Effects on Toxicity and on Enzyme Activity of the Interactions Between Aldrin, Chlordane, Piperonyl Butoxide, and Banol in Rats (34984)

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(Introduced by B. J. Vos)
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We found previously that when rats were pretreated with either aldrin[1] or chlordane[2] the acute toxicity of the carbamates Banol[3] or Mobam[4] was significantly decreased (1). When serum and liver aliesterase (AliE) activities were measured after such pretreatment, the evidence that AliE had a primary role in the protective action of the chlorinated hydrocarbons was not convincing. Aldrin and chlordane are known inducers, not only of AliE but also of many "processing" or drug-metabolizing enzymes in liver microsomes; therefore, investigations into the role of these enzymes in the protective action against carbamate toxicity might help in elucidating the mechanisms involved. One approach is through a study of the effect on the toxicity of carbamate in animals pretreated with both an organochlorine pesticide and an agent which blocks the processing enzymes. Leeling and Casida (2) reported that piperonyl butoxide[5] blocked the *in vitro* activity of rat liver microsomal enzymes which metabolize carbaryl. Nakatsugawa *et al.* (3) found that piperonyl butoxide inhibited the *in vitro* activity of rabbit liver aldrin epoxidase. Falk and Kotin (4) found that the liver benzpyrene hydrolase was inhibited 6 hr after piperonyl butoxide was administered to rats, but the activity was normal 24 hr after administration.

The present study describes the effects of the concurrent pretreatment of rats with piperonyl butoxide and either aldrin or chlordane upon the toxicity of single oral doses of Banol, and upon the activities of serum AliE and liver AliE, aniline hydroxylase, and nitroreductase.

Materials and Methods. Four groups of female Osborne–Mendel rats (150–200 g), bred in our laboratory, were used. They were housed individually and fed Purina chow and water *ad libitum*. Group A was injected ip with either aldrin (50 mg/kg) or chlordane (300 mg/kg) on day 1; Group B was injected with aldrin or chlordane on day 1 and subsequently treated orally with piperonyl butoxide (500 mg/kg) on days 2, 3, 6, and 7; Group C was treated orally on days 2, 3, 6, and 7 with piperonyl butoxide (500 mg/kg) alone; and Group D was given corn oil, the vehicle used for the pesticide administrations. Four hours after administration of either piperonyl butoxide or corn oil on day 7, Banol was given per os to all four groups of rats (63.2 mg/kg, 2 × LD_{50}) and the mortality was recorded for a 24-hr period. The volume of liquid administered was always 0.002 ml/g body weight. This experiment was replicated once using five rats for Group D and ten rats for the other groups each time.

[1] Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene) is manufactured by Shell Chemical Company, New York, New York.

[2] Chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3α,4,7,7α-hexahydro-4,7-methanoindene) was obtained from Velsicol Chemical Corporation, Chicago, Illinois.

[3] Banol is a trademark of the Upjohn Company, Kalamazoo, Michigan 49001, for 6-chloro-3,4-xylyl methylcarbamate.

[4] Mobam (4-benzothienyl methylcarbamate) is an experimental compound manufactured by Mobil Chemical Company, Metuchen, New Jersey.

[5] Piperonyl butoxide (3,4-methylenedioxy-6-propylbenzyl-*n*-butyl diethyleneglycol ether) was purchased from K & K Laboratories, Inc., Plainview, New York.

For the enzyme studies, another four groups were pretreated as above and were killed 4 hr after administration of piperonyl butoxide or corn oil on day 7. An additional four groups were intubated with Banol (63.2 mg/kg) 4 hr after piperonyl butoxide administration and were decapitated 10 min later. Tremors, when present after carbamate administration, appeared within this time. Blood was collected in centrifuge tubes, allowed to clot, and centrifuged at 5°, and the serum was removed for AliE measurements. The livers were quickly excised and stored at −20° until used. The number of animals in both the mortality and enzyme experiments are recorded in the tables. The experiment was replicated once for each organochlorine pesticide, using five rats for Groups C and D and seven rats for Groups A and B each time. The doses used were determined by a series of range-finding tests to ascertain the time intervals and dose level yielding the desired toxicity pattern.

Serum AliE was determined by the Warburg manometric method with triacetin as substrate; AliE in liver homogenates was measured by the automatic titrator method of Jensen-Holm *et al.* (5), with triacetin as substrate. The reaction mixtures used have been described previously (6).

Nitroreductase activity in whole liver homogenates was measured according to a modification of the method of Fouts and Brodie (7). The incubation mixture, contained in screw-capped tubes, consisted of 0.5 ml of a 20% liver homogenate prepared in isotonic KCl containing 0.25% nicotinamide, 1.0 ml of glucose-6-phosphate (G-6-P, 3.3 μmoles), 1.0 ml of 0.1 *M* KH_2PO_4 buffer (pH 7.4), 1.0 ml of *p*-nitrobenzoic acid (3.5 μmoles) as substrate, and 0.1 ml of nicotinamide adenine dinucleotide phosphate (NADP, 0.24 μmoles). Nitrogen was bubbled through the mixture for 1 min; the tubes were then capped and incubated in a 37.5° water bath for 15 min. One milliliter of 30% trichloracetic acid was added, and the precipitated protein was centrifuged. The *p*-aminobenzoic acid formed was measured in the supernatant fluid colorimetrically according to the procedure of Bratton and Marshall (8), and read in a Bausch and Lomb Spectronic 20 at 630 mμ.

Aniline hydroxylase was determined by an adaptation of the method described by Imai

TABLE I. Acute Oral Toxicity of Banol After Pretreatment with Aldrin, Chlordane, and Piperonyl Butoxide.[a,b]

Pretreatment and route	Dose (mg/kg)	Days of dosing	Banol treatment[c]	Percent mortality[d]
Corn oil, ip, po	0	1, 2, 3, 6, 7	4 hr	85
Aldrin, ip	60	1	8 days	25
Aldrin, ip	60	1		
+			4 hr	82
Piperonyl butoxide, po	500	1, 2, 3, 6, 7		
Chlordane, ip	300	1	8 days	32
Chlordane, ip	300	1		
+			4 hr	67
Piperonyl butoxide, po	500	1, 2, 3, 6, 7		
Piperonyl butoxide, po	500	1, 2, 3, 6, 7	4 hr	85

[a] LD_{50} values determined were: aldrin, 150 mg/kg; chlordane, 560 mg/kg (8 days); Banol, 31 mg/kg. Oral LD_{50} of piperonyl butoxide in rats as reported by Sarles *et al.* (10) is 7.5–10.0 ml/kg.

[b] Banol administered at 63.2 mg/kg.

[c] Banol administered on day 7 to all rats; to those dosed on day 7, administration was 4 hr later.

[d] Twenty-four-hour observation, 10–20 rats for each treatment.

et al. (9). A 30% liver homogenate was prepared in isotonic KCl containing 0.25% nicotinamide and centrifuged at 3000 rpm for 10 min in a PR-2 centrifuge, and the supernatant fluid was used as the source of the enzyme. The incubation mixture contained 0.5 ml of the supernatant fluid, 0.5 ml of G-6P (1.1 μmoles), and 0.5 ml of aniline (25 μmoles) as substrate, and was incubated for 1 hr in 15-ml beakers in air at 37.5° in a Dubnoff shaking incubator. One milliliter of 20% trichloracetic acid was then added and the precipitate was centrifuged. The amount of *p*-aminophenol in the supernatant fraction was measured by adding 1 ml of 10% Na_2CO_3 and 2 ml of a phenol solution (2 g/100 ml of 0.2 *N* NaOH), allowing the color to develop for 30 min, and reading at 630 mμ with a Bausch and Lomb Spectronic 20.

Results and Discussion. Mortality studies (Table I) show that repeated administration of 1/15 the LD_{50} of piperonyl butoxide to rats pretreated with 2/5 the LD_{50} of aldrin or with approximately 1/2 the LD_{50} of chlordane blocked the protective action which the same dose of aldrin or chlordane alone had against the toxic effects of 63.2 mg/kg of Banol ($2 \times LD_{50}$). Aldrin pretreatment decreased the Banol-induced mortality from 85 to 25%, while chlordane pretreatment decreased it from 85 to 32%. When rats were pretreated with the combination of aldrin and piperonyl butoxide the mortality increased to 82%; when pretreated with the combination of chlordane and piperonyl butoxide the mortality increased from 32 to 67%. Mortality in rats pretreated with piperonyl butoxide alone and then given Banol was equal to that of the controls given corn oil followed by Banol.

Signs of cholinergic intoxication after Banol were shown by all the rats in Groups B, C, and D, but only by an occasional rat in Group A.

Enzyme activities after pesticide administration are shown in Table II. Comparisons are given of the activities measured in a group of rats pretreated with aldrin or chlordane and piperonyl butoxide as well as those in a

TABLE II. Rat Serum and Liver Enzyme Activities[a] After Treatment with Chlordane, Aldrin, Piperonyl Butoxide, and Banol.

	Serum		Liver					
	AliE (μmoles acid/ml/min)		AliE (μmoles acid/g/min)		Aniline hydroxylase (μg *p*-aminophenol/100 g)		Nitroreductase (μg *p*-aminobenzoic acid/100 mg)	
Pretreatment[b]	None	Banol	None	Banol	None	Banol	None	Banol
Corn oil	3.5 ± 0.2	3.1 ± 0.2	86.2 ± 2.5	40.6 ± 1.7	8.6 ± 0.6	7.5 ± 2.8	6.5 ± 0.8	5.2 ± 0.4
Chlordane	6.0 ± 0.3	7.4 ± 0.6	91.2 ± 3.2[c]	51.1 ± 4.0	18.5 ± 0.8	15.6 ± 0.7	16.1 ± 2.2	17.5 ± 2.7
Chlordane + piperonyl butoxide	5.6 ± 0.2	6.7 ± 0.4	102.1 ± 3.9	54.4 ± 6.2	15.9 ± 0.8	15.1 ± 0.8	17.5 ± 1.6	14.4 ± 2.3
Piperonyl butoxide	4.5 ± 0.2	4.9 ± 0.2	100.0 ± 5.1	47.2 ± 3.9	11.9 ± 0.7	10.4 ± 0.6	9.7 ± 1.2	7.0 ± 1.3
Corn oil	3.3 ± 0.3	3.3 ± 0.4	90.6 ± 2.7	36.2 ± 3.4	9.5 ± 1.1	7.3 ± 0.4	9.1 ± 0.5	11.5 ± 1.2
Aldrin	5.7 ± 0.5	6.3 ± 0.3	109.9 ± 4.5	54.5 ± 5.3	15.5 ± 0.9	15.9 ± 0.8	14.3 ± 1.6	21.8 ± 2.9
Aldrin + piperonyl butoxide	5.2 ± 0.4	5.6 ± 1.1	119.8 ± 6.1	59.4 ± 6.7	18.2 ± 0.7	15.5 ± 1.2	20.4 ± 1.7	21.4 ± 2.2
Piperonyl butoxide	4.3 ± 0.7[c]	4.1 ± 0.6[c]	98.5 ± 5.1[c]	58.9 ± 5.7	13.5 ± 0.7	10.3 ± 1.0	15.0 ± 2.0	13.8 ± 0.6

[a] Each value represents the mean ± SE of 10–14 female rats.
[b] Doses, frequency, and route of administrations as listed in Table I.
[c] Increase not significantly different from control value. Others significant at 5% level or below by Student *t* test.

group of similarly pretreated animals which had also been given a toxic dose of Banol. Serum AliE activity was increased about 65% above control activity after treatment with either aldrin, chlordane, or organochlorine plus piperonyl butoxide. After piperonyl butoxide alone, the serum AliE was increased, but the increase was less than when organochlorines were given. The administration of Banol had no inhibitory effect upon the serum AliE in animals which had been given similar pretreatments. Liver AliE activity was significantly increased after the administration of aldrin alone and aldrin plus piperonyl butoxide, but not after piperonyl butoxide alone. The liver AliE was significantly increased above values for control rats after chlordane plus piperonyl butoxide and after piperonyl butoxide alone, but not after chlordane alone. Administration of Banol to rats given these various pretreatments resulted in a decreased activity in all cases, and the amount of the inhibition was approximately 50% in each instance.

Aldrin and chlordane both caused an induction of aniline hydroxylase and nitroreductase activity; the additional pretreatment with piperonyl butoxide had no effect upon the induction. Livers from rats treated only with piperonyl butoxide had significantly elevated aniline hydroxylase and nitroreductase activities, but the increases were not so great as those in the organochlorine-treated rats. Administration of Banol to the pretreated rats had no significant effect upon the activity of these two liver-processing enzymes.

Organochlorine pesticides act on liver drug-metabolizing enzymes in the same way as do drugs such as phenobarbital, and it has been postulated that the enzymes stimulated are those which detoxify carbamate and organophosphate pesticides (11). The well-known synergistic action of piperonyl butoxide is due to its ability to inhibit these drug or pesticide detoxifying enzymes and thereby increase the toxicity of these agents. In the present study, piperonyl butoxide reversed the protective effect of aldrin or chlordane on Banol toxicity in rats, an action which could be expected from the known inhibitory effect of piperonyl butoxide on drug-metabolizing enzymes. One would also have expected to note an effect of piperonyl butoxide upon one of the three liver enzyme systems tested (an esterase, a nitroreductase, and a ring hydroxylase). Many pesticides form glucuronides *in vivo* and are excreted as such in this reaction. Our next efforts will be directed toward the action of piperonyl butoxide on the liver enzyme involved in glucuronide formation (uridine diphosphate glucuronyl transferase) in an effort to elucidate the mechanism of interaction between the organochlorine and carbamate pesticides.

Summary. Rats were administered aldrin, chlordane, piperonyl butoxide, aldrin plus piperonyl butoxide, or chlordane plus piperonyl butoxide, and the toxicity and effects on some serum and liver enzyme activities after subsequent Banol administration was investigated. Aldrin and chlordane pretreatment decreased the mortality after a single oral dose of Banol ($2 \times LD_{50}$) from 85 to 35%. Pretreatment with the organochlorines and piperonyl butoxide together abolished the protective action of the organochlorines alone. Piperonyl butoxide pretreatment alone had no effect on the toxicity of Banol.

Serum aliesterase (AliE) and liver aniline hydroxylase and nitroreductase activities were markedly increased after organochlorine pretreatment and also after organochlorine–piperonyl butoxide pretreatment, while liver AliE was less markedly increased and increases in enzyme activity after piperonyl butoxide pretreatment were minimal. Administration of Banol to rats similarly pretreated decreased liver AliE, but not serum AliE or liver aniline hydroxylase or nitroreductase.

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