounds are tumor promoters (*14*), and there is evidence that this promotion is mediated at least in part by reactive oxygen species such as superoxide or hydrogen peroxide (*15*). The mechanism by which this effect occurs remains to be elucidated, but it appears to be AhR mediated (*13*). Markers of oxidative stress were evaluated because PCBs and dioxins induce oxidative stress (*16*), which has been implicated in the promotion of cancer (*17*) and developmental toxicity (*12*); thus, it is important to evaluate both dioxin-like and non–dioxin-like PCBs for similar activity. In the Aroclor mixtures there

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are both dioxin-like and non–dioxin-like PCB congeners so measures of oxidative stress were used to evaluate biochemical and toxic effects.

2,3,7,8-TCDD is never found alone in the environment, but rather as a component of a complex mixture of compounds, many of which share a common AhR-mediated mechanism of action and which elicit similar biological responses. Because of this, a relative potency-ranking scheme was created for hazard and risk assessment of complex mixtures of dioxin-like compounds, including the coplanar and mono-*ortho* PCBs (*1,2,4,18*). The toxic equivalency factor (TEF) approach assigns a relative potency value to polyhalogenated aromatic hydrocarbons based on four criteria, one of which is the mechanism of action via the AhR [see Van den Berg (*1*) for discussion of the criteria]. TCDD is the most potent of the structurally related polyhalogenated aromatic hydrocarbons and thus has a TEF of 1. The TCDD toxic equivalence (TEQ) of a mixture is determined from the assigned TEF and the concentrations of the given individual congeners, as shown in the following equation:

$$TEQ = \Sigma\,[(congener_i \times TEF_i)_n],$$

where n is the number of congeners.

The TEQ approach works well when only PCDDs, PCDFs, and dioxin-like PCBs are present. For example, the induction of cleft palate in mice follows a strictly dose-additive pattern (*19*), as does immunotoxicity (*20*). Recent studies have shown that decreases in hepatic retinoid levels, especially retinyl palmitate, also behave in a strictly additive manner (*21*). However, when non–dioxin-like PCBs are present, application of the TEF approach must be used with caution. Results from laboratory animal and wildlife studies suggest the predictive value of TEFs for PCBs may be both species and response dependent (*5*). This is due to both synergistic and antagonistic interactions that have been observed with PCB mixtures. In the case of an antagonist, the TEF approach would overestimate the toxicity of a PCB mixture. For example, Smialowicz et al. (*20*) found that cotreatment with TCDD (1 µg TCDD/kg) and PCB 153 (358 mg/kg) resulted in no change in the plaque-forming cell response relative to corn oil, but treatment with TCDD alone led to significant suppression and treatment with PCB 153 alone led to enhancement of the plaque-forming cell response. More important, and of greater concern for the protection of human health and the environment, is that the TEF approach could significantly underestimate the toxicity of a mixture if synergism is occurring, as demonstrated in the induction of hepatic porphyrins (*22*). Current approaches focus on the toxicity of individual congeners, assuming additivity, and do not take into account possible interactions.

Aroclors are complex commercial mixtures of PCBs. Aroclor 1254 is defined by the weight percentage of chlorine, but the PCB congener composition varies from lot to lot. Two lots (AccuStandard, New Haven, CT) that have been used in toxicity studies and are representative of commercial Aroclor mixtures, 124-191 and 6024, were analyzed for their congener composition (*23*). Lot 6024 had approximately 10 times the TEQ of lot 124-191 (Table 1). The purpose of this study was to determine if the difference in the TEQ for the two lots explained the different biological responses seen on a weight basis. To test whether the difference in TEQ could explain the differential effects observed, male Long-Evans rats were treated via oral gavage with graded doses of 0–1,000 mg/kg of each lot (Table 2). Enzymatic markers of cytochrome P450 1A1 and 1A2 induction, ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-deethylase (MROD), were used as they are AhR-mediated responses and are common to TCDD and dioxin-like PCBs. Because many of the non–dioxin-like PCB congeners act in a phenobarbital-like manner (*8*) and induce CYP2B, the pentoxyresorufin *O*-deethylase (PROD) assay was used to measure this induction (*24*). Serum T_4 concentrations and measures of oxidative stress were also determined, and the degree of response was compared on a mass basis and on a TEQ basis.

Table 1. Summary of dioxin-like PCB congeners for TEQ comparison.

	Lot 124-191			Lot 6024		
Congeners	Concentration (mg/g)	TEF	TEQ (µg/g)	Concentration (mg/g)	TEF	TEQ (µg/g)
Mono-*ortho*						
105	51	0.0001	5.1	130	0.0001	13
118	127	0.0001	12.7	124	0.0001	12.4
123	0.57	0.0001	0.057	2.14	0.0001	0.214
131/114/122	0.05	0.0005	0.025	0.78	0.0005	0.39
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
Coplanar						
77	0.01	0.0001	0.001	27.2	0.0001	2.72
81	0.01	0.0001	0.001	0.28	0.0001	0.028
126	0.167	0.1	16.7	3.24	0.1	324
169	0.013	0.01	0.13	0.022	0.01	0.22
Furans						
4 Cl	0.001678	0.1	0.1678	0.001693	0.1	0.1693
5 Cl	0.002933	0.5	1.4665	0.014151	0.5	7.0755
6 Cl	0.004744	0.1	0.4744	0.017191	0.1	1.7191
7 Cl	0.001649	0.01	0.01649	0.004724	0.01	0.04724
8 Cl	0.0000356	0.0001	0.00000356	0.000946	0.0001	0.0000946
Grand total			39.41919356			400.63323

Materials and Methods

Chemicals. Aroclor lots 124-191 and 6024 (99% purity) were obtained from AccuStandard (New Haven, CT). Ethoxyresorufin and pentoxyresorufin were purchased from Sigma (St. Louis, MO). Methoxyresorufin was purchased from Molecular Probe (Eugene, OR). Radioimmunoassay kits for thyroid hormone measurements were purchased from Diagnostic Products, Inc. (Los Angeles, CA). Other chemicals were purchased from commercial sources (Sigma) and were of the highest grade commercially available.

Animal treatment. Male Long-Evans rats (Charles River Laboratories, Inc., Raleigh, NC) were used in order to compare the results with those obtained previously in experiments conducted with Aroclor 1254 in this laboratory. The animals were obtained at 60 days of age and acclimatized for 10 days before the study. They were maintained according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (*25*) in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Animals were housed individually in clear polycarbonate cages with pine shavings (North Eastern Products Inc., Warrensburg, NY) and given food (Rodent Chow; Purina, St. Louis, MO) and tap water *ad libitum*. The room was maintained at 21 ± 2°C with 55 ± 5% relative humidity. The lights were on a 12-hr light:dark cycle with lights on at 0600 hr.

Table 2. Dose schedule for two lots of Aroclor 1254 based on weight and TEQ values.

	Dose (µg TEQ/kg)	
Dose (mg/kg)	Lot 124-191	Lot 6024
0	0	0
1	—	0.4
3	—	1.2
10	0.4	4
30	1.2	12
100	4	40
300	12	120
1,000	40	400

Each dose group consisted of five animals, weighing between 300 and 400 g each. They were treated via oral gavage with corn oil (2 mL/kg). Dose levels were chosen based on the results of Nishida et al. (*26*) because that experiment was conducted successfully using the more potent lot 6024. The doses for lot 124-191 were 0, 10, 30, 100, 300, or 1,000 mg/kg body weight, and the doses for lot 6024 were 0, 1, 3, 10, 30, 100, 300, or 1,000 mg/kg body weight. To compare the dose levels on a TEQ basis, two dose groups of 1 and 3 mg/kg of lot 6024 were added, which equates on a TEQ basis to 10 and 30 mg/kg of lot 124-191, respectively (Table 2). Calculated TEQs were based on consensus TEFs from the World Health Organization (*1*) and the congener concentrations present in the two lots were determined by electron-capture detection and gas chromatography/mass spectrometry (Table 1) (*23*). Three days after treatment, the animals were euthanized by carbon dioxide asphyxiation. We collected blood (1–2 mL) for serum triiodothyronine (T_3)/T_4 analysis; the livers were weighed and saved for enzyme analysis and determination of oxidative stress; and thymic weights were obtained. Organs were snap frozen in liquid nitrogen and stored at –80°C until analyzed. Hepatic microsomal suspensions were prepared as previously described (*27*).

Enzymatic assays. For cytochrome P450 activity measurements, hepatic microsomes were appropriately diluted to provide linearity of the reaction with protein concentration and time. The assay conditions were previously reported by our laboratory (*28*).

EROD assay. Cytochrome P450 1A1 enzyme activity was measured using the EROD activity assay. EROD activity was determined from the rate of formation of resorufin from ethoxyresorufin as described by DeVito (*28*).

MROD assay. The MROD activity assay, a measure of the activity of CYP1A2, was carried out as for EROD except that methoxyresorufin was used as the substrate (*28*).

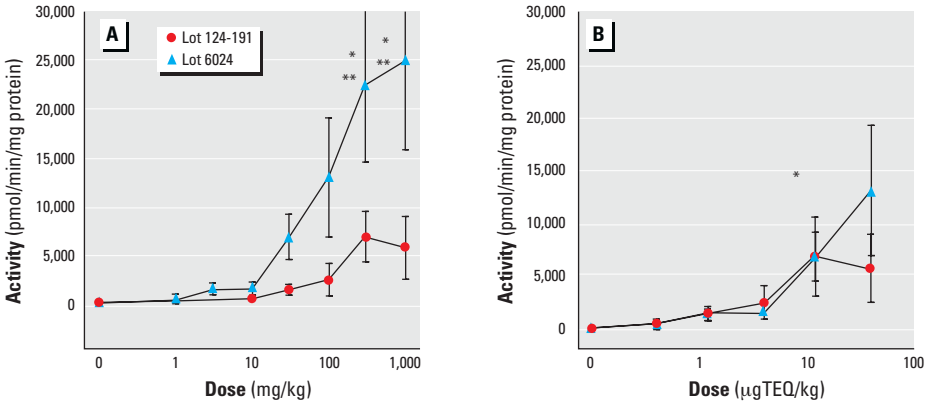

Figure 1. Comparison of EROD enzymatic activity based on (*A*) weight and (*B*) TEQ. On a weight basis, lot 6024 induces higher EROD activity than lot 124-191, as predicted based on mono-*ortho* and TCDD-like PCB congener composition. On a TEQ basis, lot 6024 has similar induction to lot 124-191, but at a lower dose level.
*Statistically significant difference from control group ($p < 0.05$); **Statistically significant difference from other lot at same dose ($p < 0.05$); $n = 5$.

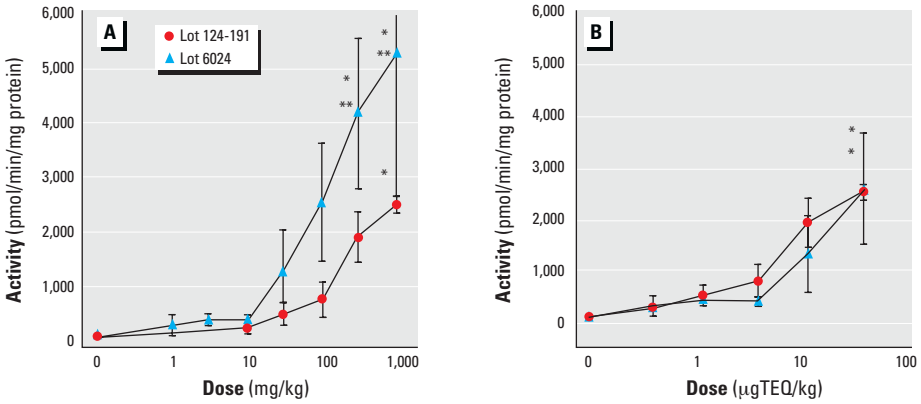

Figure 2. Comparison of MROD enzymatic activity based on (*A*) weight and (*B*) TEQ. On a weight basis, lot 6024 induces higher MROD activity than lot 124-191, as predicted based on mono-*ortho* and TCDD-like PCB congener composition. On a TEQ basis, lot 6024 has similar induction to lot 124-191, but this occurs a lower dose level.
*Statistically significant difference from control group ($p<0.05$) **Statistically significant difference from other lot at same dose ($p<0.05$); $n = 5$.

PROD assay. The PROD activity assay, a measure of CYP2B activity, was carried out as for EROD except that pentoxyresorufin was used as the substrate instead of ethoxyresorufin as described by van Birgelen et al. (*24*).

Circulating thyroid hormones. Blood was collected and allowed to clot on ice for 1 hr. Blood samples were centrifuged at 750 × *g* for 15 min to separate serum. Serum samples were stored at –80°C until radioimmunoassay as described previously (*28*). Total T_4 and T_3 levels were determined with standard radioimmunoassay kits (Coat-A-Count Total T_3 and Total T_4; Diagnostic Products Corp, Los Angeles, CA) based on competitive protein binding.

Cytochrome c reduction assay. Production of superoxide anion by the reduction of cytochrome c was measured by the method of Babior et al. (*30*). Reduced cytochrome c was collected for spectrophotometric measurement on a Novaspec II spectrophotometer (Pharmacia Biotech, Cambridge, England). Although this is not an entirely specific method because other intracellular substances also reduce cytochrome c, it has been used to estimate super oxide anion production (*31*). A 10% (w/v) tissue homogenate was prepared in Tris-KCl buffer (0.05 M Tris and 1.15% w/v KCl, pH 7.4). Each sample for analysis contained 2 mL phosphate-buffered saline (pH 7.2), 45 nmol cytochrome c, and 20 μL tissue homogenate. Absorbance was determined at 550 nm and converted to nanomoles of cytochrome c reduced per minute, using an extinction coefficient of 2.1×10^4/M/cm.

Determination of ascorbic acid and uric acid. Ascorbic acid and uric acid concentrations were determined as described by Ghio et al. (*32*). Between 50 and 100 mg of tissue was homogenized and acidified with 3 mL of 60% perchloric acid and centrifuged at 20,000 × *g* for 30 min at 4° C. Using HPLC (Waters RCM; Millipore Corporation, Marlborough, MA) with electrochemical detection (BAS model LC-4B; Bioanalytical Systems, West Lafayette, IN), the supernatant was assayed for ascorbate and urate.

Total glutathione. Total glutathione (GSH) was determined by a modification of the DTNB-GSSG reductase recycling assay previously described by Anderson (*33*). This assay was modified for use on the COBAS FARA II (Hoffman-La Roche, Branchbury, NJ) centrifugal spectrophotometer.

Protein concentration assay. Microsomal protein concentrations were determined according to the method of Bradford (*34*) using a Bio-Rad (Richmond, CA) microprotein assay kit with bovine serum albumin as the standard. Protein concentrations from GSH and ascorbic acid/uric acid samples were determined using Pierce Commassie Plus Protein assay reagent (Pierce, Rockford,

IL) (*32*). Sample protein concentration was determined from a standard curve using bovine serum albumin standards.

Statistical analysis. Data are presented as means ± standard deviations. Intergroup comparisons were performed by a two-factor analysis of variance with a Bonferroni correction to the *p*-value to control for experimental error. Differences between groups were considered statistically significant when $p < 0.05$.

Results

Both lots demonstrated increased EROD activity on a weight basis. Lot 6024 showed a significantly greater dose-dependent increase over lot 124-191 (Figure 1A). On a TEQ basis, both lots induced a similar level of activity (Figure 1B).

On a weight basis, lot 6024 induced significantly greater MROD activity than lot 124-191 (Figure 2A). On a TEQ basis, both lots induced a similar level of activity (Figure 2B).

On a weight basis, lot 6024 induced significantly greater PROD activity than lot 124-191 (Figure 3A). However, when compared on an equivalent TEQ basis, lot 124-191 was a more potent inducer of PROD (Figure 3B) than was lot 6024.

On a weight basis, the two PCB mixtures resulted in the same dose–response curve for the decrease in total T_4 (Figure 4A), and there were no consistent dose–response findings for T_3 (data not shown). When compared on the basis of TEQ, lot 124-191 was more potent for the decrease in total T_4 (Figure 4B).

Effects on cytochrome c reduction, a measure of superoxide anion production, were not seen for either lot (data not shown). There were no consistent dose–response findings for GSH (data not shown). Ascorbic acid levels increased only at the highest mass of both mixtures, with a greater effect caused by lot 6024 (Figure 5). When compared on a TEQ basis, there is no effect seen on lot 6024 when at the same TEQ as lot 124-191, but at a 10 times lower mass. Uric acid showed a significant decrease from control at the highest dose level for both lots (Figure 6). On a weight basis, lot 6024 showed a greater decrease than lot 124-191 (Figure 6A). On an equivalent TEQ basis, lot 124-191 shows a greater decrease in uric acid levels than lot 6024 (Figure 6B).

Discussion

The purpose of this study was to determine if the difference in the TEQ for the two Aroclor lots 6024 and 124-191 explained the different magnitude of biological responses seen on a weight basis. We found that the differences in TEQ were able to explain induction levels of EROD and MROD. The higher amount of dioxin-like PCBs present in lot 6024 gave rise to greater enzyme induction as measured by EROD and MROD. However, the PROD effects were not due to dioxin-like congeners and thus could not be predicted by the TEQ.

Enzymatic markers of cytochrome P450 1A1 and 1A2 induction were used because they are AhR-mediated responses and are common to TCDD and dioxin-like PCBs. Because many of the non–dioxin-like PCB congeners act in a phenobarbital-like manner (*8*) and induce CYP2B, the PROD assay was used to measure this induction (*24*). PCB 153 has been shown to increase PROD induction (*35*), but both lots 124-191 and 6024 have comparable amounts [31.8 mg/g and 33.93 mg/g, respectively (*23*)]. PCB 126, on the other hand, causes a marked induction of CYP1A2 activity (*36*). Lot 6024 has several times more PCB126 than lot 124-191. The greater effect on PROD by lot 6024, which also had a higher TEQ, may be due to the excess of congeners 105, 138, 156, and 157 as compared with lot 124-191 (Table 1) (*23*). These congeners have phenobarbital-like induction activity.

The decrease in T_4 is likely due to multiple mechanisms such as induction of several uridine diphosphate glucuronysyltransferases (UDPGTs) and binding to T_4 transport protein, transthyretin (TTR) (*11,37*). Cheek et al. (*11*) found that hydroxylated PCBs are potent ligands for TTR, having affinities in the 1 nM range, 50-fold greater than that of T_4. Recently, Chauhan et al. (*37*) studied the structure–activity relationships of 49 PCB congeners and demonstrated differential binding activity on T_4–TTR binding. When compared on the basis of TEQ, the greater mass of PCB congeners in lot 124-191 was more effective for the decrease in total T4 (Figure 4B). Thus, TEQ alone is not predictive of the decrease in T_4.

PCBs are known to be developmental toxicants at environmentally relevant concentrations (*38*). Abnormalities associated with low-level PCB exposure in humans include hypoactivity and impaired learning

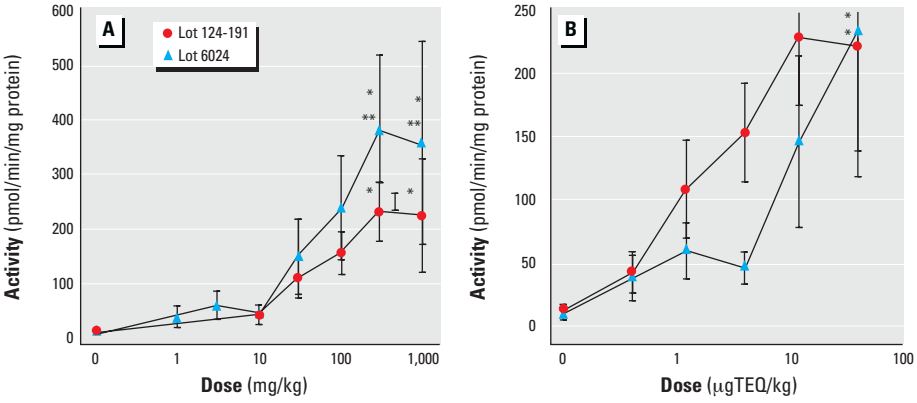

Figure 3. Comparison of PROD enzymatic activity based on (*A*) weight and (*B*) TEQ. On a weight basis, lot 6024 induces higher PROD activity than lot 124-191, as predicted based on non-*ortho* congener composition. However, on a TEQ basis, lot 124-191 was more potent for PROD than was lot 6024.
*Statistically significant difference from control group ($p<0.05$). **Statistically significant difference from other lot at same dose ($p<0.05$); $n = 5$.

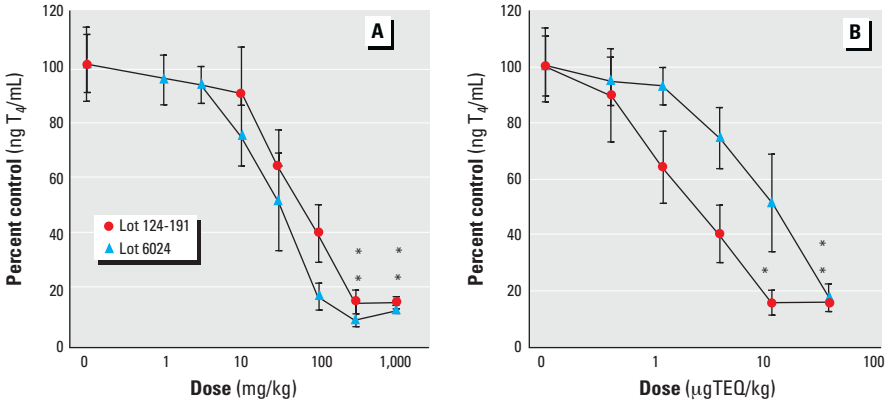

Figure 4. Comparison of total T_4 activity based on (*A*) weight and (*B*) TEQ. The two PCB mixtures resulted in the same dose–response curve for the decrease in total T_4 on a weight basis (*A*), and there were no consistent dose–response findings for T_3 (data not shown). When compared on the basis of TEQ, lot 124-191 was more potent for the decrease in total T_4 (*B*).
*Statistically significant difference from control group ($p < 0.05$); $n = 5$.

(*9,39,40*). In the Netherlands, PCB levels in women were inversely related to serum total T_4 in their newborn children, as well as birth size and early growth rate (*40*). Some congeners, especially the non-*ortho* substituted congeners such as PCB 126, are particularly potent at reducing circulating T_4 levels, but they do not become concentrated in brain tissue. In contrast, some *ortho*-substituted congeners such as PCB 153 appear to concentrate in brain tissues and may bind to the thyroid receptor but are not as potent at reducing circulating T_4 levels (*9*).

No effects of acute PCB exposure were seen for GSH. This may be surprising at first because GSH plays a fundamental role in the antioxidant biology of mammals. Severe GSH depletion has been associated with such pathological consequences as susceptibility to the development of lipid peroxidation. It plays a role in the regeneration of ascorbic acid (vitamin C) and reduction of the oxidized form of tocopherol (vitamin E) (*16*). In this study, the animals were terminated at day 4, which may be enough time for the GSH pools in the body to be replenished. During a subchronic study, it would be reasonable to expect GSH depletion as a consequence of TCDD exposure as observed by Slezak et al. (*16*).

Recent studies indicate that production of reactive oxygen species after TCDD exposure is not solely dependent on TCDD concentration in the tissue. Although acute, low-dose exposure to TCDD does not appear to cause oxidative stress, very low doses of TCDD that result in tissue concentrations similar to human background levels produced an effect on an oxidative stress endogenous defense system (*16*). Previous studies demonstrate that oxidative stress is seen only after high-dose, acute exposure to TCDD (*12,16*). A dose of 1,000 mg/kg of lot 124-191 results in a dose of about 800 mg/kg of non–dioxin-like PCBs. However, non–dioxin-like PCBs can also induce oxidative stress (*41*). This suggests that there are multiple mechanisms which lead to a greater than additive effect of the individual congeners resulting in higher than expected levels of oxidative stress as measured by glutathione, ascorbic acid, and uric acid, markers of antioxidant defense. The presence of oxidative stress at the highest mass is likely due to non–dioxin-like PCBs or to interactions.

It is apparent that the contrast in the TEQ explains the variances in the dioxin-like effects, but the non–dioxin-like congeners cause other responses that are not associated with the AhR. Use of TEFs assumes that polyhalogenated aromatic hydrocarbon congeners act in an additive manner. Assumption of additivity is only valid if the dioxin-like chemical has no mechanism of action other than binding to the AhR and the induction of response by different chemicals occurs by the same mechanism of action. This appears to hold true for all of the laterally substituted polyhalogenated dibenzo-*p*-dioxins and PCDFs, and the coplanar PCBs, especially PCB 126. It is not true with the mixed-inducer PCBs (i.e., the mono-*ortho* substituted congeners such as PCBs 105, 118, and 156).

However, when non–dioxin-like PCBs are present, application of the TEF approach must be used with caution. The potential exists for synergistic and antagonistic interactions, which have been observed with PCB mixtures, although these potentials have not been fully explored. In the case of synergism, the congeners act via multiple mechanisms to produce a greater than additive effect which the TEQ would thus underpredict. Van Birgelen et al. (*42*) found clear evidence of synergism for increases in both hepatic and urinary porphyrins as well as significant decreases in total T4 (*36,43*).

In the case of antagonism, the congeners would work against each other to cause a less than additive effect that the TEQ would then overpredict. For example, Morrissey et al. (*44*) found that combinations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153; 125–500 mg/kg) and TCDD (15 µg/kg) decreased the incidence of cleft palate in C57BL/6N mice induced by TCDD alone. Antagonism of hydronephrosis also occurred in this study (500 mg PCB-153/kg and 15 µg/kg TCDD). It is believed that these examples of nonadditivity are due to multiple mechanisms of action that can result in a common phenotype.

Kodavanti et al. (*23*) described the *in vitro* effects of Aroclor 1254 on neurochemical end points, in particular protein kinase C (PKC) translocation and intracellular Ca^{2+} buffering. Lot 124-191 was more potent in PKC translocation than lot 6024, whereas lot 6024 was slightly more active on Ca^{2+} bufferings than lot 124-191 on a mass basis. The difference in results for PKC translocation

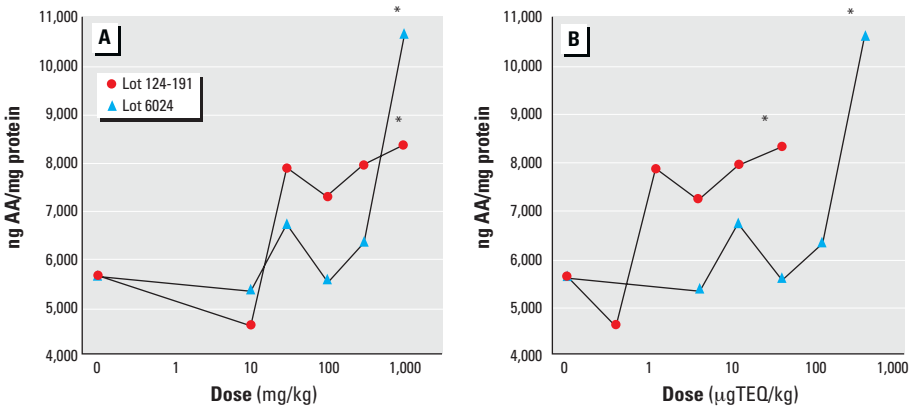

Figure 5. Comparison of ascorbic acid (AA) levels based on (*A*) weight and (*B*) TEQ. AA levels increased only at the highest mass of both mixtures, with a greater effect caused by lot 6024. When compared on a TEQ basis, there is no effect seen on lot 6024 when at the same TEQ as lot 124-191, but at a 10 times lower mass.
*Statistically significant difference from control group ($p < 0.05$); $n = 5$.

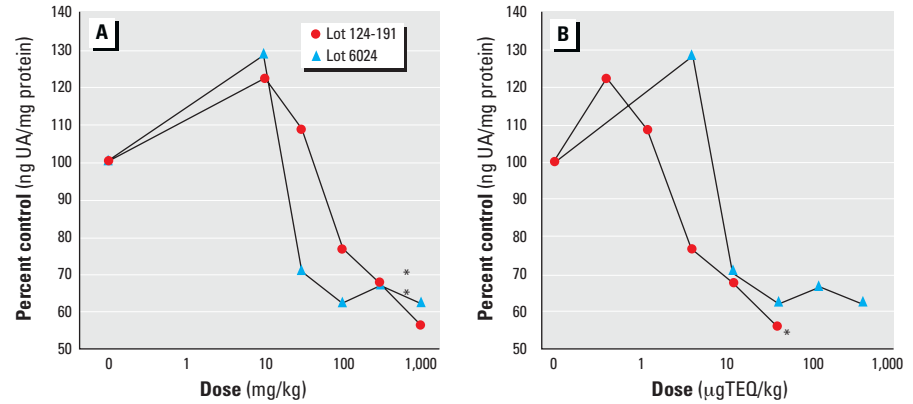

Figure 6. Comparison of uric acid (UA) levels based on (*A*) weight and (*B*) TEQ. Uric acid showed a significant decrease from control at the highest dose level for both lots. On a weight basis, lot 6024 showed a greater decrease than lot 124-191 at the same dose level (*A*). On an equivalent TEQ basis, lot 124-191 shows a greater decrease in uric acid levels than lot 6024 (*B*).
Statistically significant difference from control group ($p < 0.05$); $n = 5$.

and intracellular Ca^{2+} buffering could not be attributed to the non-*ortho* congeners or PCDFs, as they are inactive in these assays (*23*). These outcomes could not be predicted by the TEQ, which indicates there are non–dioxin-like congeners present in higher amounts in lot 124-191 driving this response. Analysis of the two lots revealed that PCBs 44, 49, 52, 66, 85, 97, 132, 135, 137/176, 149, 174, and 187 were higher in lot 124-191, and PCBs 40, 47-48, 70, 74, 77, 81, 92, 99, 105, 110, 123, 126, 138, 156, and 157 were higher in lot 6024.

For protection of human health and the environment, current regulations rely on the toxicity of complex mixtures and do not take into account possible interactions. TEFs were developed to facilitate the estimation of the toxicity of these complex mixtures, but this assumes that the dose–response curves are parallel for a given response between chemicals and that combined effects are additive. Overall toxicity of complex mixtures cannot be entirely predicted based on the TEQ values, and in the future it is vital that the lot number in studies conducted with Aroclor 1254, as well as the congener composition of the mixture, are reported. It is important for researchers to be aware that differences exist from lot to lot of Aroclor in terms of biochemical and toxic responses. Laboratory results may not predict environmental impact when the congener compositions deviate. Clearly, depicting environmental or biological samples as resembling Aroclor mixtures does not adequately account for effects because congener composition varies.

TEQ determines the amount of dioxin-like activity in a mixture. However, because only a small portion of the total mass of PCB mixtures are coplanar non-*ortho* congeners that elicit dioxin-like activities, the TEF approach based on AhR-mediated responses cannot be applied for the risk assessment of non–AhR-mediated toxic effects (*7*). For a complete evaluation of risks due to PCBs exposure, consideration of the effects of both *ortho*- and non-*ortho* substituted congeners is also required.

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