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Acute Behavioral Toxicity of Carbaryl and Propoxur in Adults Rats[1,2]

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RUPPERT, P. H., L. L. COOK, K. F. DEAN AND L. W. REITER. *Acute behavioral toxicity of carbaryl and propoxur in adult rats*. PHARMACOL BIOCHEM BEHAV **18**(4) 579–584, 1983.—Motor activity and neuromotor function were examined in adult CD rats exposed to either carbaryl or propoxur, and behavioral effects were compared with the time course of cholinesterase inhibition. Rats received an IP injection of either 0, 2, 4, 6 or 8 mg/kg propoxur or 0, 4, 8, 16 or 28 mg/kg carbaryl in corn oil 20 min before testing. All doses of propoxur reduced 2 hr activity in a figure-eight maze, and crossovers and rears in an open field. For carbaryl, dosages of 8, 16 and 28 mg/kg decreased maze activity whereas 16 and 28 mg/kg reduced open field activity. In order to determine the time course of effects, rats received a single IP injection of either corn oil, 2 mg/kg propoxur or 16 mg/kg carbaryl, and were tested for 5 min in a figure-eight maze either 15, 30, 60, 120 or 240 min post-injection. Immediately after testing, animals were sacrificed and total cholinesterase was measured. Maximum effects of propoxur and carbaryl on blood and brain cholinesterase and motor activity were seen within 15 min. Maze activity had returned to control levels within 30 and 60 min whereas cholinesterase levels remained depressed for 120 and 240 min for propoxur and carbaryl, respectively. These results indicate that both carbamates decrease motor activity, but behavioral recovery occurs prior to that of cholinesterase following acute exposure.

Carbamates Carbaryl Propoxur Motor activity Cholinesterase inhibition Behavioral toxicology

CARBARYL (1-napthyl N-methylcarbamate) and propoxur (0-isopropoxyphenyl N-methylcarbamate) are two structurally related carbamate insecticides. Carbaryl is mainly applied to food crops while propoxur is primarily used as a household insecticide and for protection of domestic animals [11]. The LD_{50} for IP administration in rats is reported to be 64–200 mg/kg for carbaryl and 30 mg/kg for propoxur [3,20]. Both carbamates are used widely because of their low mammalian toxicity compared to other classes of insecticide. The proposed mechanism of acute carbamate toxicity is a rapid and brief inhibition of acetylcholinesterase (AChE) activity. For carbaryl, maximal inhibition of total brain cholinesterase (ChE) activity was seen 30 min after IP injection of rats; the ED_{50} was 10 mg/kg [7]. Brain ChE activity following 5 mg/kg carbaryl returned to control levels 2 hr after injection [6]. For propoxur, 2 mg/kg reduced blood and brain ChE to 49% and 47% of normal values, respectively [20].

Behavioral effects, including disruption of schedule-controlled responding, avoidance conditioning, and motor activity, have been reported in rats following acute exposure to carbaryl. Response rate on a variable interval schedule of reinforcement was decreased by 3 mg/kg carbaryl [2], while 7 mg/kg carbaryl decreased the number of food reinforcements obtained on a variable ratio schedule [6]. Following 10 mg/kg carbaryl, rats did not suppress bar pressing for water which was paired with footshock [18], while 5 mg/kg increased the number of shocks taken in a signalled avoidance task [6,7]. The effects of carbaryl on motor activity are less clear. For example, Singh [19] reported decreases in running wheel activity of rats with 0.56 mg/kg carbaryl, while Albright and Simmel [1] found that 10 mg/kg carbaryl increased activity in a selective activity meter but decreased activity in a novel environment.

In the present experiment, rats were tested for motor activity in both an open field and a figure-eight maze to clarify the effects of carbaryl on activity, and to compare these effects with those of a structurally similar carbamate, propoxur. Animals were also tested for neuromotor function using the landing foot-spread technique of Edwards and Parker [4]. In a second experiment, the time course for alterations in motor activity and in total ChE levels in blood and brain was determined in order to correlate the behavioral effects of these carbamates with one of their prominent biochemical effects.

METHOD

Subjects and Apparatus

Adult CD male rats (Charles River), 85–120 days of age,

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were housed in groups of 3 in cages measuring 45×24×20 cm. Purina Lab Chow and water were available ad lib throughout the experiment. The animal room was controlled for temperature (22°C±2) and humidity (50%±10), and was maintained on a 12 hr light cycle (0600–1800 hr). All data were collected during the light portion of the cycle.

Locomotor activity was measured in a figure-eight maze consisting of 10×10 cm alleys converging on a central arena [15]. Activity was automatically detected by 8 phototransistor/photodiode pairs, which registered a count each time an infrared light beam was interrupted. Activity was also measured in an open field (90×90×60 cm) divided into sixteen 22.5 cm squares. Both crossovers (counted each time an animal entered a square with all four feet) and rears (counted each time an animal raised both front paws) were scored. Each animal was initially placed in the center of the field, and activity was monitored on closed-circuit television by observers in an adjacent room. Both the mazes and open field were located in sound-attenuated rooms and testing was conducted under normal fluorescent lighting.

In order to determine foot-spread [4], each hindpaw was dipped in ink, the animal was suspended in a horizontal position 30 cm above a table, and was dropped twice. The distance between the midpoint of the prints produced by the hindpaws was measured to the nearest mm.

Acute Behavioral Toxicity Experiment

Rats (N=10/group) received a single IP injection of either 0, 2, 4, 6 or 8 mg/kg propoxur, (Baygon, Chemagro, Kansas City, MO, 97% pure) or 0, 4, 8, 16 or 28 mg/kg carbaryl, (Sevin, Union Carbide, South Charleston, WV, 99% pure) in corn oil 20 min before testing. The injection volume was 0.5 ml/kg except for 28 mg/kg carbaryl (1.0 ml/kg). An additional control group of non-injected animals was tested for each carbamate (N=10/group). Each animal was tested for 2 hr in a figure-eight maze, and activity counts were tabulated both for the first 5 min of the test and the entire 2 hr. Immediately afterward landing foot-spread was determined. One week later, all animals again received a single injection of the same treatment as before, and were tested for 5 min in an open field, followed again by measurement of landing foot-spread.

Time Course Experiment

Rats (N=9/group/time period) received a single IP injection of either corn oil (0.5 ml/kg), 2 mg/kg propoxur or 16 mg/kg carbaryl, and were tested for 5 min in a figure-eight maze either 15, 30, 60, 120 or 240 min after injection. Immediately after testing, animals were decapitated, brains were removed and trunk blood was collected in heparinized vials. Total levels of cholinesterase in both brain and blood were analyzed from frozen samples using the colorimetric method of Ellman *et al.* [5]. This includes both true and pseudo cholinesterase and is referred to here as ChE.

Data Analysis

For the acute behavioral toxicity experiment, the effect of treatment for carbaryl and baygon was analyzed using multivariate [13] statistical analysis (MANOVA) available through the Statistical Analysis System (SAS), 1979. The dependent variables were: the first 5 min and 2 hr figure-eight maze activity, crossovers and rears in the open field, landing foot-spread and body weights (for both days of testing). Separate ANOVAs were performed for individual variables when MANOVA indicated significant treatment effects.

For the time-course experiment, the effects of treatment, time, and the interaction of treatment by time were analyzed by MANOVA for the dependent variables: maze activity, and blood and brain ChE. Individual dependent variables were then analyzed using ANOVA. When significant treatment by time interactions were found, separate analyses by time were performed for the main effect of treatment.

For both experiments, when significant effects were found, post-hoc comparisons of means were made using Tukey's (a) test [22].

TABLE 1

STATISTICAL TABLE FOR ACUTE EXPERIMENT

A. Overall MANOVA

Propoxur	$F(40,207)=4.45$, $p<0.0001$
Carbaryl	$F(40,207)=4.84$, $p<0.0001$

B. ANOVA Values for Individual Variables

Dependent Variables	Propoxur F(5,54)	Propoxur $p<$	Carbaryl F(5,54)	Carbaryl $p<$
Maze Activity				
5 min	51.61	0.0001	23.98	0.0001
2 hr	36.98	0.0001	21.99	0.0001
Open Field				
Crossovers	31.96	0.0001	25.44	0.0001
Rears	36.85	0.0001	19.67	0.0001
Landing Foot-Spread				
Week 1	1.17	0.3338	1.30	0.2787
Week 2	1.58	0.1791	1.46	0.2190
Body Weight				
Week 1	0.73	0.6046	1.71	0.1460
Week 2	0.39	0.8535	2.75	0.0275

RESULTS

For the acute toxicity experiment, MANOVA indicated a significant overall treatment effect for both carbaryl and propoxur (Table 1A). Activity in the figure-eight maze was significantly decreased during the first 5 min and the entire 2 hr test period for both carbaryl and propoxur (Table 1B). For propoxur, all dose groups were less active than either control group for both the first 5 min in the maze and the entire 2 hr test (Fig. 1). For carbaryl, only the 28 mg/kg group was less active than the control groups for the first 5 min, but all groups receiving 8 mg/kg or greater were less active than controls over the entire 2 hr test (Fig. 1).

Both crossovers and rears in the open field were significantly decreased by both carbaryl and propoxur (Table 1B). For carbaryl, the 16 and 28 mg/kg groups differed from both control groups. For propoxur, all dose groups differed from control groups (Fig. 2).

There were no differences in landing foot-spread for

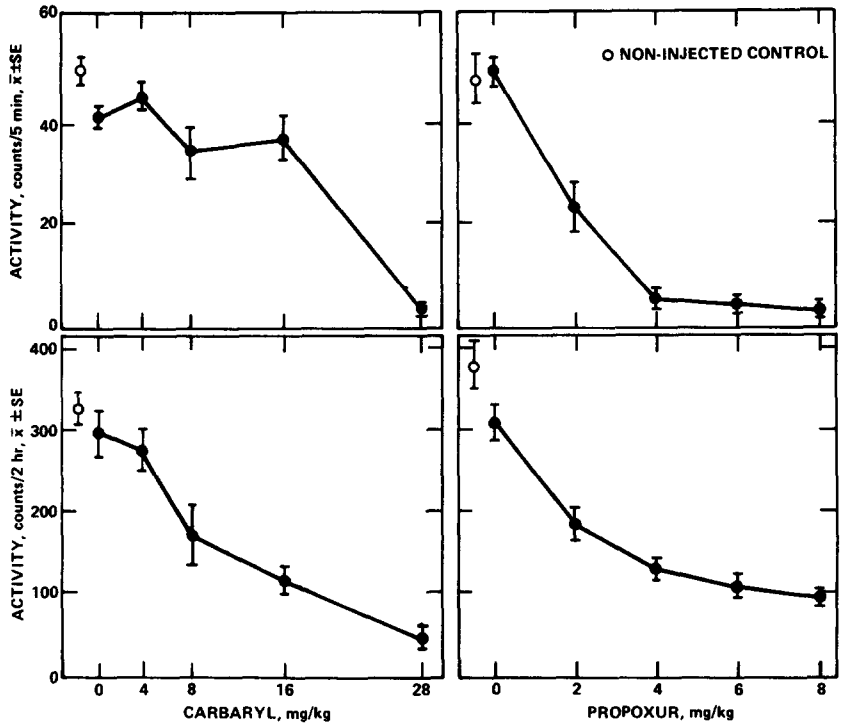

FIG. 1. Figure-eight maze activity of adult male rats following acute exposure to carbaryl and propoxur. Testing was performed 20 min after a single IP injection. Values are expressed at mean±SE for 10 animals/group.

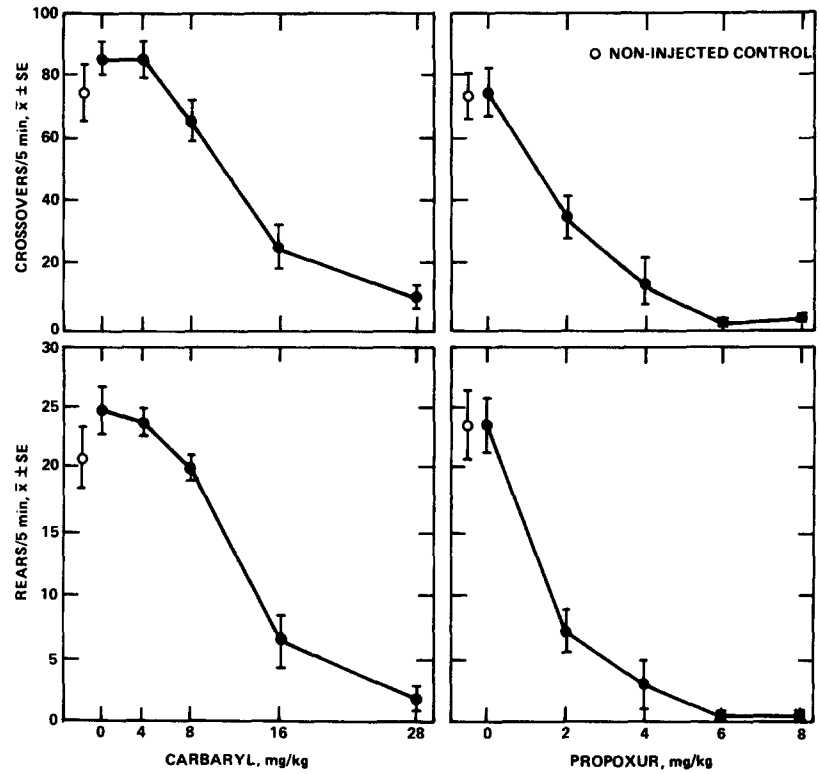

FIG. 2. Crossovers and rears in an open field for adult male rats following acute exposure to carbaryl and propoxur. Testing was performed 20 min after a single IP injection. Values are expressed at mean±SE for 10 animals/group.

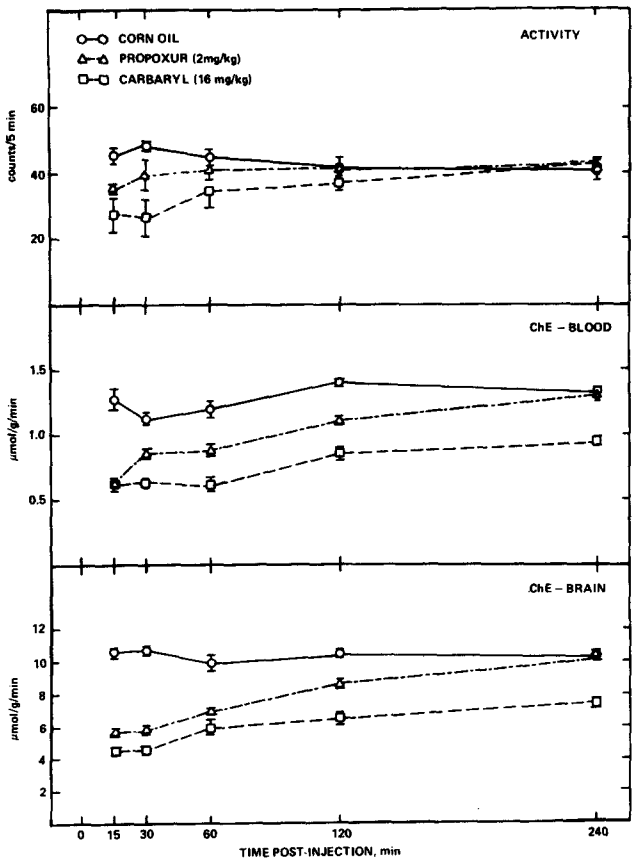

FIG. 3. Figure-eight maze activity and blood and brain ChE levels in adult male rats at varying times following acute exposure to carbaryl and propoxur. Values are mean±SE for 9 animals/group/time period.

either day of testing. For carbaryl only, there was a significant effect on body weight on the second injection day (Table 1B). There were no significant differences in body weight between any of the dosed animals and the controls, although the 28 mg/kg animals lost weight (13.7±3.0 g) while all other animals gained in the week between treatments.

Data for the time course of effects on maze activity and ChE levels in blood and brain are shown in Fig. 3. ANOVA indicated significant effects of treatment and a treatment-by-time interaction for activity and blood and brain ChE (Table 2B). For maze activity, there were significant differences at 15 min (Table 2C) and 30 min after injection. At 15 min, both carbaryl- and propoxur-treated groups were less active than controls while only the carbaryl group was significantly different from controls at 30 min. Activity at 60, 120 or 240 min after injection was not significantly different from controls.

Blood and brain ChE levels were significantly different from controls at all times sampled (Table 2C). Blood and brain ChE levels for carbaryl-treated rats were significantly lower than controls at all treatment times. Blood and brain ChE levels of propoxur-treated rats were significantly lower than controls at 15–20 min after injection but at 240 min ChE in both blood and brain had returned to control levels.

DISCUSSION

Both carbaryl and propoxur decreased motor activity in a dose-related fashion following acute exposure. This indicates that these structurally similar carbamate insecticides produce similar effects on locomotor activity. Behavioral changes were seen at the lowest dose of propoxur, 2 mg/kg, and at 8 or 16 mg/kg carbaryl, depending on the particular measure of activity. These data are in agreement with several reports which have documented other behavioral effects following acute exposures of 3–10 mg/kg carbaryl [2, 6, 7, 18]. Albright and Simmel [1] reported that carbaryl decreased activity in a novel environment but increased activity measured by a selective activity meter. Data from the novel environment are more comparable to the present study since the animals were not exposed to the apparatus prior to dosing, and ambulation was directly measured. Although Singh [19] had found decreases in running wheel activity with 0.56 mg/kg carbaryl, this dose is far below those found to alter behavior in other experiments. As suggested by Anger and Wilson [2], this may be an aberrant finding.

Decreases in motor activity produced by carbaryl and propoxur correspond to the general effects of carbamates in decreasing rates of behavior. Physostigmine, for example, does not disrupt the pattern or rate of responding on multiple fixed-ratio, fixed-interval schedules below dosages which non-selectively eliminate responding [21]. Both carbaryl and propoxur decrease the rate of arm entries in a radial-arm maze task, but do not alter accuracy or the patterning of response choices [8]. In the present experiment, even when activity was decreased by 86% and 69% for the highest dosages of carbaryl and baygon, respectively, the pattern of photocell interruptions was not altered, i.e., the animals continued to ambulate through all areas of the maze. These rate-decreasing effects of the carbamates contrast with the effects of anticholinergic drugs which produce increases in activity and exploration in a variety of test situations [9].

Both carbaryl and propoxur produced rapid and short-acting inhibition of ChE. Within 15 min after IP injection, maximal enzyme inhibition was found, corresponding to maximal decreases in motor activity. Brain ChE was reduced to 42% and 53% of control levels for 16 mg/kg carbaryl and 2 mg/kg propoxur, respectively. Blood ChE activity consistently paralleled the time course for brain ChE, and was reduced to 46% of control levels for both carbamates. The longer duration of ChE inhibition for carbaryl compared to propoxur is in agreement with differences in the time course for signs of poisoning [20]. Although the time of maximum effect for decreases in motor activity and inhibition of ChE coincided, motor activity returned to control levels by 60 min for propoxur and by 120 min for carbaryl, while ChE levels in blood and brain remained depressed for both carbamates. This implies a dissociation of behavioral and biochemical effects.

For the organophosphate insecticides, which also inhibit AChE activity, behavioral effects are maximal at the time of peak enzyme inhibition, but behavioral recovery also occurs prior to recovery of AChE activity. For example, Reiter *et al.* [14] reported deficits in passive avoidance learning in mice at doses of parathion which reduced AChE activity to 30–55% of control levels. Although blood and brain AChE levels remained depressed for 24 hr, effects on behavior were only seen during periods of maximal enzyme inhibition, 30 min to 2 hr after dosing. In the present study, motor activity was decreased only during time periods when both blood and brain ChE levels were depressed to 42–53% of

TABLE 2
STATISTICAL TABLE FOR TIME-COURSE EXPERIMENT

A. Overall MANOVA

Treatment	$F(6,234)=59.74$; $p<0.0001$
Time	$F(12,309)=12.01$; $p<0.0001$
Treatment*Time	$F(24,339)=4.76$; $p<0.0001$

B. ANOVA Values for Individual Variables

Dependent Variables		Maze		Blood ChE		Brain ChE	
Source	*df*	F	*p*<	F	*p*<	F	*p*<
Treatment	2,119	12.44	0.0001	173.01	0.0001	236.19	0.0001
Time	4,119	1.67	0.1607	36.04	0.0001	28.44	0.0001
Treatment*Time	8,119	2.01	0.0510	6.31	0.0001	11.09	0.0001

C. ANOVA Values for Effect of Treatment

Time (min)	*df*		Maze	Blood	Brain
15	2,24	F	6.30	54.76	166.13
		p	0.0063*†	0.0001*†	0.0001§
30	2,24	F	6.71	34.81	80.78
		p	0.0048*	0.0001§	0.0001*†
60	2,24	F	2.30	37.95	23.98
		p	0.1222	0.0001§	0.0001§
120	2,24	F	1.11	38.82	34.45
		p	0.3460	0.0001§	0.0001§
240	2,24	F	0.14	25.28	32.74
		p	0.8678	0.0001*‡	0.0001*‡

Significant post-hoc comparisons: *control vs. carbaryl; †control vs. propoxur; ‡carbaryl vs. propoxur; §all comparisons.

control. For all three indices of toxicity, the duration of action was longer for carbaryl than for baygon.

Since all animals were tested in both the figure-eight maze and open field, the present study allows a direct comparison of the sensitivity of these tests in detecting changes in activity. Two factors influence the measurement of locomotor activity: the method of detection, which determines which aspects of activity are being measured, and also the influence of the test environment itself (size, illumination, spatial complexity, etc.) on the animal [16]. Photocell interruptions, which are automatically recorded in figure-eight mazes, and crossovers, which are manually scored in the open field, both detect similar activity. Both record ambulation, not directly or continuously, but as distance travelled. In the maze, an animal has to move along the corridor far enough to pass from one photocell to another, while in the open field, an animal must traverse a sufficient distance so that all four paws enter a new square. Figure-eight mazes and the open field do differ, however, in spatial complexity. The maze is a connected series of narrow corridors while the open field is an uninterrupted space. Because of this difference in complexity, habituation in figure-eight mazes requires 1–2 hr for control animals, whereas control animals habituate in the open field within 15 min.

Although both tests revealed consistent decreases in activity for both carbamates, the figure-eight maze was a more sensitive indicator of toxicity. This is directly related to the differences in habituation of activity between the two devices. If data for the first 5-min period in the maze are compared with the 5-min open field data, no differences in sensitivity were found for propoxur, which is a very short-acting AChE inhibitor. For carbaryl, only 28 mg/kg produced significant differences in activity in the maze, while 16 and 28 mg/kg did so in the open field. However, ChE levels remained depressed for at least 4 hr for carbaryl, and, over the total 2 hr of testing, the maze detected differences for 8–28 mg/kg. These data indicate that measures of motor activity in an open field may be unsuitable for acute toxicity testing since the brief time window of testing must be carefully tied to the time frame of the effect (cf. [16]).

In the open field, there was no dissociation between rearing and crossovers (ambulation). Animals receiving the higher doses were obviously ataxic, and with motor incoordination it might be anticipated that rearing would decrease before crossovers. Many of the animals which received 8 mg/kg propoxur or 28 mg/kg carbaryl did not move at all in the maze or open field, or showed tremors or unsteady gait when they moved. Despite obvious signs of intoxication in these animals, landing foot-spread was not affected. Landing foot-spread has been used successfully as a measure of

toxicant-induced disruptions in neuromotor function [4, 10, 17]; although it is a useful technique for detecting specific peripheral neuropathies, it may be insensitive as a general indicator of ataxia.

Although the carbamates do not produce irreversible neurotoxicity [12], acute pharmacological effects which are reversible, such as the decreases in locomotor activity reported here, are produced at dosages much lower than the LD_{50} values reported for either carbaryl or propoxur. Since the common reference point for acute toxicity is the LD_{50}, these results indicate that behavioral changes can be detected at exposure levels substantially below those producing overt toxicity.

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