Acute Intraperitoneal Toxicity of DDT and PCB's in Mice Using Two Solvents

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Polychlorinated biphenyl compounds (PCB's) are abundant contaminants in the global ecosystem (1), and have recently been found in human food (2) and tissue (3). Most reports of PCB's in wildlife indicate that these compounds are found in conjunction with DDT and its metabolites. While some toxicological information already exists with regard to DDT (4) little has been published with regard to the effects of PCB's on vertebrates. The limited early studies of PCB's dealt mainly with mammalian pathology (5,6) while later workers investigated avian pathology (7,8).

Most recent PCB research has emphasized possible subtle sublethal effects (9,10,11,12,13,14,15) but toxicity studies have been limited in number (16,17,18,19). Not only are PCB toxicity data incomplete but also toxicity data for DDT show many inconsistencies (4,20). This comparative study was undertaken to determine the acute toxicity of PCB's (Aroclor 1254)[1] and p,p'-DDT to mice. Aroclor 1254 was used as a representative mixture of PCB isomers as it lies centrally in the PCB series with respect to substituted chlorine and seems similar to PCB's found in some wildlife studies (24,25). Four strains of laboratory mice as well as a group of wild type mice were used to isolate any differences in toxicity data which could be related to mouse strains.

The solvent used in this comparison was dimethyl sulfoxide (DMSO) but since it has been suggested that DMSO may alter the physiological effects of compounds dissolved in it (21,22,23) 1 strain of mice was selected to evaluate acute toxicities of p,p'-DDT and PCB's using corn oil as the vehicle.

METHODS

Four strains of laboratory mice (ALAS; BALB/cAnNs (BALB); CBA/T_6T_6/Wn (CBA); C57BL/Wn (C57) and a group of wild type mice (*Peromyscus maniculatus*) were used. A group of wild type *Mus musculus* was used in one DDT toxicity test only. The ALAS strain of mice was developed by Mr. J. Thomsen of Bioscience Animal Services of the University of Alberta and maintained in a closed random bred colony as were the other 3 strains of laboratory mice.

[1]Manufactured by the Monsanto Company, St. Louis, Missouri.

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The *Peromyscus maniculatus* were supplied from a closed random bred colony maintained by Bioscience Animal Services and the wild type *Mus musculus* were live trapped at the University of Alberta farm.

p,p'-DDT and Aroclor 1254 dissolved in corn oil were administered to a total of 302 ALAS mice by i.p. injections. Injection volumes for DDT in corn oil were in the range of 0.08-0.56 ml for a 25 mg/ml solution and 0.02-0.40 ml for a 100 mg/ml solution. For PCB's injection volumes were 0.12-1.45 ml for a 100 mg/ml solution and 0.18-0.54 ml for a 250 mg/ml solution. These volumes were calculated to attain desired dose levels in milligrams per kilogram (mg/kg) of body weight. As larger doses of pure solvent had no effect on control animals, solvent volumes were not adjusted to be identical at each dose level. Ten mice injected with 0.2 ml, 5 mice with 0.6 ml, and 5 mice with 1.0 ml of corn oil served as controls. Litters were mixed randomly and held in wire topped polypropylene cages (28.5 x 17.5 x 12 cm) and 5 mice were used at each dose level except for the 100 mg/ml DDT experiment where 10 mice were used. All mice were in the 20-25 g weight range with a 2:3 sex ratio alternating at each dose level. Five day LD50 values were calculated for the 100 mg/ml p,p'-DDT and 250 mg/ml Aroclor 1254 experiments by the method of Litchfield and Wilcoxon (26) and verified by probit analysis (27).

Five strains (332 mice) were given i.p. injections of either p,p'-DDT or Aroclor 1254 dissolved in DMSO at a concentration of 250 mg/ml. Injected volumes were in the range of 0.03 ml to 0.18 ml again to give desired dose levels in mg/kg of body weight. A minimum of 5 mice of each strain, selected randomly with respect to sex, was used at each dose level for each chemical. Five control mice were injected with 0.125 ml of DMSO only. Following injections behavioral observations were made every 4-8 hours and after 5 days each experiment was terminated and the LD50 value calculated as described previously. LD50 values of different strains for the same chemical and each strain for the 2 chemicals were compared statistically ($P < .05$) by the method of Litchfield and Wilcoxon (26).

RESULTS

The results of the experiments using corn oil as the solvent are in Table 1. For ALAS mice the LD50 for 100 mg/ml of p,p'-DDT in corn oil (near the upper limit of solubility) is 280 mg/kg, but for a 250 mg/ml solution of PCB's in corn oil an LD50 of 2840 mg/kg was observed. Thus DDT is significantly more toxic than PCB's, when administered in corn oil.

Dilution of the corn oil solution did affect the LD50 data. Whereas the LD50 for PCB's at 250 mg/ml in corn oil is 2840 mg/kg the 100 mg/ml solution had yielded no higher than 60% mortality with injections up to 5800 mg/kg at which point the injection volumes became unreasonably large. Similarly a 25 mg/ml solution

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of p,p'-DDT in corn oil had yielded no mortality with injections as high as 700 mg/kg.

TABLE 1

Acute i.p. LD50 Data for DDT and PCB's in Corn Oil

Compound	Solution concentration	Mouse strain	5 day LD50 (mg/kg)[1]	95% confidence range (mg/kg)[1]
p,p'-DDT	100 mg/ml	ALAS	280	220- 360
	25 mg/ml	ALAS	> 700	-
Aroclor 1254	100 mg/ml	ALAS	>5800	-
	250 mg/ml	ALAS	2840	2560-3150

[1]All values are rounded to the nearest 10 mg/kg. LD50 and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (26) and verified by probit analysis (27).

From Table 2 it can be seen that the response to p,p'-DDT in DMSO did not vary with the strain of mouse except in the case of the CBA strain where the LD50 was significantly less ($P < .05$) than that of the *Peromyscus* mice. The LD50 values from these experiments are similar to those found in a study using methoxytriglycol as the solvent where the strain of mouse was not identified (28). For PCB's it can be seen that the CBA strain LD50 was significantly less ($P < .05$) than that of the ALAS and BALB strains but that the other LD50 values did not differ statistically ($P < .05$).

The LD50 values for DDT and PCB's when compared within each strain did not differ significantly ($P < .05$) except in the case of the BALB strain where DDT was more toxic than PCB's.

Mice which died after treatment with p,p'-DDT exhibited the expected hyperexcitability, tremors and convulsions before death. Mice which died after treatment with Aroclor 1254 did not exhibit any of the above symptoms but were inactive prior to death. Control mice injected only with DMSO were inactive for 4-6 hours but then resumed normal behavior. Control mice injected only with corn oil resumed normal behavior immediately. There was no mortality in the control groups.

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TABLE 2

Acute i.p. LD50 Data for DDT and PCB's in DMSO

Compound	Solution concentration	Mouse strain	5 day LD50 (mg/kg)[1]	95% confidence range (mg/kg)[1]
		ALAS	960	780-1180
		BALB	800	660- 980
p,p'-DDT	250 mg/ml	CBA	730	550- 960
		C57	980	740-1290
		Wild *Mus*	850	660-1100
		Peromyscus	1200*	840-1720
		ALAS	1200*	1010-1430
		BALB	1080*	970-1200
Aroclor 1254	250 mg/ml	CBA	880	790- 990
		C57	1000	830-1210
		Peromyscus	970	800-1180

[1]All values are rounded to the nearest 10 mg/kg. LD50 and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (26) and verified by probit analysis (27).

*LD50 value is significantly ($P < .05$) higher than that of the CBA strain which had the lowest LD50 value for both DDT and PCB's.

DISCUSSION

From the results it is apparent that in the case of DMSO as a vehicle for i.p. injections of DDT and Aroclor 1254, these chlorinated hydrocarbons have similar LD50 values in mice. Only 1 strain (BALB) showed significant differences in LD50 values for the two compounds.

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The similarity in LD50's for all strains treated with each compound (only the extremes showed statistically significant difference) indicates that mouse type has little effect on DDT or PCB toxicity when DMSO is used as a solvent.

For corn oil as a vehicle the LD50 values for Aroclor 1254 and DDT differed markedly from each other as well as from the values obtained with DMSO. These differences are probably the result of factors such as differing rates of absorption of the vehicle and/or solute from the coelom. Additionally, these results are complicated by the finding that the toxicities of DDT and PCB's in corn oil seem directly proportional to solute concentration.

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