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Regional Induction of CYP1A1 in Rat Liver Following Treatment with Mixtures of PCB 126 and PCB 153

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ABSTRACT

Liver enzyme induction has been shown previously to be regional with clear borders between induced and uninduced regions in vivo, and cells either fully induced or not induced in vitro. The current study examined this phenomenon in vivo by evaluating enzyme induction after exposure to PCB 126 and PCB 153 in female Fisher 344 (F344) and male Sprague–Dawley (SD) rats. IHC revealed a regional induction of CYP1A1 after exposure to PCB 126, apparent in the centrilobular region at lower doses and progressing to panlobular with higher doses. PCB 153 exposure induced CYP2B1/2 in the centrilobular region, which spread to the midzonal region as the dose increased, but never became panlobular even at the highest dosage tested. In rats treated with PCB 126 in combination with high doses of PCB 153, induction of CYP1A1 occurred preferentially in the periportal region, a reversal from the pattern seen with PCB 126 alone. This CYP1A1 induction pattern reversal is a unique example of complex biological interactions between coplanar (PCB 126) and noncoplanar (PCB 153) halogenated aromatic hydrocarbons.

Keywords. PCB 126; PCB 153; Liver; Cytochrome P450 1A1; Coplanar; Noncoplanar; Mixtures; Cytochrome P450 2B1/2.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are halogenated aromatic hydrocarbons that were widely used in industry and are ubiquitous in the environment. The over 200 PCB congeners have a number of diverse actions at multiple receptors and there are numerous structure activity relationships among different groups of congeners (McFarland and Clarke, 1989; Safe, 1994; Wong et al., 1997; Hansen, 1998; Tilson and Kodavanti, 1998). Many PCB congeners with 4 or more chlorine atoms are known tumor promoters and inducers of cytochrome P450s (Ganem et al., 1999), which are involved in the biotransoformation of various drugs, carcinogens, and steroid hormones (Estabrook, 1996). PCB congeners considered "coplanar" have substitutions in both the para positions and at least 2 meta positions and relate structurally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These coplanar congeners act similarly to TCDD, binding to the aryl hydrocarbon receptor (AhR) to induce CYP1A1 and 1A2 enzymes (Safe, 1990). PCBs with at least 2 meta and 2 para, but no more than 1 ortho chlorine atom are called "mono-ortho congeners" and these congeners, although coplanar, have a lower affinity for the AhR, but are often quite potent inducers of CYPs 2B/3A (Bandiera, 2001; Hansen, 1998; Safe, 1994). PCBs with 4 or more chlorine atoms, with 2 or more of these in ortho positions, are considered "noncoplanar" and may induce CYP2B1/2 and/or CYP3A (Safe, 1994; Bandiera, 2001).

PCBs in the environment exist as mixtures of congeners that differ in physical and biological characteristics. Understanding the mechanism of interaction(s) between the coplanar 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) and the noncoplanar 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) is of importance because they are among the most environmentally important congeners, are known hepatotoxins (Kimbrough, 1995) and are found in significant quantities in human tissues (Cogliano, 1998; Hansen, 1998). Individually, PCB 126 and PCB 153 have both been shown to be promoters of carcinogenesis as indicated by enhancement of preneoplastic GST-P (glutathione-S-transferase placental form) positive liver foci in F344 rats (Dean et al., 2002). However, treatment with a mixture of PCB 126 and 153 resulted in antagonistic GST-P positive foci formation. These findings underscore the complexity of biological interactions of PCBs in chemical mixtures.

The induction of CYP1A1 in the liver appears to occur in response to chemical exposure in a dose dependent pattern. IHC staining of liver for CYP1A1 after low doses of TCDD treatment have shown a centrilobular induction of CYP1A1 with the pattern spreading toward the periportal region as the dosage increased (Tritscher et al., 1992; Andersen et al., 1995). Interestingly, clear boundaries are found between induced and uninduced regions indicating that hepatocytes are either fully induced or are not induced at all (Bars and Elcombe, 1991; Bars et al., 1989).

This study was designed to assess in vivo effects of PCB 126 and PCB 153, individually or in mixture, on CYP1A1 and CYP2B1/2 induction patterns in rat hepatocytes. Our finding that coexposure with PCB 126 and 153 altered the pattern of CYP1A1 expression from a centrilobular to a periportal induction is a novel example of a potentially complex biological interaction between coplanar and noncoplanar PCBs in mixtures.

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Abbreviations: PCB 126, 3,3′,4,4′,5-pentachlorobiphenyl; PCB 153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CYP1A1, Cytochrome P450 1A1 isoform; CYP2B1/2, Cytochrome P450 2B1, 2B2 isoforms.

MATERIALS AND METHODS

Chemicals: PCB 126 was purchased from Accustandard (New Haven, CT), PCB 153 was purchased from Ultra Scientific (North Kingstown, RI), and diethylnitrosamine (DEN) was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade.

Animals and Care: Female Fisher-344 (F344) rats and male Sprague–Dawley (SD) rats, 28–30 days of age, were purchased from Harlan Sprague Dawley (Indianapolis, IN) and allowed to acclimate to a 5,000 ft altitude (Fort Collins, CO) for 4 weeks. Rats were then randomized by weight and divided into treatment groups. All animals were housed (3 per cage) in polycarbonate cages with corncob bedding and stainless steel wire tops. The room was maintained at 25°C with 55% humidity and lighting maintained on a 12-hour light/12-hour dark cycle. All animals were given NIH-07 diet (Harlan Teklad, Madison, WI) and deionized water ad libitum. The clinical status of the rats was observed daily and food, water consumption, and body weight were evaluated 3 times per week. This study was conducted in accordance with the National Institutes of Health guidelines for the care of laboratory animals. Animals were housed in facilities fully accredited by the Association for Accreditation of Laboratory Animal Care.

In Vivo Study: After a 4-week acclimation period, F344 rats were given an intraperitoneal injection of either DEN, 200 mg/kg, or saline for control animals. DEN is a known initiator of carcinogenesis and was used because the animals on this study were also part of a Medium Term Bioassay to assess the carcinogenicity of PCB 126 and PCB 153 alone and in combination (Dean et al., 2002). Two weeks after initiation, gavage treatment was started using PCB 126 or PCB 153 in corn oil or a mixture of PCB 126 and PCB 153, or corn oil alone for control animals, 3 times/week for 6 weeks. Doses of PCB's are shown in the figure legends. Treatment groups consisted of 6 rats each except for the corn oil control group that consisted of 3 rats. SD rats used in the study were not initiated with DEN, but were gavaged with the same concentrations of PCB 126 and PCB 153 for the same amount of time (6 weeks) as the F344 rats. At necropsy, all animals

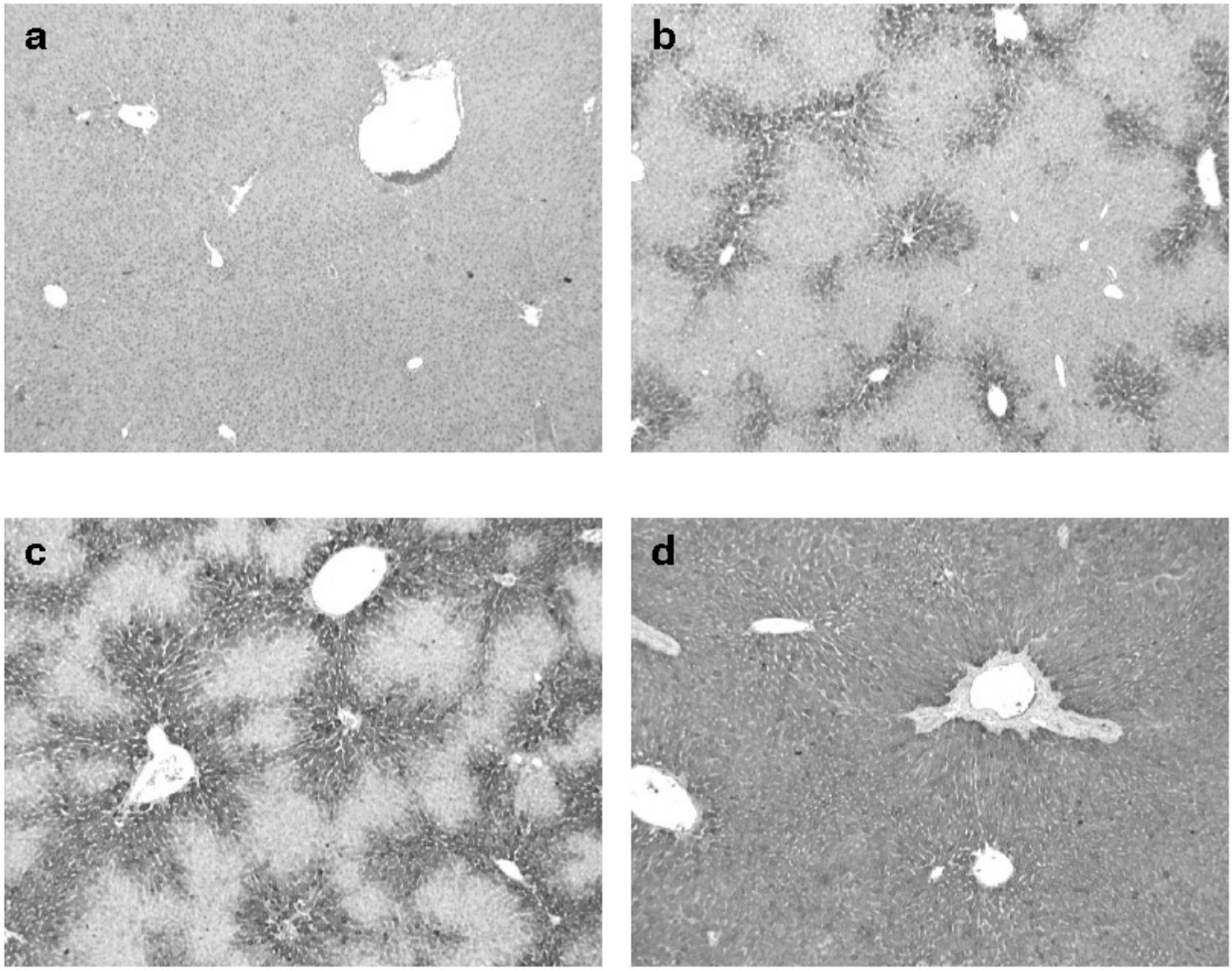

FIGURE 1.—Centrilobular regional induction of CYP1A1 was observed in female F344 rats treated with (b) 0.1 μg/kg/day PCB 126, which progressed to panlobular induction in rats treated with (d) 10 μg/kg/day PCB 126. There was no induction in the (a) control animals and an intermediate induction in the (c) 1.0 μg/kg/day PCB 126 treated animals. All images were photographed at 4× magnification (color photomicrographs available upon request from the corresponding author).

were anesthetized deeply with isoflurane followed by exsanguination, and liver tissue was collected.

Liver sections from the F344 rats were fixed in 10% neutral buffered formalin and paraffin embedded. The liver sections were cut, 5-μm-thick, using a standard microtome and placed on glass slides for use in IHC staining for CYP1A1 or CYP2B1/2.

Liver sections from the SD rats were frozen in liquid nitrogen and stored at -80°C. Eight-μm-thick sections were cut from frozen tissue on a cryostat and placed on glass slides. The sections were allowed to dry overnight at 37°C to be stained for CYP1A1 by IHC.

Immunohistochemistry (IHC): The formalin fixed, paraffin embedded sections were deparaffinized through xylene, 100%, 95%, and 70% ethanol, and put into deionized water. The sections from frozen liver were fixed in 1% paraformaldehyde for 10 minutes, washed in phosphate buffered saline (PBS, pH 7.5) for 10 minutes and then put into deionized water for 5 minutes. The following protocol was then employed for both sets of slides. Endogenous peroxidase activity was quenched by incubating slides in 0.3% hydrogen peroxide for 5 minutes. Slides were washed for 5 minutes in deionized water and antigen retrieval was performed using Citra Antigen Retrieval Solution (ARS) (Biogenex, San Ramon, CA) in a microwave set for 30% power for 10 minutes. The slides were allowed to cool for 25 minutes in Citra ARS, washed for 5 minutes in deionized water and placed back into PBS for 5 minutes. A Vectastain Elite Rabbit IgG ABC kit (Vector Laboratories, Burlingame, CA) was used for staining. Sections were incubated 20 minutes with normal goat serum, 15 minutes with polyclonal rabbit anti-rat CYP1A1 antibody (Chemicon, Temecula,CA) diluted 1:1,000 in PBS, washed in PBS for 5 minutes, incubated 30 minutes with biotinylated anti-rabbit IgG (Vector Laboratories ABC kit), washed for 5 minutes in PBS, and incubated 30 minutes in ABC (Vector ABC kit) reagent. Slides were washed 5 minutes in PBS and AEC

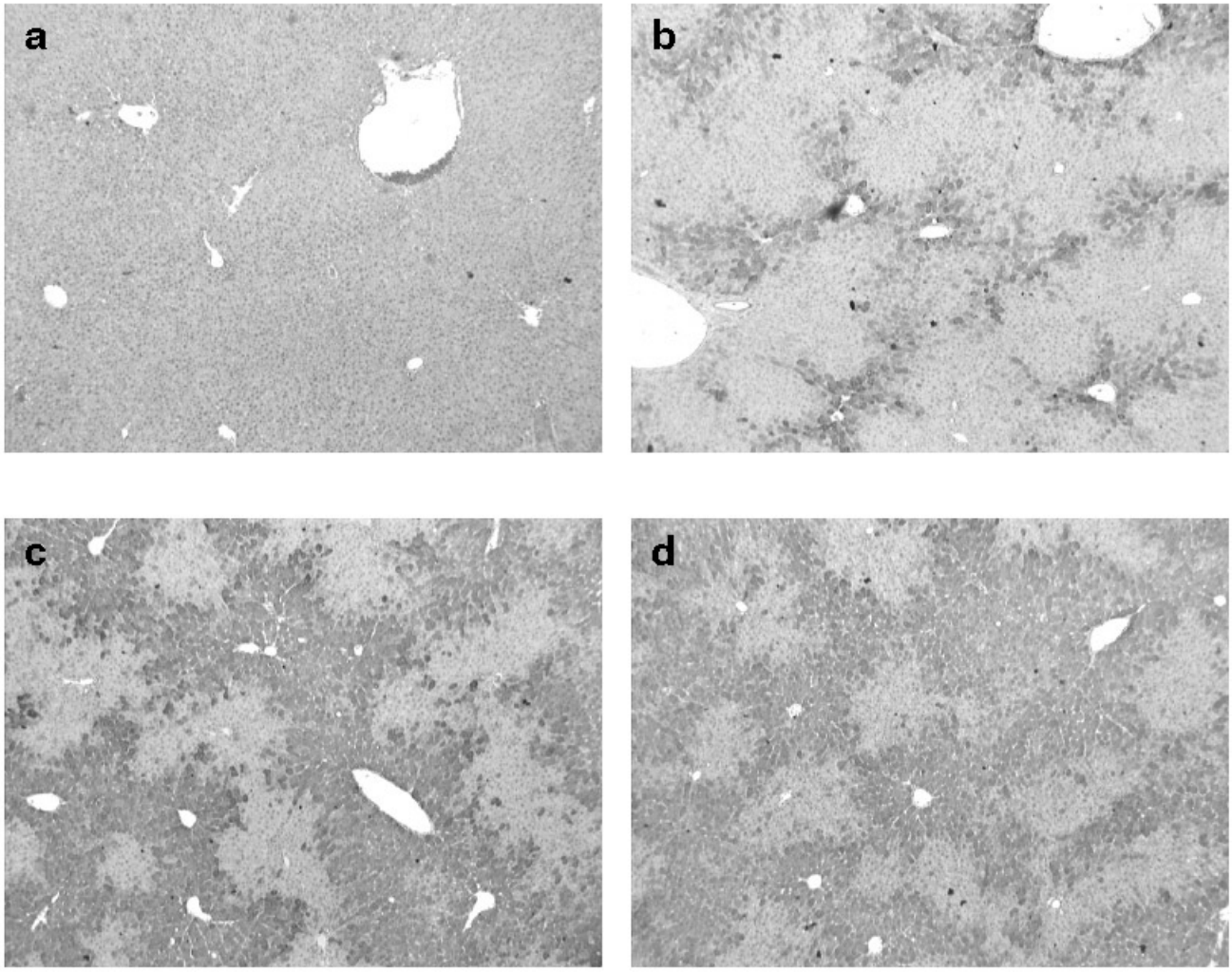

FIGURE 2.—Sections stained for CYP2B1/2 show a dose-dependent increase in staining in the centrilobular region in female F344 rats treated with PCB 153. The pattern shifts from no induction in the (a) control to increasing area of centrilobular induction at (b) 1000 μg/kg/day, (c) 5,000 μg/kg/day, and (d) 10,000 μg/kg/day. Induction never reaches the periportal regions, even at the highest dose of PCB 153. All images were photographed at 4× magnification (color photomicrographs available upon request from the corresponding author).

(9-amino-3-ethylcarbazole) (Biomeda, Foster City, CA) was added for 10 minutes. Slides were stained for 30 sec in Gill's Hematoxylin, washed in running tap water for 10 minutes then mounted with a glycerol/water based mounting media.

The same protocol was employed for CYP2B1/2 except for the following changes. The polyclonal goat anti-rat CYP2B1/2 antibody (BD Gentest, Woburn, Massachusetts) was applied (1:1,000) instead of the CYP1A1 antibody. A Vectastain Elite Goat IgG ABC kit was used in place of the Vectastain Elite Rabbit IgG ABC kit.

RESULTS

Immunohistochemistry (IHC): PCB 126 induced CYP1A1 in a dose dependent regional manner in both F344 and SD rats. PCB 126 induced CYP1A1 protein in the centrilobular region of F344 rats at the lowest dose of 0.1 μg/kg/day with no induction in control animals (Figures 1a, b). At 1.0 μg/kg/day, the pattern of CYP1A1 induction increased outward toward the periportal regions (Figure 1c), becoming panlobular at the highest dose, 10 μg/kg/day (Figure 1d).

PCB 153 induced CYP2B1/2 in the centrilobular region of F344 rats at the lowest dose of 1,000 μg/kg/day (Figure 2b) spreading out midzonally as the dose increased to 5,000 μg/kg/day (Figure 2c). However, CYP2B1/2 induction did not become completely panlobular even at the highest dose tested, 10,000 μg/kg/day (Figure 2d). There was no CYP2B1/2 induction in control animals treated with corn oil (Figure 2a).

Patterns of induction of both CYP1A1 and CYP2B1/2 were qualitatively analyzed for animals treated with a mixture of PCB 126 and 153. PCB 126 did not alter the pattern of induction for CYP2B1/2 observed with PCB 153 alone in rats treated with a mixture of PCB 126 and 153, even at the highest doses (Figure 3). Interestingly, the border of induced/noninduced cells was more distinct with the highest mixture dose (Figure 3d). PCB 153 did, however, alter the

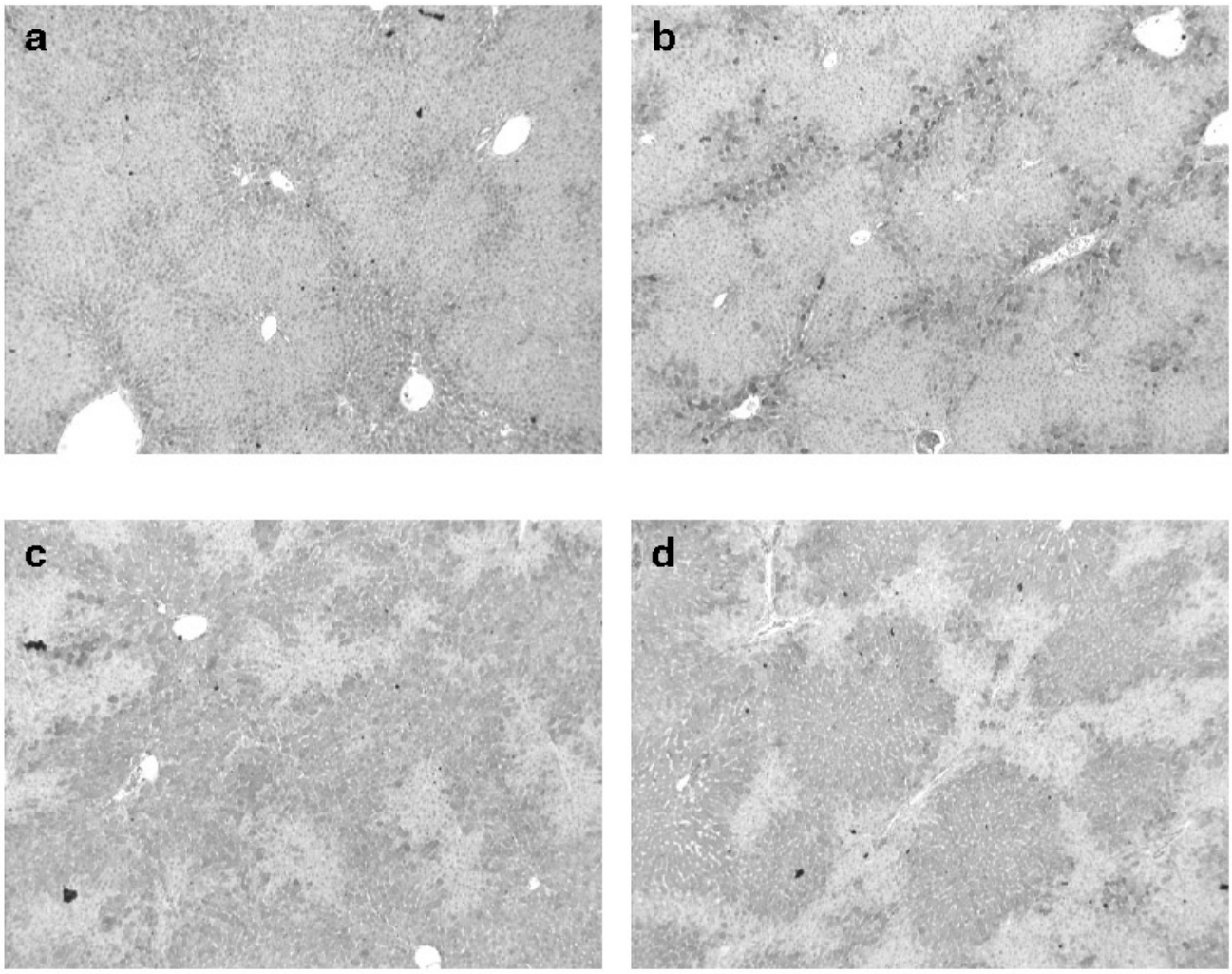

FIGURE 3.—Sections from F344 rats treated with mixtures of PCBs and stained for CYP2B1/2 show a dose dependent increase in staining in the centrilobular region from doses of (a) 1.0 μg/kg/day PCB 126 + 100 μg/kg/day PCB 153, (b) 10 μg/kg/day PCB 126 + 1000 μg/kg/day PCB 153, and (c) 10 μg/kg/day PCB 126 + 5,000 μg/kg/day PCB 153. The pattern at the highest dose, (d) 10 μg/kg/day PCB 126 + 10,000 μg/kg/day PCB 153, was not changed but has more distinct borders between induced and uninduced regions. All images were photographed at 4× magnification (color photomicrographs available upon request from the corresponding author).

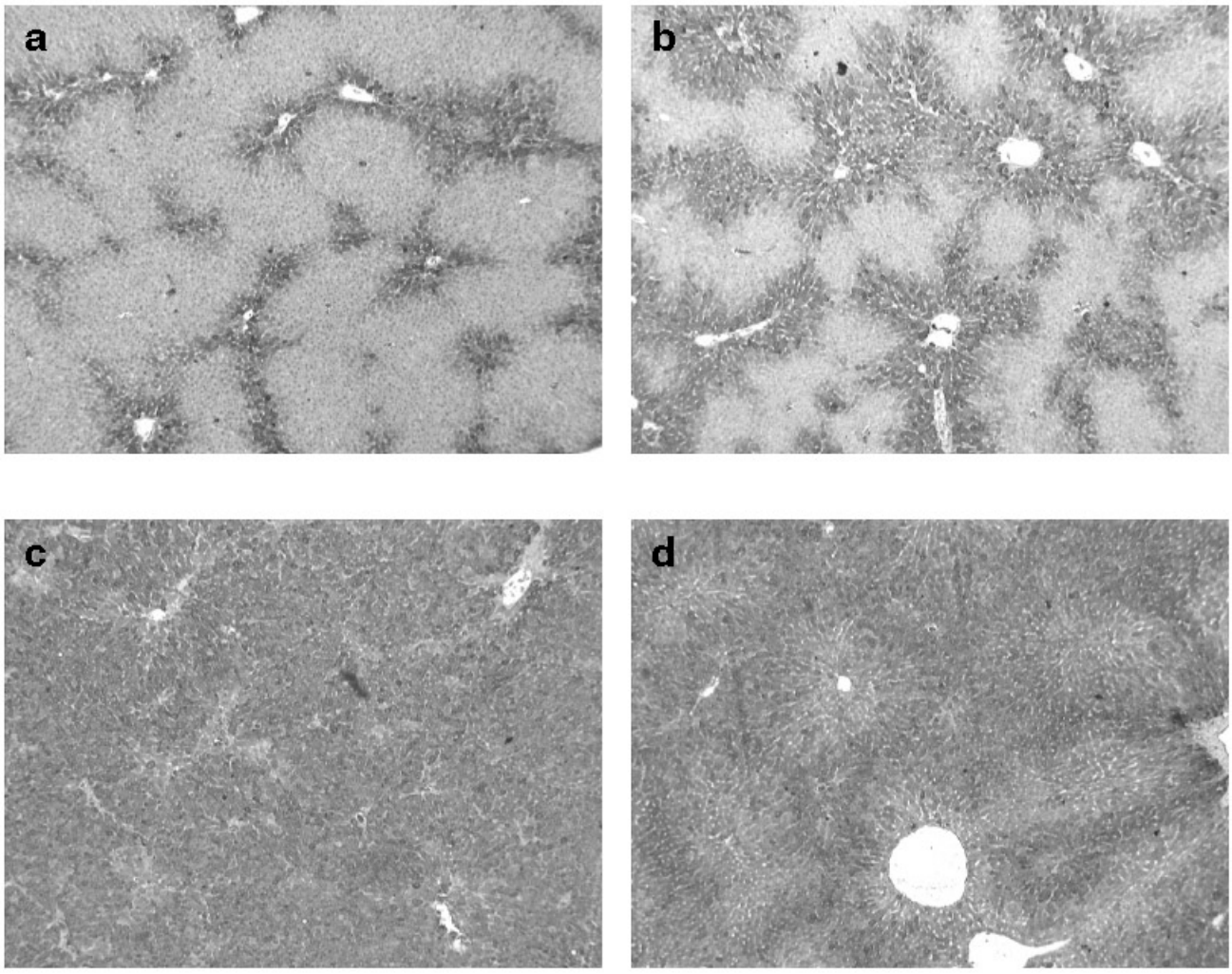

FIGURE 4.—When PCB treatments were administered to F344 rats at (a) 0.1 μg/kg/day PCB 126 + 10 μg/kg/day PCB 153, (b) 1.0 μg/kg/day PCB 126 + 100 μg/kg/day PCB 153 (c) 10 μg/kg/day PCB 126 + 1000 μg/kg/day PCB 153, the pattern of CYP1A1 induction observed was the same as with PCB 126 alone. The pattern reversed to more periportal induction in rats treated with the highest doses of PCB 126 and PCB 153, (d) 10 μg/kg/day PCB 126 + 10,000 μg/kg/day PCB 153. All images were photographed at 4× magnification (color photomicrographs available upon request from the corresponding author).

pattern of CYP1A1 induction observed with PCB 126 alone in rats treated with PCB 126 and 153 at the highest dose (Figure 4). The lower doses showed the same dose dependent response in CYP1A1 induction as with PCB 126 alone (Figure 4a, b, c), but the highest dose (10 μg/kg/day PCB 126 + 10,000 μg/kg/day PCB 153) resulted in a reversal of the pattern of induction with the most intense staining in the periportal region and less intense staining in the centrilobular area (Figure 4d). The identical pattern of induction of CYP1A1 and pattern reversal with the highest dose of the PCB 126 and 153 mixture was also seen in SD rats which were not treated with DEN (data not shown).

DISCUSSION

This observation of CYP1A1 induction pattern reversal due to a mixture of coplanar and noncoplanar PCBs is of interest as it demonstrates complex biological interactions between PCBs known to act via different pathways. PCB 126 has been shown to interact predominantly with the AhR (Hestermann et al., 2000), and PCB 153 activity is mediated via the nuclear receptor known as the constitutive androstane receptor (CAR) (Kawamoto et al., 1999; Zelko and Negishi, 2000), as outlined in more detail below. This pattern of CYP1A1 induction reversal could be explained by several scenarios, including competition for the AhR at high doses of ligand (Petrulis and Bunce, 2000), distribution of ligand in the liver (Dean et al., 2002), differential localization of AhR in the centrilobular versus periportal regions of the liver (Forkert, 1997), or perhaps through interactions of signal transduction cascades initiated by the different PCBs.

AhR is a cytosolic receptor bound to two 90 kDa heat shock protein (hsp90) molecules: an immunophilin-related AIP/XAP/ARA9 protein, and a p23 chaperone protein. After ligand binding, AhR dissociates from this complex, translocates into the nucleus and dimerizes with Arnt. The ligand-bound AhR-Arnt complex acts as a transcription factor complex and binds to specific dioxin response elements (DREs) located in the enhancer/promoter region of dioxin responsive genes such as CYP1A1 (Ma, 2001; Whitlock, 1999).

Unproductive binding of PCB 153 to the rat AhR has been demonstrated (Petrulis and Bunce, 2000). At high doses PCB 153 can bind to the AhR, but for unknown reasons this complex does not bind to a DRE sequence, resulting in sequestration of the AhR. The binding of an unproductive ligand (PCB 153) to the AhR could lead to competition with an effective agonist such as PCB 126 or TCDD. This competition for the AhR could explain the CYP1A1 induction pattern reversal at the high dose of 10 μg/kg/day PCB 126 and 10,000 μg/kg/day PCB 153.

Coplanar and noncoplanar compounds are known to affect the mutual, whole organ distribution of each other when administered in combination (van Birgelen et al., 1996; Dean et al., 2002). An increase in PCB 153 concentrations in the liver when administered in combination with PCB 126 compared with PCB 153 alone has been demonstrated (Dean et al., 2002). The CYP1A1 induction pattern reversal observed could be partially explained by the increased liver levels of PCB 153 due to concomitant administration of PCB 126.

Differential localization of AhR and CYP1A1 have been shown in livers of C57BL/6J and DBA/2J mice by IHC after exposure to 3-methylcholanthrene (Forkert, 1997). Interestingly, subcellular AhR staining was primarily cytoplasmic in the centrilobular hepatocytes and nuclear in the periportal hepatocytes. CYP1A1 induction was shown to be predominantly centrilobular, the area in which the AhR was cytoplasmic and thus available for ligand binding. This pattern of centrilobular CYP1A1 induction following exposure to low doses of an AhR agonist is consistent with our findings. It would be interesting to assess AhR localization in vivo after a more chronic exposure to an AhR agonist and compare these findings to CYP1A1 induction across the acinus.

PCB 153 is a phenobarbital-like inducer of CYP2B1/2 metabolizing enzymes (Ganem et al., 1999), inducing CYP2Bs via the CAR (Kawamoto et al., 1999; Sueyoshi et al., 1999). Following exposure, CAR translocates to the nucleus, forms a heterodimer with the retinoid X receptor, and binds to a phenobarbital response element (PBRE) in the upstream region of responsive genes (Zelko and Negishi, 2000). CAR-mediated transcription is further enhanced by the steroid coactivator 1 (SRC-1) and the ubiquitous transcription factor Sp1 (Muangmoonchai et al., 2001). CAR is also know to exhibit constitutive activation in the absence of any known ligand (Zelko and Negishi, 2000).

Interaction or competition between key components of the AhR and CAR receptor pathways, or between pathways activated independently of the receptors, may also play a role in the CYP1A1 induction pattern reversal we have observed. Common pathways activated by coplanar PCBs and noncoplanar PCBs and dioxin-like compounds (such as TCDD) include ERK1/2 (extracellular signal regulated kinase) (Canesi et al., 2003; Machala et al., 2003; Tan et al., 2002; Zelko and Negishi, 2000), Protein kinase C and intracellular calcium alterations (Hanneman et al., 1996; Wong and Pessah, 1996; Wong et al., 1997; Tilson and Kodavanti, 1998; Kodavanti and Tilson, 2000). Furthermore, both AhR and CAR have been shown to interact with the coactivators SRC-1 and Sp1 (Wang et al., 1999; Muangmoonchai et al., 2001; Beischlag et al., 2002).

In conclusion, we have observed a novel biological interaction between a coplanar (PCB 126) and noncoplanar (PCB 153) polychorinated aromatic hydrocarbon. The molecular mechanisms underlying this complex interaction are currently unknown, but may be explained by ligand competition for the AhR, differential subcellular localization of the AhR within zonal areas of the hepatic acinus/lobule, differential ligand distribution patterns in the liver, or by interactions between receptors and or simultaneously activated pathways. This phenomenon will require further study to more fully understand the mechanisms which will explain the complex biological interactions we have seen with coplanar and noncoplanar PCBs in mixtures.

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REFERENCES

Andersen, M. E., Mills, J. J., Jirtle, R. L., and Greenlee, W. F. (1995). Negative selection in hepatic tumor promotion in relation to cancer risk assessment. *Toxicology* **102**, 223–37.

Bandiera, S. M. (2001). Cytochrome P450 enzymes as biomarkers of PCB exposure and modulators of toxicity. In: Recent Advances in the environmental toxicology and health effects of PCBs (L. W. Robertson and L. G. Hansen, eds). pp. 185–92. University of Kentucky Press, Lexington.

Bars, R. G., and Elcombe, C. R. (1991). Dose-dependent acinar induction of cytochromes P450 in rat liver. Evidence for a differential mechanism of induction of P450IA1 by beta-naphthoflavone and dioxin. *Biochem J* **277**, 577–80.

Bars, R. G., Mitchell, A. M., Wolf, C. R., and Elcombe, C. R. (1989). Induction of cytochrome P-450 in cultured rat hepatocytes. The heterogeneous localization of specific isoenzymes using immunocytochemistry. *Biochem J* **262**, 151–8.

Beischlag, T. V., Wang, S., Rose, D. W., Torchia, J., Reisz-Porszasz, S., Muhammad, K., Nelson, W. E., Probst, M. R., Rosenfeld, M. G., and Hankinson, O. (2002). Recruitment of the NCoA/SRC-1/p160 family of transcriptional coactivators by the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator complex. *Mol Cell Biol* **22**, 4319–33.

Canesi, L., Ciacci, C., Betti, M., Scarpato, A., Citterio, B., Pruzzo, C., and Gallo, G. (2003). Effects of PCB congeners on the immune function of Mytilus hemocytes: alterations of tyrosine kinase-mediated cell signaling. *Aquat Toxicol* **63**, 293–306.

Cogliano, V. J. (1998). Assessing the cancer risk from environmental PCBs. *Environ Health Perspect* **106**, 317–23.

Dean, C. E., Jr., Benjamin, S. A., Chubb, L. S., Tessari, J. D., and Keefe, T. J. (2002). Nonadditive hepatic tumor promoting effects by a mixture of two structurally different polychlorinated biphenyls in female rat livers. *Toxicol Sci* **66**, 54–61.

Estabrook, R. W. (1996). The remarkable P450s: a historical overview of these versatile hemeprotein catalysts. *FASEB J* **10**, 202–4.

Forkert, P. G. (1997). Coexpression of Ah receptor and CYP1A1 in hepatocytes of C57BL/6J and DBA/2J mice. *Toxicol Appl Pharmacol* **142**, 69–78.

Ganem, L. G., Trottier, E., Anderson, A., and Jefcoate, C. R. (1999). Phenobarbital induction of CYP2B1/2 in primary hepatocytes: endocrine regulation and evidence for a single pathway for multiple inducers. *Toxicol Appl Pharmacol* **155**, 32–42.

Hanneman, W. H., Legare, M. E., Barhoumi, R., Burghardt, R. C., Safe, S., Tiffany-Castiglioni, E. T. (1996). Stimulation of calcium uptake in cultured rat hippocampal neurons by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin *Toxicol* 112(1), 19–25.

Hansen, L. G. (1998). Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect* **106 Suppl 1**, 171–89.

Hestermann, E. V., Stegeman, J. J., and Hahn, M. E. (2000). Relative contributions of affinity and intrinsic efficacy to aryl hydrocarbon receptor ligand potency. *Toxicol Appl Pharmacol* **168**(2), 160–72.

Kawamoto, T., Sueyoshi, T., Zelko, I., Moore, R., Washburn, K., and Negishi, M. (1999). Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. *Mol Cell Biol* **19**, 6318–22.

Kimbrough, R. D. (1995). Polychlorinated biphenyls (PCBs) and human health: an update. *Crit Rev Toxicol* **25**, 133–63.

Kodavanti, P. R., and Tilson, H. A. (2000). Neurochemical effects of environmental chemicals: in vitro and in vivo correlations on second messenger pathways. *Ann NY Acad Sci* **919**, 97–105.

Ma, Q. (2001). Induction of CYP1A1. The AhR/DRE paradigm: transcription, receptor regulation, and expanding biological roles. *Curr Drug Metab* **2**, 149–64.

Machala, M., Blaha, L., Vondracek, J., Trosko, J. E., Scott, J., and Upham, B. L. (2003). Inhibition of gap junctional intercellular communication by noncoplanar polychlorinated biphenyls: inhibitory potencies and screening for potential mode(s) of action. *Toxicol Sci* **76**, 102–11.

McFarland, V. A., and Clarke, J. U. (1989). Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ Health Perspect* **81**, 225–39.

Muangmoonchai, R., Smirlis, D., Wong, S. C., Edwards, M., Phillips, I. R., and Shephard, E. A. (2001). Xenobiotic induction of cytochrome P450 2B1 (CYP2B1) is mediated by the orphan nuclear receptor constitutive androstane receptor (CAR) and requires steroid coactivator 1 (SRC-1) and the transcription factor Sp1. *Biochem J* **355**, 71–8.

Petrulis, J. R., and Bunce, N. J. (2000). Competitive behavior in the interactive toxicology of halogenated aromatic compounds. *J Biochem Mol Toxicol* **14**, 73–81.

Safe, S. (1989). Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. *Mutat Res* **220**, 31–47.

Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicol* **21**, 51–88.

Safe, S. H. (1994). Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* **24**, 87–149.

Sueyoshi, T., Kawamoto, T., Zelko, I., Honkakoski, P., and Negishi, M. (1999). The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *J Biol Chem* **274**, 6043–6.

Tan, Z., Chang, X., Puga, A., and Xia, Y. (2002). Activation of mitogen-activated protein kinases (MAPKs) by aromatic hydrocarbons: role in the regulation of aryl hydrocarbon receptor (AHR) function. *Biochem Pharmacol* **64**, 771.

Tilson, H. A., and Kodavanti, P. R. (1998). The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology* **19**, 517–25.

Tritscher, A. M., Goldstein, J. A., Portier, C. J., McCoy, Z., Clark, G. C., and Lucier, G. W. (1992). Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a rat tumor promotion model: quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. *Cancer Res* **52**, 3436–42.

van Birgelen, A. P., Ross, D. G., DeVito, M. J., and Birnbaum, L. S. (1996). Interactive effects between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,2′,4,4′,5,5′-hexachlorobiphenyl in female B6C3F1 mice: tissue distribution and tissue-specific enzyme induction. *Fundam Appl Toxicol* **34**, 118–31.

Wang, F., Wang, W., and Safe, S. (1999). Regulation of constitutive gene expression through interactions of Sp1 protein with the nuclear aryl hydrocarbon receptor complex. *Biochemistry* **38**, 11490–500.

Whitlock, J. P., Jr. (1999). Induction of cytochrome P4501A1. *Annu Rev Pharmacol Toxicol* **39**, 103–25.

Wong, P. W., Brackney, W. R., and Pessah, I. N. (1997). Ortho-substituted polychlorinated biphenyls alter microsomal calcium transport by direct interaction with ryanodine receptors of mammalian brain. *J Biol Chem* **272**, 15145–53.

Wong, P. W., and Pessah, I. N. (1996). Ortho-substituted polychlorinated biphenyls alter calcium regulation by a ryanodine receptor-mediated mechanism: structural specificity toward skeletal- and cardiac-type microsomal calcium release channels. *Mol Pharmacol* **49**, 740–51.

Zelko, I., and Negishi, M. (2000). Phenobarbital-elicited activation of nuclear receptor CAR in induction of cytochrome P450 genes. *Biochem Biophys Res Commun* **277**, 1–6.