DIFFERENTIAL EFFECTS OF TWO LOTS OF AROCLOR 1254[R]: CONGENER ANALYSIS AND NEUROCHEMICAL ENDPOINTS

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Introduction

Polychlorinated biphenyls (PCBs) are widely used in industry as heat transfer and dielectric fluids for transformers and capacitors[1]. PCBs were commercially produced as Aroclor[R] mixtures in USA by the chlorination of biphenyl. Although all 209 congeners can be synthesized, the reaction conditions 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 numerical designation [1-2].

PCBs cause a wide range of effects in humans including chloracne, diverse hepatic effects, decreased birth weight in the offsprings, decreased pulmonary function, subtle endocrine disturbances, cancer, and learning and memory deficits[3-7]. Several of these effects have been demonstrated in animals following exposure to commercial PCB mixtures such as Aroclor 1254[3-5]. This information has been used in the risk/exposure assessment of PCBs and related chemicals[1,5]. However, recent reports indicate substantial differences in the congener composition among Aroclor lots[6, 7]. Toxicity studies with Aroclor 1254 have given varied results[8, 9], but these differences were attributed to the animal species or dosing rather than to the chemical composition. The current study was designed to study the effects of two lots of Aroclor 1254 (Lot #s 6024 and 124-191) on the neurochemical endpoints *in vitro* and compare the effects with their PCB congener composition and other contaminants.

Materials and Methods

Chemicals. Aroclor 1254 (>99% purity) with lot numbers 6024 and 124-191 were purchased from AccuStandard, New Haven, CT. For neurochemical experiments, stock solutions were prepared in dimethyl sulfoxide (DMSO). DMSO alone (2 µl/ml) had no significant effect on the endpoints.

Congener-Specific Analysis of PCBs, Dioxins (PCDDs), Furans (PCDFs), and Napthalenes (PCNs) in Two Lot s of Aroclor 1254. Congener-specific analysis of PCBs was performed according to previously established method[10]. Non-*ortho* PCBs were separated from other dominant *ortho*-PCBs for their trace level determination using Cosmosil 5-PYE column-HPLC method and analyzed in high resolution multidimensional gas chromatography-electron capture detector (HRMDGC-ECD). The PCDD, PCDF, and PCN analyses were performed as per the methods described previously[11, 12].

Neurochemical endpoints. Adult rat cerebella were fractionated to obtain microsomes and mitochondria[13]. Intracellular Ca^{2+} buffering was determined by measuring the uptake of $^{45}Ca^{2+}$ by microsomes and mitochondria[14]. Granule cells from cerebellum of 7 day old rats were isolated by the enzymatic disruption of cells[15]. These cells were maintained for 7 days *in vitro* in culture and used for protein kinase C (PKC) translocation studies, determined by measuring ^{3}H-phorbol ester binding[16].

Statistics. The neurochemical data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Dunnett=s test. The IC_{50} (concentration that inhibits control activity by 50%) and E50 (concentration that increases control activity by 50%) values for $^{45}Ca^{2+}$ -uptake and ^{3}H-phorbol ester

binding, respectively, were calculated from the regression line fit to the linear portion of the curve and were compared using student=s t-test between two lots. The significance was set at $p < 0.05$.

Results and Discussion

Results from this study indicate that the composition of PCB congeners were significantly different between the two lots of Aroclor 1254 (Table 1). Dioxins were not detected in either lot (< 2 parts per billion), as anticipated. PCNs were similar in both lots. However, PCDFs were detectable and Lot # 6024 has 3.4 times more than Lot # 124-191 (data not shown). Differences in the congener composition among different lots of Aroclor mixtures have been previously reported[6,7]. However, this is the first detailed report about the congener-specific analysis along with the contaminants of these two lots of Aroclor 1254 (*Source*: AccuStandard, Inc), a widely used commercial mixture in USA for conducting scientific research. Of the two lots of Aroclor 1254 tested, Lot # 6024 has more non-*ortho* PCBs and PCDFs contributing to higher TEQ values. Recently, Frame[7] discussed the manufacturing process of several Aroclors. Lot # 124-191 of Aroclor 1254 has the typical PCB congener distribution. Lot #6024 has been traced back to Monsanto Lot # KI-6024 and represents the late (1974-1976) production which used a two-stage chlorination procedure resulting in greatly increased levels of non-*ortho*, mono-*ortho* congeners and PCDFs with high TEF values[7].

The biological activity of two lots of Aroclor 1254 was tested on two previously established neurochemical endpoints such as intracellular Ca^{2+} buffering and PKC translocation[17]. Intracellular Ca^{2+} buffering is essential in the maintenance of normal calcium homeostasis. When intracellular free Ca^{2+} levels increase, PKC may translocate from cytosol to the membrane where it gets activated. PCBs have been shown to increase intracellular free Ca^{2+} in a number of cell systems[15,18]. When these two lots of Aroclors were tested, the effects were significantly different (Table 2). 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 compared to Lot # 124-191. This difference in the potency increased several fold when the concentration is transformed to TEQ values demonstrating that the greater effect with Lot # 6024 is not due to the greater Ah-receptor binding activity alone. PKC translocation measured as ^{3}H-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 Ca^{2+} buffering, the difference in the potency also increased several fold when the concentration is transformed to TEQ (ng/ml) 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 endpoints were not explained by the differences in their TEQs. However, other endpoints such as hepatic induction of EROD and MROD were explained by their TEQs[19]. These results suggest that overall toxicity of complex mixtures can not be entirely predicted based on the TEQ values and caution should be used when making risk assessment decisions about chemical mixtures which involve both Ah-receptor-dependent and -independent mechanisms.

In general, current data indicate that the effects of two lots of Aroclor 1254 on selected neurochemical endpoints could not be explained entirely either by total mass or by TEQ. These effects could not be attributed to the differences in non-*ortho* PCBs or dibenzofurans, since they were inactive on these two parameters[4,15]. These effects also could not be attributed to the interactions among the congeners and contaminants, since our previous studies indicated that these inactive congeners did not interfere with the activity of other congeners and the interactions between two active congeners seem to follow additivity[20]. However, the differential effects could be due to differences in the composition of *ortho*-congeners, since *ortho*-lateral PCBs exhibited different activities on these neurochemical endpoints[17]. Because of these differential effects between different lots, the composition of Aroclor mixtures used in investigations should be disclosed. (This abstract does not necessarily reflect USEPA policy).

Table 1. Congener-Specific Analysis of of PCBs in Two Lots of Aroclor 1254. Values are in mg/g.

PCBs	IUPAC #	Lot 124-191	Lot 6024	PCBs	IUPAC #	Lot 124-191	Lot 6024
2,2',3,3'	40	2.11	3.36	2,3,3',4,4'	105	15.82	49.71
2,2',3,4	{41 +	9.71	9.81	2,3,3',4',6	110	76.57	87.15
2,3,4',6	64}			2,3,4,4',5	{114 +	0.05	0.78
2,2',3,5	44	25.18	10.46	2',3,3',4,5	122 +		
2,2',4,4'	{47 +	-	5.96	2,2',3,3',4,6	131}		
2,2',4,5	48}			2,3',4,4',5	118	134.25	138.48
2,2',4,5'	49	21.36	4.07	2',3,4,4',5	123	0.57	2.14
2,2',4,6	50	-	-	3,3',4,4',5	126	0.17	3.24
2,2',5,5'	52	35.06	7.73	2,2',3,3',4,4'	128	7.04	9.67
2,2',5,6'	53	-	-	2,2',3,3',4,5	129	8.70	9.34
2,2',6,6'	54	-	-	2,2',3,3',4,5'	130	-	-
2,3',4,4'	{66 +	90.78	75.98	2,2',3,3',4,6'	132	33.03	25.82
2,2',3,5',6	95}			2,2',3,3',5,6'	135	12.30	6.69
2,3',4',5	70	25.24	63.57	2,2',3,4,4',5	{137 +	4.18	-
2,4,4',5	74	4.36	23.47	2,2',3,3',4,6,6'	176}		
3,3',4,4'	77	0.01	27.20	2,2',3,4,4',5'	138	58.70	71.80
3,3',5,5'	80	-	-	2,2',3,4,5,5'	{141 +	12.18	12.86
3,4,4',5	81	0.01	0.28	2,2',3,3',5,6,6'	179}		
2,2',3,3',4	{82 +	22.02	20.98	2,2',3,4,6,6'	145	-	-
2,2',3,5,5',6	151}			2,2',3,4',5,5'	146	10.29	8.44
2,2',3,3',5	83	4.29	4.30	2,2',3,4',5',6	149	41.76	14.37
2,2',3,3',6	84	-	-	2,2',4,4',5,5'	153	31.80	33.93
2,2',3,4,4'	85	7.16	-	2,3,3',4,4',5	156	4.80	51.00
2,2',3,4,5	86	-	-	2,3,3',4,4',5'	157	0.36	26.30
2,2',3,4,5'	{87 +	42.21	47.22	3,3',4,4',5,5'	169	0.01	0.02
2,3,4,4',6	115}			2,2',3,3',4,4',5	170	4.02	3.39
2,2',3,4',5	{90 +	72.28	61.60	2,2',3,3',4,5,6	173	0.77	1.74
2,2',4,5,5'	101}			2,2',3,3',4,5,6'	174	31.12	18.32
2,2',3,4',6	91	20.24	22.54	2,2',3,3',4',5,6	177	1.59	0.88
2,2',3,5,5'	92	28.34	36.00	2,2',3,4,4',5,5'	180	5.18	4.51
2,2',3',4,5	97	20.33	-	2,2',3,4,4',5',6	183	1.97	1.36
2,2',4,4',5	99	24.97	37.10	2,2',3,4',5,5',6	187	3.51	0.39

PCBs without any values are below the detection limit, < 0.05% (w/w).

Table 2. Differential effects of two lots of Aroclor 1254 on Intracellular Ca^{2+} buffering and PKC translocation in brain. Values represent IC50s for Ca^{2+} buffering and E50s for PKC translocation.

Neurochemical endpoint	Concentration (μg/ml)		TEQ (ng/ml)	
	Lot # 124-191	Lot # 6024	Lot # 124-191	Lot # 6024
Intracellular Ca^{2+} buffering (IC50):				
Microsomes	1.65 ± 0.05	1.23 ± 0.05*	0.058 ± 0.002	0.489 ± 0.019*
Mitochondria	1.78 ± 0.07	1.47 ± 0.08	0.062 ± 0.002	0.585 ± 0.032*
PKC translocation (E50):				
	11.03 ± 1.29	28.01 ± 6.01*	0.386 ± 0.045	11.14 ± 2.39*

*Significantly different from Lot 124-191 at $p < 0.05$. Values are mean ± SE of 4-6 experiments.

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