EFFECTS OF TWO LOTS OF AROLOR 1254 ON HEPATIC RETINOID CONCENTRATION IN RATS

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Introduction

Vitamin A (retinol) is necessary for normal vision, growth, reproduction, cell differentiation, embryonic development and immune function. The liver is the primary storage organ for vitamin A, containing about 80% of total body stores[1]. In the body, the majority of the vitamin A is in the form of retinyl esters.

Decreased hepatic vitamin A storage has been reported in animals exposed to various chlorinated aromatic compounds. A single oral dose of 10 μg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg bw to female Sprague-Dawley rats decreased vitamin A in the liver, lungs, intestines and adrenal glands while increasing its concentration in serum and kidneys and urine[2]. Like TCDD, dioxin-like 3,3',4,4'-tetrachlobiphenyl (PCB 77), 3,3'4,4',5,5'-hexachlorobiphenyl (PCB 169), and 3,3',4,4',5,5'-Hexabromobiphenyl (HBB) also cause decreased hepatic and increased renal levels of vitamin A in rats[3]. Nondioxin-like PCB congeners have no effect on vitamin A tissue levels[4].

Previous studies by our laboratory on two lots of Aroclor 1254 with different dioxin toxic equivalents (TEQ) have shown that the contrast explains the variances in the dioxin-like effects such as cytochrome P450 1A1 and 1A2 activity. The nondioxin-like congeners present in the lots cause other responses that are not associated with the Ah receptor such as P450 2B1 activity. In addition, supra-additive effects also occur in the mixture (T4, oxidative stress)[5]. Thus, overall toxicity cannot be entirely predicted based on the TEQ values. As retinoid levels have not been shown to be affected by nondioxin-like PCBs, any differences in the two lots should be predicted by the TEQ.

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 using corn oil as vehicle (See Table 1 for doses). Three days after treatment, animals were killed by CO_2 asphyxiation. Livers were removed and total liver whole homogenate fractions were taken and frozen at –80°C until analysis.

Vitamin A analysis: Liver retinoids were extracted from 100 µl aliquots of liver homogenates with 250 µl of ethyl acetate containing 0.1% BHT as an antioxidant. The samples were then centrifuged at 15,000 rpm for 1-2 minutes. Two hundred microliters of the ethyl acetate layer was removed and evaporated using the speedvac at full vacuum, medium chamber heat for 30 minutes. Samples were resuspended with 250 µl of mobile phase (95% methanol/ 5% ethyl acetate) and a 200 µl aliquot was removed for analysis[6].

Twenty-five microliters of the 200 µl sample were analyzed with HPLC using a C-18 reverse-phase analytical column (Rainin Microsorb ODS, 4.6 mm x 25 cm, 5 µm particle size) with a guard column (Ultrasphere ODS, 4.6 mm x 4.5 cm, 5 µm particle size). A wavelength of 326 nm with a 4 nm bandwith was used for the detection of retinoids[6].

Statistical analysis: Retinol and retinyl palmitate levels were analyzed independently using a one-way analysis of variance (ANOVA) and the Fisher's PLSD ($p<0.05$)

Results and Discussion

Previous studies in rats have shown decreased hepatic retinol and retinyl palmitate concentrations[2,3,4,6] in response to exposure with chlorinated aromatic compounds. There appears to be a decrease in retinyl palmitate levels with both lots on a weight (Figure 1) and a TEQ basis (Figure 2). Retinol does not demonstrate a clear response for either lot (data not shown). In the case of retinyl palmitate, the TEQ appears to be predictive of the effect when dealing with mixtures of dioxin-like and nondioxin-like PCB congeners.

Studies have shown that PCB 77 markedly decreases serum retinol levels. The binding of PCB 77 to transthyretin causes a conformational change that results in less transthyretin bound to the retinol-retinol binding protein (RBP) complex. Since retinol-RBP is small enough to be filtered by the kidneys, serum levels of both retinol and RBP decrease following exposure to PCB 77. Lot 6024 has 27.2 mg/g PCB 77 while Lot 124-191 has only 0.01 mg/g PCB 77. Future experiments should look at renal and serum retinoid concentrations.

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Literature

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Table 1 – Dose Schedule for Two Lots of Aroclor based on Weight and TEQ values

Lot 124-191			**Lot 6024**	
Dose mg/kg	Dose µg TEQ/kg		Dose mg/kg	Dose µg TEQ/kg
0.00	0.000		0.00	0.000
10.00	0.033		1.00	0.033
30.00	0.099		3.00	0.099
100.00	0.330		10.00	0.330
300.00	0.991		30.00	0.991
1000.00	3.305		100.00	3.305
			300.00	10.48
			1000.00	34.93

Figure 1 – Comparison of Retinyl Palmitate Levels on a Weight basis
Control Retinyl Palmitate level for Lot 124-191 is 568.89 µg/g tissue
Control Retinyl Palmitate level for Lot 6024 is 644.65 µg/g tissue

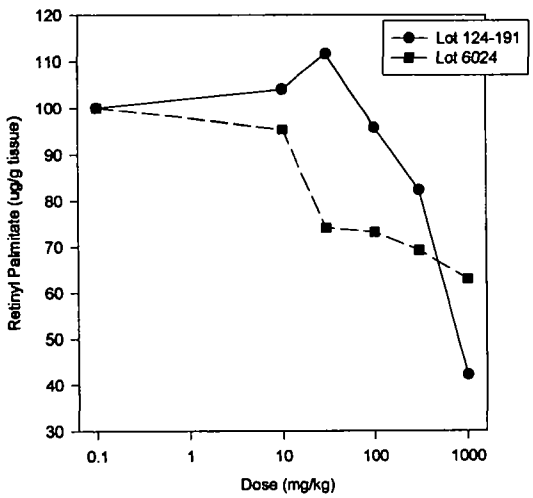

Figure 2 – Comparison of Retinyl Palmitate Levels on a TEQ basis
Control Retinyl Palmitate level for Lot 124-191 is 568.89 μg/g tissue
Control Retinyl Palmitate level for Lot 6024 is 644.65 μg/g tissue