CYP1A1 AND CYP1B1 EXPRESSION IN SPRAGUE-DAWLEY RATS FED AROCLORS 1016, 1242, 1254, AND 1260 FOR 6 MONTHS

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Introduction

The hepatic tumorigenicities of four formerly commercial PCB compositions were recently determined by Battelle Laboratories for General Electric and reported by Mayes *et al.*[1]. In that study, female Sprague-Dawley rats were found to be much more sensitive to tumor development than males. Female Sprague-Dawley rats that had been fed diets containing Aroclors 1016, 1242, 1254, or 1260 at dietary levels ranging from 25 to 200 ppm for up to 2 years had maximal tumor incidences of 12, 30, 56, and 48% respectively. Only the highest dose of Aroclor 1260 produced a significantly elevated tumor incidence in males (20%). PCB accumulations in hepatic and adipose tissue lipids and their respective congener distributions were also determined at various intervals during the study. Modeling efforts indicated that PCB hepatic tumorigenicity in females was dependent on a combination of two factors: the concentrations of coplanar PCB congeners, or TEQ, in liver lipid and the total PCB body burden as measured in adipose lipid. In contrast, hepatic tumorigenicity in males was solely dependent on the latter. This observation suggested a tumorigenic mechanism in females that involved the interaction of a female sex specific factor with an aromatic hydrocarbon receptor (AhR) dependent response(s). Thus, it seemed appropriate to examine the possibility that PCBs had altered the normal metabolism of estrogen in some manner. It also seemed appropriate to compare our results with those of others who have been investigating the role of estrogen metabolism in TCDD carcinogenesis [2-4].

The 2- and 4-hydroxylation of 17β-estradiol (E_2) are carried out by AhR-regulated CYP1A1 and CYP1B1 enzymes[5,6]. Some investigators have suggested that elevated expression of CYP1B1 and the consequent formation of additional 4-OH-E_2 are markers for increased human cancer risk[7]. One possible carcinogenic pathway could be through the excessive generation of 4-OH-E_2, followed by the formation of semiquinone and quinone metabolites of estradiol[8]. Since these metabolites can redox cycle and produce superoxide[8] it is possible that a state of oxidative stress could develop that would favor the development of hepatic tumors.

Evidence for redox cycling activities in the Aroclor-fed SD rats of this study was detected when it was found that a low molecular weight component of the hepatocyte cytosolic fraction was able to produce superoxide when incubated with control microsomes[9]. In fact, there was a good correlation (r^2=0.74-0.89) at all time points between the ability to produce superoxide and Aroclor hepatic tumorigenicity. This correlation extended across both sexes, all doses, including controls, and all Aroclors. To investigate the possibility of a role for CYP1B1 in the observed tumorigenesis in SD rats, the expressions of CYP1A1 and CYP1B1 were determined in rats fed Aroclors for six

months. CYP1A1 and CYP1B1 protein expression was then compared to both dietary intake of TEQ and hepatic TEQ concentration.

Materials and Methods

Microsomes were prepared[10] from SD rat livers kept frozen at –20°C since collected at necropsy. Microsomal CYP1A1 protein was quantitated against standard curves using a colorimetric ELISA assay (Amersham, Life Science, RPN 269). The mean values of three animals per dose group are shown in Table 1. CYP1B1 was determined in 50 μg samples of SD rat microsomal protein by immunoblot analysis[2] using anti-His_6-CYP1B1 antibody detected by HRP-linked anti-rabbit IgG and an enhanced chemiluminescence method (SuperSignal, Pierce). For this preliminary study, CYP1B1 was determined, individually, for only 27 animals. Samples were selected to allow for the detection of differences in CYP1B1 expression among the Aroclors and between the sexes, and the possible detection of a dose-response relationship for Aroclor 1254-dosed female rats, the group most likely to be CYP1B1-induced (based on the relatively high TEQ of Aroclor 1254.) TEQ calculations were based on the toxic equivalency values (TEF) for PCB congeners recommended by Ahlborg *et al.* [11] in 1994.

Results and Discussion

As shown in Table 1, Aroclor 1016 did not induce CYP1A1 above constitutive levels in females but induced it about twofold in males, indicating a sex-dependency and greater sensitivity in males, even when adjusted for their 30% greater dietary intake. Aroclors 1242, 1254, and 1260, each induced females about 16 to 18-fold and induced males about 26 to 32-fold. For each of these Aroclors, males were about twofold more induced than females. Except for Aroclor 1254, which showed a dose-response relationship for both sexes, each Aroclor appeared to have produced a saturated level of CYP1A1 protein. Note that the 2 year tumor response correlates poorly with hepatic CYP1A1 protein at six months for both sexes. CYP1B1 protein was not detected in any animals above the detection limit of 0.02 pmol/mg microsomal protein, although the relative stabilities of these proteins during storage is not yet known.

When Walker *et al.*[2] compared the induction of hepatic CYP1A1, CYP1A2, and CYP1B1 in female SD rats administered TCDD for 30 weeks, using a protocol that was similar to our Aroclor dosing protocol in most respects, they found a relatively low level of CYP1B1 expression compared to that of either CYP1A1 or CYP1A2. Based on CYP1A1 expression alone, we should not have expected to see any CYP1B1, since our CYP1A1 values were in the range of about 50-60 pmol/mg protein. This is about the level at which Walker *et al.* [2] had just begun to see CYP1B1 expression. However, from a dietary exposure perspective, they observed CYP1A1 and CYP1A2 induction at dose levels as low as 10.7 ng TCDD/kg/day but saw CYP1B1 induction only at dosages at and above 35.7 ng TCDD/kg/day. In contrast, in our study, rats dosed at rates up to 331 ng TEQ/kg/day, ten times the amount needed for TCDD, still showed no signs of CYP1B1 expression. Furthermore, based on liver tissue TCDD levels, they first saw CYP1B1 expression at a liver tissue TCDD concentration of about 6 ppb wet weight. Interestingly, we did not observe CYP1B1 induction at hepatic lipid TEQ levels of up to 1118 ppb. This is equivalent to about 60 ppb on a wet weight basis, again, ten times more than needed with TCDD. This absence of CYP1B1 expression may be due to the inhibition of AhR-regulated gene expression by the *ortho* chlorinated PCBs as reported by van der Plas[12]. Thus, it is clear that TEQs in either PCB dietary exposure or tissue levels greatly overestimates this particular hepatic effect.

Thus, although a possible role for CYP1B1 in the chemical carcinogenesis of TCDD and PCBs has been investigated by others[2)] and by ourselves, it is apparent that the data now available does not support this earlier hypothesis[13)]. It is also important to recognize that although TCDD can induce hepatic CYP1B1 expression, the PCBs administered in our study have not induced CYP1B1 expression, even when administered at TCDD-equivalent doses. This may mean that CYP1B1 is, as other have suggested[4,14)], regulated differently from the other P450s. More important, however, is the recognition that although CYP1B1 may contribute to human cancer[15)], the causation of cancer through the induction of this particular P450 by PCBs now seems unlikely. This lack of parallelism between the effects of TCDD and PCBs points out the need to be cautious in drawing inferences about later stage biologic responses based on similarities found at the early receptor binding stages. In summary, the actions of complex mixtures of PCBs may not be as simply predicted as those of single compounds like TCDD.

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Table 1. Hepatic P450 and dietary and tissue TEQ levels of SD rats fed four Aroclors for 6 months and their tumor responses at 2 years.

		FEMALE					MALE				
		TEQ		P450			TEQ		P450		
	Dietary PCB	Dietary consumption	Liver lipid	pmol/mg protein		%	Dietary consumption	Liver lipid	pmol/mg protein		%
	(ppm)	(ng/kg/day)	(ppb)	CYP1A1	CYP1B1	Tumor	(ng/kg/day)	(ppb)	CYP1A1	CYP1B1	Tumor
control	0	0	0	3.3	<0.02	1	0	0	2.9	<0.02	7
A1016	50	0.4	0.04	3.0	ND	2	0.3	0.04	7.6	ND	4
A1016	100	0.8	0.07	3.9	<0.02	12	0.6	0.05	8.7	ND	4
A1016	200	1.6	0.13	4.7	ND	10	1.2	0.07	8.5	ND	8
A1242	50	27	120	55.2	ND	22	20.3	60	110.1	ND	2
A1242	100	55	253	55.4	<0.02	30	40.7	113	98.7	ND	8
A1254	25	82	240	18.0	<0.02	38	63.0	169	38.1	ND	8
A1254	50	166	516	46.2	<0.02	56	126.5	309	79.2	ND	8
A1254	100	331	1118	58.5	<0.02	56	253.5	479	93.5	<0.02	12