IMMUNOTOXICITY OF HIGHER CHLORINATED DIPHENYL ETHERS

L. Howie, R. Dickerson and S. Safe*

Department of Veterinary Physiology and Pharmacology

College of Veterinary Medicine, Texas A&M University

College Station, Texas 77843

ABSTRACT

The effects of 2,2',3,3',4,4',5,5',6-nonachlorodiphenyl ether (Cl_9-DPE) and decachlorodiphenyl ether (Cl_{10}-DPE) on the splenic plaque-forming cell (PFC) response to sheep red blood cells (SRBCs) were determined in C57BL/6 mice. Both compounds caused a dose-response decrease in PFCs/spleen and PFCs/10^6 viable cells and significant immunotoxic effects were observed at doses as low as 2.5 μmol/kg. Both compounds also induced hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) activities however, higher doses were required to cause the induction compared to the immunotoxic responses.

INTRODUCTION

Polychlorinated diphenyl ethers (PCDEs) have been identified as contaminants of industrial chlorinated phenols (1). Recent studies in our laboratory (2) have shown that PCDEs cause immunotoxicity and induce AHH and EROD activity in C57BL/6 mice. The effects caused by the PCDEs were similar to those observed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related toxic halogenated aromatic hydrocarbons. However, the results of recent studies have shown that for the lower chlorinated PCDEs, their structure-activity relationships differed from those reported for the corresponding polychlorinated biphenyls (PCBs). In most cases, the PCDEs were more potent than the corresponding PCB congeners; moreover, it was apparent that for the PCDEs, ortho-chloro substituents are less effective in reducing potency compared to the effect of ortho-substituents on the activity of PCBs.

Table 1. Dose Response Activities of Cl_9-DPE and Cl_{10}-DPE in C57BL/6 Mice[a]

CONGENER	DOSE (μmol/ kg)	PFCs/ SPLEEN (x 10^5)	PFCs/10^6 VIABLE CELLS	AHH (pmol/ min/mg)	EROD (pmol/ min/mg)
Cl_9-DPE	0	1.21	1380	363	142
	2.5	0.47[a]	612[a]	228	144
	10	0.55[a]	699[a]	306	167
	25	0.30[a]	362[a]	237	197
	100	0.11[a]	131[a]	394	398[a]
	400	0.24[a]	274[a]	1510[a]	1740[a]
Cl_{10}-DPE	0	2.1	1530	227	119
	2.5	0.98[a]	761[a]	159	112
	10	0.57[a]	478[a]	345	283
	25	0.43[a]	329[a]	311	324[a]
	100	0.40[a]	364[a]	488[a]	599[a]
	400	0.43[a]	350[a]	862[a]	956[a]

[a]Significantly different ($p < 0.01$) than animals treated with corn oil (vehicle control, 0 dose).

Current studies on the relative toxic potencies of other higher chlorinated PCDEs and PCBs are in progress since these compounds are among the most persistent of all the halogenated aromatic environmental contaminants.

ACKNOWLEDGEMENTS

The financial assistance of the National Institutes of Health (P42-ESO4917) and the Texas Agricultural Experiment Station are gratefully acknowledged. S. Safe is a Burroughs Wellcome Toxicology Scholar.

REFERENCES

1. Rappe C., Buser H.R. and Bosshardt H-R (1979) *Ann. N.Y. Acad. Sci.* 321:1.
2. Howie L., Dickerson R., Davis D. and Safe S. (1990) *Toxicol. Appl. Pharmacol.*, in press.
3. Jerne K.K. and Nordin A.A. (1963) *Science* 140:405.
4. Cunningham A.J. and Szenberg A. (1968) *Immunology* 14:599.
5. Nebert D.W. and Gelboin H.V. (1968) *J. Biol. Chem.* 242:6242.
6. Pohl R.J. and Fouts J.R. (1980) *Anal. Biochem.* 107:150.

MATERIALS AND METHODS

Animals and Biochemicals:

Male C57BL/6 mice were purchased at 7-8 weeks of age from Harlan Sprague Dawley, Inc., Houston, Texas. Sheep erythrocytes in Alsevar's solution were obtained from M.A. Bioproducts, Maryland. Lyophilized guinea pig complement, RPMI 1640 media, Earle's balanced salt solution, and Alsevar's solution were purchased from Gibco Laboratories, New York.

Immunological Studies:

The method used was the "Cunningham" modification of the Jerne plaque-forming cell assay (3,4). Upon arrival, the animals were allowed to acclimate for 5-7 days before they were injected (i.p.) with the appropriate chemical dissolved in the vehicle (corn oil) or the vehicle alone. Five days after chemical injection, the animals were injected (i.p.) with 4 x 10^8 sheep red blood cells in 0.1 ml of Earle's balanced salt solution. Four days after antigen challenge, the animals were killed by cervical dislocation. The spleens were removed and immediately placed in 5 ml of cold RPMI 1640 media. A single cell suspension was made by mashing each spleen between two frosted microscope slides. Each spleen cell suspension was then spun at 220 x g for 10 minutes, and the resulting pellet was resuspended in 2 ml of fresh media. Appropriate dilutions of the suspension were then made and 0.1 ml of the dilution was mixed with 0.2 ml of 20% sheep red blood cell (SRBC) solution and 0.1 ml of 20% guinea pig complement solution in phosphate-buffered saline. Microliter aliquots of these mixtures were placed in a "Cunningham" slide chamber by capillary action and incubated for 1 hour at 37° C. Viable cell counts were determined by trypan blue staining. Statistical differences between treatment groups were determined by the Student's t test.

Enzyme-induction Studies:

Following the removal of the spleens from the above animals, the livers were perfused with cold saline-EDTA and hepatic microsomes were prepared as described (2). The hepatic microsomal enzyme fraction was obtained and the microsomal AHH and EROD activities were measured using flurometric methods (5,6). Statistical differences between treatment groups were determined by the Student's t test.

RESULTS AND DISCUSSION

Table 1 summarizes the immunotoxic and enzyme induction activity of Cl_9-DPE and Cl_{10}-DPE in C57BL/6 mice. The results show that both the Cl_9-DPE and Cl_{10}-DPE congeners were immunotoxic at doses as low as 2.5 μmol/kg and these activities were comparable to the effects observed for lower chlorinated PCDE and PCB congeners which contained only one ortho-chloro substituent. It was also apparent that Cl_9-DPE and Cl_{10}-DPE congeners induced hepatic microsomal AHH and EROD activities at higher dose levels.