Differential Effects of Two Lots of Aroclor 1254: Congener-Specific Analysis and Neurochemical End Points

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Aroclor 1254 is a widely studied commercial polychlorinated biphenyl (PCB) mixture which, by definition, contains 54% chlorine by weight. Recent reports indicate substantial differences in the congener composition among Aroclor lots and hence their biologic effects. We designed the current study to compare the effects of two lots of Aroclor 1254 (lots 6024 and 124-191). We analyzed these two lots for PCB congeners, polychlorinated dibenzoarufans (PCDFs), polychlorinated naphthalenes (PCNs), and polychlorinated dibenzodioxins (PCDDs). We used previously established techniques for analyzing intracellular Ca²⁺ buffering and protein kinase C (PKC) translocation to test their biologic activity in neuronal preparations. PCB congener-specific analysis indicated that ortho- and non-ortho congeners in these two lots varied in their percent contribution. Among all congeners, the percentages of non-ortho congeners (PCBs 77, 81, 126, and 169) were higher in lot 6024 (2.9% of total) than in lot 124-191 (0.02% of total). We detected no dioxins in these two lots (< 2 ppb). Although there are some differences in the congener composition, total PCNs were similar in both lots: 171 ppm in lot 6024 and 155 ppm in lot 124-191. However, total PCDDs were higher in lot 6024 (38.7 ppm) than in lot 124-191 (11.3 ppm). When we tested these two Aroclors on Ca²⁺ buffering and PKC translocation in brain preparations, the effects were significantly different. Although lot 124-191 was more potent on PKC translocation than lot 6024, lot 6024 was slightly more active on Ca²⁺ buffering than lot 124-191. These effects could not be attributed to the differences in the percentage of non-ortho congeners or PCDFs because they were inactive on these two parameters. The effects could not be attributed to PCNs because the levels were almost similar. The effects seen with two lots of Aroclor 1254 in neuronal cells were also not predicted based on the TCDD toxic equivalents for PCB congeners and commercial PCB mixtures 

Polychlorinated biphenyls (PCBs) belong to a large group of halogenated aromatic hydrocarbons and consist of 209 theoretically possible congeners with different numbers and positions of chlorines (1,2). These compounds were commercially produced as Aroclor mixtures in the United States by the chlorination of biphenyl, which produces technical mixtures containing a given chlorine content depending on the duration of the chlorination process. Although all 209 congeners can be synthesized, the reaction conditions in the commercial processes favor certain substitution reactions leading to particular composition of the technical mixtures, which are identified by the weight percentage of chlorine content. For example, Aroclor 1254 contains 54% chlorine by weight, as indicated by the last two digits in the numeric designation (1–5).

These compounds were used widely in industry as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and capacitors, flame retardants, plasticizers, and sealants and in carbonless copy paper because of their chemical and thermal stability, dielectric properties, and miscibility with organic compounds. These same properties have now contributed to their ability to cause environmental and human health problems (6–9). PCBs are distributed throughout the entire ecosystem including soil, air, and water (10). They are also highly likely to bioaccumulate in the food chain because of their lipophilicity, and they therefore belong to a class of environmental chemicals called persistent bioaccumulative toxicants (PBTs) (11,12). PCBs have a wide range of effects in humans, including chloracne, diverse hepatic effects, decreased birth weight in the offspring of occupationally exposed mothers, decreased pulmonary function, eye irritation, subtle endocrine disturbances, cancer, and learning and memory deficits (10–13). Several of these effects have also been demonstrated in animals during adult and developmental exposure to commercial PCB mixtures such as Aroclor 1254 (6,7,9,11,14,15). In animal models, some individual PCB congeners and commercial PCB mixtures have shown tumor-promoting activity (16). The information from these animal studies with commercial PCB mixtures has been used in the risk/exposure assessment of PCBs and related environmental chemicals (2,15). However, recent reports using high-resolution gas chromatography (HRGC) indicate substantial differences in the congener composition among Aroclor lots (17,18); hence their biologic effects could be different. Toxicity studies with Aroclor 1254 have produced varied results (19,20), but these differences were attributed to the animal species or dosing paradigm rather than to the composition of Aroclor 1254. We designed the current study to study the effects of two lots of Aroclor 1254 (lots 6024 and 124-191) obtained from AccuStandard
(New Haven, CT, USA) on the neurochemical end points that were previously reported to be sensitive to PCBs in neuronal preparations (21–23) and to compare the effects with the PCB congener composition and other contaminants in these two lots of Aroclor 1254. These two lots of Aroclor 1254 have been used widely by investigators for the last several years.

Materials and Methods

Chemicals. Aroclor 1254 (> 99% purity) with lot numbers 6024 and 124-191 were purchased from AccuStandard. For neurochemical experiments, we prepared stock solutions of two Aroclor 1254 lots by dissolving them in dimethyl sulfoxide (DMSO). A 2-µL aliquot of stock solution (different concentrations) was added to the buffer to yield the desired final concentrations. DMSO (2 µL/mL) had no significant effect either on 

$^{45}$Ca$^{2+}$ uptake or $^{3}$H-phorbol ester binding.

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was monitored using a HRMDGC-ECD. We determined the remaining PCB congeners applying our routine procedure to environmental samples. Using the specially prepared technical standard, we determined the presence of every PCB congener. When a congener was present at levels < 0.05%, we considered it undetectable. Heart-cuts were made for those congeners that coeluted usually (27).

**Analysis of PCDFs, PCDDs, and PCNs in two lots of Aroclor 1254.** We performed the polychlorinated dibenzo-β-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and polychlorinated naphthalene (PCN) analyses as per the modified methods described previously (28,29). Isomer-specific analyses were done using HRGC (HP6890) low-resolution mass spectroscopy (HRGC-LRMS). We used an HP6890 (gas chromatograph)–HP5973 (mass spectrometer) (Hewlett-Packard 5973MS; Hewlett Packard, Tokyo, Japan).

PCB samples (50–100 mg of Aroclor 1254 with lot numbers 6024 and 124-191) dissolved in 1–5 mL hexane were chromatographed on basic alumina (300 mesh, Japan). Analyses were done using HRGC (HP6890) as an internal standard and a syringe spiking standard. Recoveries of tetra-PCDD, pentachloro-PCDD, hexa-PCDD, hepta-PCDD and octa-PCDD congeners through the analytic procedures were 66–140%, 83–129%, 70–112%, 62–121%, and 41–95%, respectively. Recoveries of PCN congeners have been reported earlier (33). PCB congener 1,2,3,4-tetra-CN was present in blank at trace amounts and therefore was not quantified.

**Neurochemical end points.** Fractionation of adult rat cerebella to obtain microsomes and mitochondria was performed according to Cotman and Matthews (32). We determined intracellular Ca$^{2+}$ buffering by measuring the uptake of $^{45}$Ca$^{2+}$ by microsomes and mitochondria as outlined by Moore et al. (33).

We isolated granule cells from cerebellum of 7-day-old rats by the enzymatic disruption of cells as outlined by Gallo et al. (34) with modifications (35). These cells were maintained for 7 days *in vitro* in culture and used for protein kinase C (PKC) translocation studies. We determined PKC translocation by measuring $^{3}H$-phorbol ester binding according to Vaccarino et al. (36).

**Statistics.** We analyzed the neurochemical data by two-way analysis of variance (ANOVA) with lot number as one factor and the concentration as the other followed by Dunnett’s post-hoc test. All analyses were performed with PROC GLM of SAS software (37). We calculated the IC$_{50}$ (concentration that inhibits control activity by 50%) and E$_{50}$ (concentration that increases control activity by 50%) values for $^{45}$Ca$^{2+}$ uptake and $^{3}H$-phorbol ester binding, respectively, from the regression line fit to the linear portion of the curve using GraphPad Instat Software (GraphPad Instat Software Inc., San Diego, CA, USA). We compared the IC$_{50}$ and E$_{50}$ values between the two lots using Student’s t-test. The accepted level of significance was set at $p < 0.05$.

**Results**

**Composition of PCB congeners and contaminant s such as PCDFs, PCDDs, and PCNs in two lots of Aroclor 1254.** A definitive statistical analysis of the quantitative performance of this congener-specific analysis requires detailed regression analysis of all standards, analysis of multiple replicates of sample, and multiple replicate injections. Tumor strains, cumbersome heart-cut analysis of non-ortho PCB congeners, and tedious analyses of contaminants precluded such analyses. The quantitative information provided in this article permits only a comparison of relative congener distribution in the two lots of Aroclor 1254.

The congener-specific analysis of Aroclor 1254 indicated that the composition of PCBs was different in both lots (Table 2). Some PCB congeners were higher in lot 6024, whereas some other congeners were higher in lot 124-191. PCBs 40, 47–48, 70, 74, 77, 81, 92, 99, 105, 110, 123, 126, 138, 156, and 157 were higher in lot 6024. On the other hand, PCBs 44, 49, 52, 66, 85, 97, 132, 135, (137+176), 149, 174, and 187 were higher in lot 124-191.

When PCB congeners were grouped based on the number of chlorines (mono- to nona-; Table 5), both lots had similar percentages of PCB congeners except the hepta-chlorinated ones, where lot 124-191 had higher levels (5.9%) than did lot 6024 (3.5%). When the congeners were grouped based on the number of *ortho*-chlorine substitutions, non-*ortho* PCBs were several-fold (about 150-fold) higher in lot 6024 than in lot 124-191. Mono-*ortho* PCBs were also significantly higher in lot 6024 (38% of total) than in lot 124-191 (24% of total).

On the other hand, tri-*ortho* PCBs were significantly higher in lot 124-191 (21% of total) than in lot 6024 (14% of total), while di- and tetra-*ortho* PCBs were almost similar in both lots (Table 6).

PCDF composition was different in both lots of Aroclor 1254 as well. Lot 6024 had higher levels of total PCDFs as well as 2,3,7,8-PCDFs than did lot 124-191 (Tables 3 and 5). Based on the amount, total as well as penta-PCDFs to octa-PCDFs were higher in lot 6024 than in lot 124-191. The contribution of tetrachloro-PCDFs was lower and penta-PCDFs was higher in lot 6024 than in lot 124-191. Dioxins were not detected in either lot of Aroclor 1254 (Tables 3 and 5). Total PCNs were similar in both lots: 171 pg/µg in lot 6024 versus 155 pg/µg in lot 124-191. However, penta- and hexa-PCNs were slightly higher in lot 6024, while hepta- and octa-PCNs were slightly higher in lot 124-191 (Table 4).

We calculated the TEQ values for both lots of Aroclor 1254 using the toxic equivalent factor (TEF) values from the World Health Organization (38). We calculated the total TEQ value for lot 124-191 as 39.42 µg/g, and the TEQ value for lot 6024 was 400.63 µg/g. The TEQ value for lot 6024 was 11 times higher than that of lot 124-191 (39).

**Neurochemical effects of two lots of Aroclor 1254.** Both lots of Aroclor 1254 inhibited microsomal $^{45}$Ca$^{2+}$ uptake in a concentration-dependent manner (Figure 1). Microsomal $^{45}$Ca$^{2+}$ uptake in control tissue is 50.5 ± 2.1 (mean ± SE) pmol/mg protein/min. The ANOVA indicated a significant interaction: The PCB levels were
plotted either as concentration (microgram per milliliter; \( F_{3,36} = 8.41; p < 0.0001 \)) or as TEQ (picograms per milliliter; \( F_{2,30} = 52.05; p < 0.0001 \)), suggesting that the response of two lots of Aroclor 1254 on microsomal \( ^{45}\text{Ca}^{2+} \) uptake is different in each case. The calculated \( IC_{50} \) values for microsomal \( ^{45}\text{Ca}^{2+} \) uptake were significantly different between two lots of Aroclor 1254; the difference was much greater when the values were transformed to represent TEQ (nanograms TEQ per milliliter) compared to the original concentrations (micrograms Aroclor 1254 per milliliter).

Both lots of Aroclor 1254 also inhibited mitochondrial \( ^{45}\text{Ca}^{2+} \) uptake in a concentration-dependent manner (Figure 2). Mitochondrial \( ^{45}\text{Ca}^{2+} \) uptake in control tissue was \( 11.2 \pm 0.4 \) (mean \( \pm \) SE) pmol/mg protein/min. The ANOVA indicated a significant interaction. However, the PCB levels were plotted as concentration (micrograms per milliliter; \( F_{3,36} = 4.04; p < 0.0052 \)) or as TEQ (picograms per milliliter; \( F_{3,30} = 46.3; p < 0.0001 \)), suggesting that the response of two lots of Aroclor 1254 on mitochondrial \( ^{45}\text{Ca}^{2+} \) uptake is different in each case. The \( IC_{50} \) values for mitochondrial \( ^{45}\text{Ca}^{2+} \) uptake were not significantly different among the two lots of Aroclor 1254 when the levels were represented as concentration (micrograms per milliliter); however, they are significantly different when the values were transformed to represent TEQ (Table 7).

Glutamate (30 \( \mu \)M), which was used as a positive control, increased \( ^{3}\text{H}-\text{PDBu} \) binding by 2-fold, and this is in agreement with previous reports (40,41). Both lots of Aroclor 1254 significantly increased \( ^{3}\text{H}-\text{PDBu} \) binding in a concentration-dependent manner (Figure 3). \( ^{3}\text{H}-\text{PDBu} \) binding in control cultures is 479 \( \pm \) 25 (mean \( \pm \) SE) fmol/mg protein.

### Table 2. Congener-specific analysis of Aroclor 1254 with two different lot numbers (mg/g).

| PCBs | IUPAC no. | No. of Cl | \( \sigma \)-Cl | Lot 124-191 | Lot 6024 |
|------|-----------|-----------|---------------|------------|----------|
| 2    | 1         | 1         | 1             | –          | –        |
| 3    | 2         | 1         | 0             | –          | –        |
| 4    | 3         | 1         | 0             | –          | –        |
| 2,2’ | 4         | 2         | 2             | –          | –        |
| 2,3  | 5         | 2         | 1             | –          | –        |
| 2,3’ | 6         | 2         | 1             | –          | –        |
| 2,4  | 7         | 2         | 1             | –          | –        |
| 2,4’ | 8         | 2         | 1             | –          | –        |
| 2,5  | 9         | 2         | 1             | –          | –        |
| 2,6  | 10        | 2         | 2             | –          | –        |
| 3,3’ | 11        | 2         | 0             | –          | –        |
| 2,4’ | 12        | 2         | 0             | –          | –        |
| 3,4  | 13        | 2         | 0             | –          | –        |
| 3,5  | 14        | 2         | 0             | –          | –        |
| 4,4  | 15        | 2         | 0             | –          | –        |
| 2,3  | 16        | 3         | 2             | –          | –        |
| 2,4  | 17        | 3         | 2             | –          | –        |
| 2,5  | 18        | 3         | 2             | –          | –        |
| 2,6  | 19        | 3         | 3             | –          | –        |
| 2,3’ | 20        | 3         | 1             | –          | –        |
| 2,4  | 21        | 3         | 1             | –          | –        |
| 2,5  | 22        | 3         | 1             | –          | –        |
| 2,6  | 23        | 3         | 1             | –          | –        |
| 3,3’ | 24        | 3         | 2             | –          | –        |
| 2,4’ | 25        | 3         | 1             | –          | –        |
| 2,5  | 26        | 3         | 1             | –          | –        |
| 2,6  | 27        | 3         | 2             | –          | –        |
| 3,4  | 28        | 3         | 1             | –          | –        |
| 3,5  | 29        | 3         | 1             | –          | –        |
| 3,6  | 30        | 3         | 2             | –          | –        |
| 3,4’ | 31        | 3         | 1             | –          | –        |
| 2,4  | 32        | 3         | 2             | –          | –        |
| 2,3’ | 33        | 3         | 1             | –          | –        |
| 2,4  | 34        | 3         | 1             | –          | –        |
| 2,3’ | 35        | 3         | 0             | –          | –        |
| 3,3’ | 36        | 3         | 0             | –          | –        |
| 3,4’ | 37        | 3         | 0             | –          | –        |
| 3,5  | 38        | 3         | 0             | –          | –        |
| 2,3’ | 39        | 3         | 0             | –          | –        |
| 2,3’ | 40        | 4         | 2             | –          | –        |
| 2,4  | 41        | 4         | 2             | 9.71       | 9.81     |
| 2,3’ | 42        | 4         | 2             | –          | –        |
| 2,3’ | 43        | 4         | 2             | –          | –        |
| 2,3’ | 44        | 4         | 2             | 25.18      | 10.46    |
| 2,3  | 45        | 4         | 3             | –          | –        |
| 2,3’ | 46        | 4         | 3             | –          | –        |
| 2,3’ | 47        | 4         | 2             | –          | –        |
| 2,3’ | 48        | 4         | 2             | –          | –        |
| 2,4  | 49        | 4         | 2             | 21.36      | 4.07     |
| 2,6  | 50        | 4         | 3             | –          | –        |
| 2,4  | 51        | 4         | 3             | –          | –        |
| 2,4  | 52        | 4         | 2             | 39.06      | 7.73     |
| 2,5’ | 53        | 4         | 3             | –          | –        |

continued
protein/15 min. The ANOVA indicated a significant interaction: Either the PCB levels were plotted as concentration ($F_{0.40} = 5.94; p < 0.0006$) or as TEQ ($F_{1,49} = 64.2; p < 0.0001$), suggesting that the response of two lots of Aroclor 1254 on 4H-PDbu binding is different in each case. The E50 values for 4H-PDbu binding were significantly different between the two lots of Aroclor 1254; the difference was much greater when the values were transformed to represent TEQ than for the original concentrations (Table 7).

In general, the data from neurochemical end points indicate that the effects of two lots of Aroclor 1254 on intracellular Ca$^{2+}$ buffering is comparable, but lot 124-191 was more effective on PKC translocation than was lot 6024. The potency difference between the two lots ranged from 1.2- to 2.5-fold when PCB levels were represented as concentration. However, the difference increased to several-fold (8- to 29-fold) when PCB levels were transformed to TEQs (Figures 1–3; Table 7).

Discussion

Health risks associated with exposure to PCBs and related chemicals have been assessed based on either total PCB concentrations or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) TEQs using the TEF concept when congener-specific data are available. U.S. EPA has adopted the TEF approach as an interim procedure that assumes additivity for the toxic effects of individual congeners in the mixtures and the common mechanism of toxicity action (e.g., ability to interact with and activate the aryl hydrocarbon receptor). In addition, assessment of risks to humans is based on the reference doses (RfDs) derived from animal studies with commercial PCB mixtures (15).

Table 2 (continued).

| PCBs | IUPAC no. | No. of Cl | No. of o-Cl | Lot 124-191 | Lot 6024 | PCBs | IUPAC no. | No. of Cl | o-Cl | Lot 124-191 | Lot 6024 |
|------|-----------|-----------|-------------|-------------|----------|------|-----------|-----------|------|-------------|---------|
| 2,3´,3´,4´,4´  | 127  | 5  | 0  | –  | –  | 2,3´,3´,4´,4´,5´  | 157  | 6  | 1  | 0.36  | 26.30  |
| 2,3´,3´,4´,5´  | 127  | 5  | 0  | 0.17 | 3.24  | 2,3´,3´,4´,5´,5´  | 159  | 6  | 1  | –  | –  |
| 2,3´,3´,4´,5´  | 127  | 5  | 0  | –  | –  | 2,3´,3´,4´,4´,5´,5´  | 170  | 7  | 2  | 0.01  | 0.02  |
| 2,3´,3´,4,4´,5´,5´  | 170  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5´,6,6´  | 172  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,6,6´  | 173  | 7  | 3  | 3.51  | 0.39  |
| 2,3´,3´,4,4´,5,6,6´  | 174  | 7  | 3  | 31.12  | 18.32  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 180  | 7  | 2  | 5.18  | 4.51  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 181  | 7  | 3  | 1.59  | 0.88  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 182  | 7  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 183  | 7  | 3  | 35.1  | 0.39  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 184  | 7  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 185  | 7  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 186  | 7  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 187  | 7  | 3  | 35.1  | 0.39  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 188  | 7  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 189  | 7  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 190  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 191  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 192  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 193  | 7  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 194  | 8  | 2  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 195  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 196  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 197  | 8  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 198  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 199  | 8  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 200  | 8  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 201  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 202  | 8  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 203  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 204  | 8  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 205  | 8  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 206  | 9  | 3  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 207  | 9  | 4  | –  | –  |
| 2,3´,3´,4,4´,5,5´,6,6´  | 208  | 10  | 4  | –  | –  |

No. of Cl, number of total chlorine substitutions; No. of o-Cl, number of ortho-chlorine substitutions. PCBs without any values are below the detection limit, < 0.05% (w/w). The PCB numbers are in agreement with IUPAC convention, but note the change in numbers for PCBs 199, 200, and 201 compared to Ballschmitter and Zell (3).
Recently, a new approach has been developed to assess the cancer risk from environmental PCB exposure, and this approach considers both toxicity and environmental processes to distinguish among environmental mixtures (42). Although current risk/exposure assessment is based on these approaches, several problems were identified with the recent literature. Several studies from our laboratory as well as others indicate that ortho-substituted PCBs that do not or weakly bind to aryl hydrocarbon receptors are highly active in neuronal and other biologic processes to distinguish among environmental samples (> 99%), lack of significant consideration of this group of PCBs in the TEF approach could lead to underestimation of the risk associated with exposure to these PCBs. Likewise, RDs derived from commercial preparations might not be accurate because the pattern of relative proportions of PCBs in environmental mixtures is variable and does not resemble the composition of the original PCB mixtures that were released into the environment. Recent reports indicate substantial differences in the congener composition among Aroclor lots (17, 18), so their biologic effects could be different (19, 20). In the present study, we report a thorough PCB congener analysis along with the contaminants such as PCDFs, PCNs, and any dioxins in two different lots of Aroclor 1254 mixtures and their effects on selected neurochemical end points that were previously reported to be sensitive to PCBs in neuronal preparations (21–23).

Results from this study indicate that the composition of PCB congeners was significantly different between the two lot numbers of Aroclor 1254. We detected no dioxins in either lot (< 2 ppb), as anticipated. PCNs were similar in both lots. Other contaminants in this mixture, such as PCDFs, were detectable, mainly because PCDFs are produced often as co-contaminants in PCB preparation (28). In the present study, lot-to-lot differences in PCDF levels have been identified, with lot 6024 having 3.4 times more PCDFs than lot 124-191. Differences in the congener composition between different lots for a variety of Aroclor mixtures have been previously reported (17, 18). However, this is the first detailed report of congener-specific analysis that includes contaminants in these two lots of Aroclor 1254, a widely used commercial mixture in the United States for conducting scientific research. Of the two lots of Aroclor 1254

### Table 3. Concentration (ng/g; ppb) of PCDFs and PCDDs in Aroclor 1254 with two different lot numbers.

| PCDFs/PCDDs | Lot 124–191 | Lot 6024 |
|-------------|-------------|----------|
| Tetra-CDF (0.0 = ND; < 2.0 ppb) | 13468 - - | - - |
| 1378/1379 | 10.0 - | - - |
| 1347 | - - | - - |
| 1468 | - - | - - |
| 1247/1367 | - - | - - |
| 1349 | - - | - - |
| 1346/1248 | 109.9 7.6 | - - |
| 1246/1268 | 25.0 - | - - |
| 1478/1369/1327 | 15.0 - | - - |
| 1678/1234 | - - | - - |
| 2468/1238/1671/1236 | 54.9 - | - - |
| 1249 | - - | - - |
| 1267/1279 | 30.0 30.4 | - - |
| 1469 | - - | - - |
| 1249 | 84.9 68.5 | - - |
| 2368 | - - | - - |
| 2467 | 15.0 15.2 | - - |
| 1229 | - - | - - |
| 2347 | 89.9 289.2 | - - |
| 1269 | - - | - - |
| 2378 | 129.9 305.1 | - - |
| 2348 | 719.2 730.6 | - - |
| 2346 | 49.9 49.5 | - - |
| 2347 | 219.7 152.2 | - - |
| 1247 | - - | - - |
| 2348 | 8.6 - | - - |
| 2467 | 235.2 292.2 | - - |
| 12479/1367 | 4.3 20.9 | - - |
| 12467 | 94.1 41.7 | - - |
| 1467/12347 | 102.6 41.7 | - - |
| Penta-CDF (0.0 = ND; < 2.0 ppb) | 12348/12378 | 295.0 1920.2 |
| 12346 | 34.2 - | - - |
| 12379 | - - | - - |
| 12367 | 8.6 167.0 | - - |
| 12469/12678 | 615.7 2838.6 | - - |
| 12679 | - - | - - |
| 12369 | - - | - - |
| 23468 | 81.2 250.5 | - - |
| 12349 | - - | - - |
| 12469 | 470.3 3757.0 | - - |
| 23467 | 821.0 4049.2 | - - |
| 12389 | - - | - - |
| 23467 | 162.5 751.4 | - - |
| Hexa-CDF (0.0 = ND; < 4.0 ppb) | 123468 | 17.1 - |
| 134678 | 85.3 - | - - |
| 134679 | - - | - - |
| 124678 | 238.9 - | - - |
| 124679 | 17.1 - | - - |
| 123478/123479 | 1638.1 4571.4 | - - |
| 123678 | 733.7 3190.5 | - - |
| 124689 | 418.1 952.4 | - - |
| 123467 | 341.3 2142.9 | - - |
| 123679 | - - | - - |
| 124698/123689 | 597.2 2238.1 | - - |
| 123789 | - - | - - |
| 12469 | 443.7 2761.9 | - - |
| 234678 | 213.3 1333.3 | - - |
| Hepta-CDF (0.0 = ND; < 4.0 ppb) | 1234678 | 581.8 1506.5 |
| 1234679 | 375.8 1286.8 | - - |
| 1234689 | 157.6 470.8 | - - |
| 1234789 | 533.3 1459.4 | - - |
| Octa-CDF (0.0 = ND; < 4.0 ppb) | 12346789 | 356.0 945.6 | - - |

| PCDDs | ND | ND |

| < 2.0 ppb | < 2.0 ppb |

ND, not detected.
tested, lot 6024 has more non-ortho PCBs and PCDFs contributing to higher TEQ values. Recently, Frame (18) discussed the manufacturing process of several lots of Aroclor 1254 and the differences in the congener composition. Lot 124-191 of Aroclor 1254 has the typical PCB congener distribution. However, lot 6024 has unusually enhanced levels of non-ortho and mono-ortho congeners and PCDFs. Lot 6024 has been traced back to Monsanto lot KI-6024 and represents the late (1974–1976) production of Aroclor 1254s, which used a two-stage chlorination procedure (18). In the first stage, biphenyl was chlorinated to 42% chlorine content by weight for Aroclor 1242 production and then fractionated to give a distillate that was sold as Aroclor 1016. In the second stage, the residue from the distillate was further chlorinated to 54% chlorine by weight, greatly increasing the levels of non-ortho and mono-ortho congeners with high TEF values (18). Caution should be used when comparing the results using this lot (lot 6024) with results from other lots of Aroclor 1254.

The biologic activity of two lots of Aroclor 1254 was tested on two previously established neurochemical end points, intracellular Ca\(^{2+}\) buffering and PKC translocation. We selected these two end points on the basis of our previous work, where intracellular Ca\(^{2+}\) buffering by endoplasmic reticulum and mitochondria as well as PKC translocation were preferentially affected by ortho-substituted PCBs (43). Intracellular Ca\(^{2+}\) buffering is essential for maintaining normal calcium homeostasis (46). When intracellular free-Ca\(^{2+}\) levels increase, PKC may translocate from cytosol to the membrane, where it gets activated. Increases in intracellular free-Ca\(^{2+}\) levels by PCBs have been reported by several investigators in several cell systems (35, 47, 48). In addition, increases in intracellular-free Ca\(^{2+}\) levels, inhibition of Ca\(^{2+}\) buffering, and PKC activation and translocation have been involved in the neurotoxicity of a variety of environmental chemicals (49–51). When these two lots of Aroclors were tested on Ca\(^{2+}\) buffering and PKC translocation in brain preparations, the effects were significantly different. Intracellular Ca\(^{2+}\) buffering by microsomes and mitochondria was significantly inhibited by both lots of Aroclor 1254 in a concentration-dependent manner. Lot 6024 seems to be more potent than lot 124-191. This difference in the potency increased several-fold when the concentration of Aroclor 1254 was transformed to TEQ values (derived from dioxin TEFs), demonstrating that the greater effect with lot 6024 is not caused by the greater ary1 hydrocarbon-receptor binding activity alone. PKC translocation, measured

Figure 1. Inhibition of rat brain microsomal \(^{45}\)Ca\(^{2+}\) uptake by Aroclor 1254 with two lot numbers. The \(^{45}\)Ca\(^{2+}\) uptake was represented as percent of control (50.5 ± 2.1 pmol/mg protein/min). Values are mean ± SEM of four preparations, assayed in triplicate.

Figure 2. Inhibition of rat brain mitochondrial \(^{45}\)Ca\(^{2+}\) uptake by Aroclor 1254 with two lot numbers. The \(^{45}\)Ca\(^{2+}\) uptake was represented as percent of control (11.2 ± 0.4 pmol/mg protein/min). Values are mean ± SEM of four preparations, assayed in triplicate.

Table 5. Different PCB/PCDF/PCDD/PCN congeners based on the number of chlorines in the Aroclor 1254 mixtures with two different lot numbers.

| Aroclor 1254 lot numbers | Units | Mono, di, and tri | Tetra | Penta | Hexa | Hepta | Octa, nona |
|--------------------------|-------|------------------|-------|-------|------|-------|------------|
| 124-191                  | PCBs  | ND               | 168.6 | 504.5 | 222.2| 56.6  | ND         |
|                          | PCDFs | 1.86 (14.3)      | 2.93 (25.8)| 4.74 (41.8)| 1.65 (14.5)| 0.36 (3.1) |
|                          | PCDDs | ND               | 0.13 (2.6) | 1.12 (22.6) | 2.58 (52.3) | 1.12 (22.5) | ND         |
|                          | PCNs  | 0.070            | 0.562 | 8.12  | 65.71| 65.81 | 14.99      |
| 6024                     | PCBs  | ND               | 194.1 | 539.5 | 274.3| 37.3  | ND         |
|                          | PCDFs | 1.89 (4.4)       | 14.15 (38.6)| 17.19 (44.4)| 4.72 (12.2)| 0.95 (2.4) |
|                          | PCDDs | ND               | 0.35 (1.9) | 5.97 (32.5) | 9.09 (49.5) | 2.97 (16.1) | ND         |
|                          | PCNs  | 0.015            | 0.319 | 23.26 | 94.99| 44.85 | 7.80       |

Table 6. PCB congeners (pg/ng) based on ortho-substitutions in the Aroclor 1254 mixtures with two different lot numbers.

| PCB congeners (pg/ng) | Total PCB congeners (pg/ng) |
|-----------------------|----------------------------|
| Non-ortho              | 0.2 (0.02%)                |
| Mono-ortho             | 230.8 (2.9%)               |
| Di-ortho               | 514.5 (43.3%)              |
| Tri-ortho              | 917.1 (13.5%)              |
| Tetra-ortho            | 14.99 (45.3%)              |

The numbers in parentheses indicate the percentage of total.
as [3H]-phorbol ester binding, was significantly increased by both lots of Aroclor 1254; lot 124-191 was significantly more active than lot 6024. As seen with Ca2+ buffering, the difference in the potency also increased several-fold when the concentration of Aroclor 1254 was transformed to TEQ, suggesting that the dioxin-like congeners are not responsible for this effect. The differential effects of two lots of Aroclor 1254 on the selected neurochemical end points could not be explained either by total mass or by TEQ. These effects could not be attributed to the differences in the percentage of non-ortho congeners or dibenzofurans because they were inactive on these two parameters. These effects also could not be attributed to the interactions among the congeners and contaminants because our previous studies indicated that inactive congeners did not interfere with the activity of active congeners, and the interactions between two active congeners seem to follow additivity (52). However, these differential effects could be caused by differences in the composition of ortho-congeners in these two mixtures, because PCBs with ortho-lateral substitutions have been shown to exhibit different activities on the selected neurochemical end points (43). Because of these differential effects between different lots, the composition of Aroclor mixtures used in investigations should be disclosed.

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