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Toxicity of Carbofuran and Lindane to the Old-Field Mouse (*Peromyscus polionotus*) and the Cotton Mouse (*P. gossypinus*)

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Carbofuran and lindane are two insecticides commonly used in forestry practices (WHITE-STEVENS 1977, ULMANN 1972, KUHR & DOROUGH 1976). Carbofuran has a high acute toxicity to mammals. The reported oral LD_{50} of 4.0 mg/kg for the laboratory mouse is about the same as the topical LD_{50} for the house fly (YU et al. 1972). The apparent mode of action is by acetylcholinesterase inhibition (YU et al. 1972, CAMBON et al. 1979). Significant effects of chronic treatment have not been reported (MCCARTHY et al. 1971). The acute toxicity of lindane to mammals is one-half to one-tenth that of carbofuran (SPECTOR 1955, ULMANN 1972, Present study). Behavioral effects at sublethal treatment levels (hyperexcitability, tremors, convulsions) have been reported (DALLEMAGNE & PHILIPPOT 1948, HULTH et al. 1976).

The objectives of this study were to identify lethal levels of these compounds to small mammals when administered in the diet and to assess the effects of ingestion at sublethal levels on survival, reproduction, development of young, and some aspects of behavior. The cotton mouse, *Peromyscus gossypinus*, a woodland form, and the old-field mouse, *Peromyscus polionotus*, a species typically found in early successional areas, were used as experimental animals.

METHODS

Animals. Both species were trapped from wild populations in Florida. *P. polionotus* were taken in Marion County and *P. gossypinus* in Okaloosa County. Adult *P. polionotus* averaged 13.8 g in weight; *P. gossypinus* averaged 29.5 g.

Mice were housed in standard plastic laboratory cages (30 X 13 X 12 cm) with stainless steel tops. Food and water were provided in excess. Cotton and ground corncobs (San-i-cell) were placed in the cages for bedding. Animals were kept as male-female pairs or with littermates according to individual protocols. Both wild-caught animals and first-generation laboratory offspring were used.

Diet formulations. The standard diet was commercial laboratory animal food formulated for rats and mice (Wayne Feed Co.). In preparing experimental diets, hexane was used as a solvent for lindane; ethanol was used for carbofuran. A measured amount of

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insecticide dissolved in its respective solvent was thoroughly mixed with a measured amount of lab. chow, and air dried to remove as much solvent as possible. Equivalent amounts of solvent alone were mixed with food and used as control diets in all tests.

Range-finding tests. An indication of lethal levels of the compounds administered in the food was obtained over a 4-day period. Dietary concentrations of 0.5, 1.0, 1.5, and 2.0 mg lindane/g food, and 0.25, 0.50, and 1.0 mg carbofuran/g food were used. Ten *P. polionotus* were used in each group.

Eight-month tests of chronic effects. Dietary concentrations for chronic treatment were chosen after preliminary range-finding tests for lethal levels (see results). Levels of 0.2 mg lindane per g of food (0.02%), and 0.1 mg carbofuran per g of food (0.01%) were used.

Animals were paired (male-female) and placed on one of the experimental or control diets for 8 mo. These diets contained 0.01% carbofuran, 0.02% lindane, or one of the two control solvents. During this period, survival, reproduction, and development of young were monitored. Male survivors of each group were subjected to behavioral testing at the end of the treatment period.

Behavioral testing. A residential maze consisting of four arms radiating from a central chamber was employed for behavioral tests (WOLFE & ESHER 1978). Number of entries into each arm of the maze was monitored and recorded electronically. These records were used as measurements of general locomotory activity and of periodicities of activity.

Two regimes were used. The first consisted of testing animals for a 3-day pre-treatment control period, beginning the experimental diet on day 4, and continuing testing for 14 additional days. Activity for each individual during the test period is expressed as percent of its activity on days 2 and 3 (mean) of the pre-treatment period (Fig. 2). These data were analyzed using a multiple regression with dummy variables (NIE et al. 1975). In the second test, male survivors of the 8-mo chronic feeding regime were placed on control diets and tested for 4 days.

Differences in activity between treated and control groups were determined by a one-way analysis of variance with the appropriate F-test. Mean separation was determined by a t-test made on contrasts between the group that had been on lindane versus the hexane control and carbofuran versus the ethanol control.

RESULTS

Range-finding tests. Mortality after 96 h on carbofuran was 20% at 0.25 mg/g and 100% on 0.50 mg/g. On lindane, there was no mortality at 0.50 mg/g, 50% at 1.00 mg/g, 80% at 1.50 mg/g and 100% at 2.00 mg/g.

TABLE 1

Reproduction and survival of litters to 14 days of age in *Peromyscus polionotus* and *P. gossypinus* while on carbofuran (C) or lindane (L) diets for 8 months.

			Control			Treated		
Species	Diet	Breeding Pairs	No. of Litters	Mean Size ($\pm$ SE)	Percent Survival	No. of Litters	Mean Size ($\pm$ SE)	Percent Survival
	C	9	29	4.1 $\pm$0.33	90	32	4.3 $\pm$0.21	62
P. polionotus								
	L	15	25	4.3 $\pm$0.30	61	38	3.8 $\pm$0.29	81
	C	7	9	3.6 $\pm$0.39	38	12	3.5 $\pm$0.37	52
P. gossypinus								
	L	13	11	4.5 $\pm$0.37	100	32	3.8 $\pm$0.33	81

TABLE 2

Age in days at appearance of selected developmental characters in mice born to parents of chronic treatment. Means and standard errors are given.

	P. gossypinus				*P. polionotus*			
	Hexane (N=12)	Lindane (N=22)	Ethanol (N=49)	Carbofuran (N=98)	Hexane (N=107)	Lindane (N=85)	Ethanol (N=65)	Carbofuran (N=87)
Appearance of Pigment	3.4 ±0.22	3.2 ±0.20	3.0 ±0.16	3.7 ±0.15	3.2 ±0.13	3.1 ±0.18	3.0 ±0.21	3.2 ±0.16
Appearance of Hair	6.6 ±0.30	7.0 ±0.27	9.0 ±0.21	6.3 ±0.19	8.2 ±0.18	7.3 ±0.20	7.3 ±0.19	7.6 ±0.18
Righting Response	7.8 ±0.36	6.8 ±0.29	9.0 ±0.22	5.7 ±0.19	7.7 ±0.17	7.5 ±0.21	6.9 ±0.30	6.8 ±0.25
Walking	10.6 ±0.39	9.8 ±0.29	11.0 ±0.41	9.3 ±0.21	11.8 ±0.45	10.3 ±0.33	10.2 ±0.40	10.6 ±0.36
Eyes Open	14.4 ±0.45	14.1 ±0.30	15.0 ±0.43	13.3 ±0.39	16.7 ±0.40	15.3 ±0.49	14.6 ±0.51	16.2 ±0.49
Ears Open	15.4 ±0.51	14.4 ±0.43	16.0 ±0.39	12.3 ±0.35	16.9 ±0.36	14.9 ±0.40	14.8 ±0.38	15.8 ±0.42

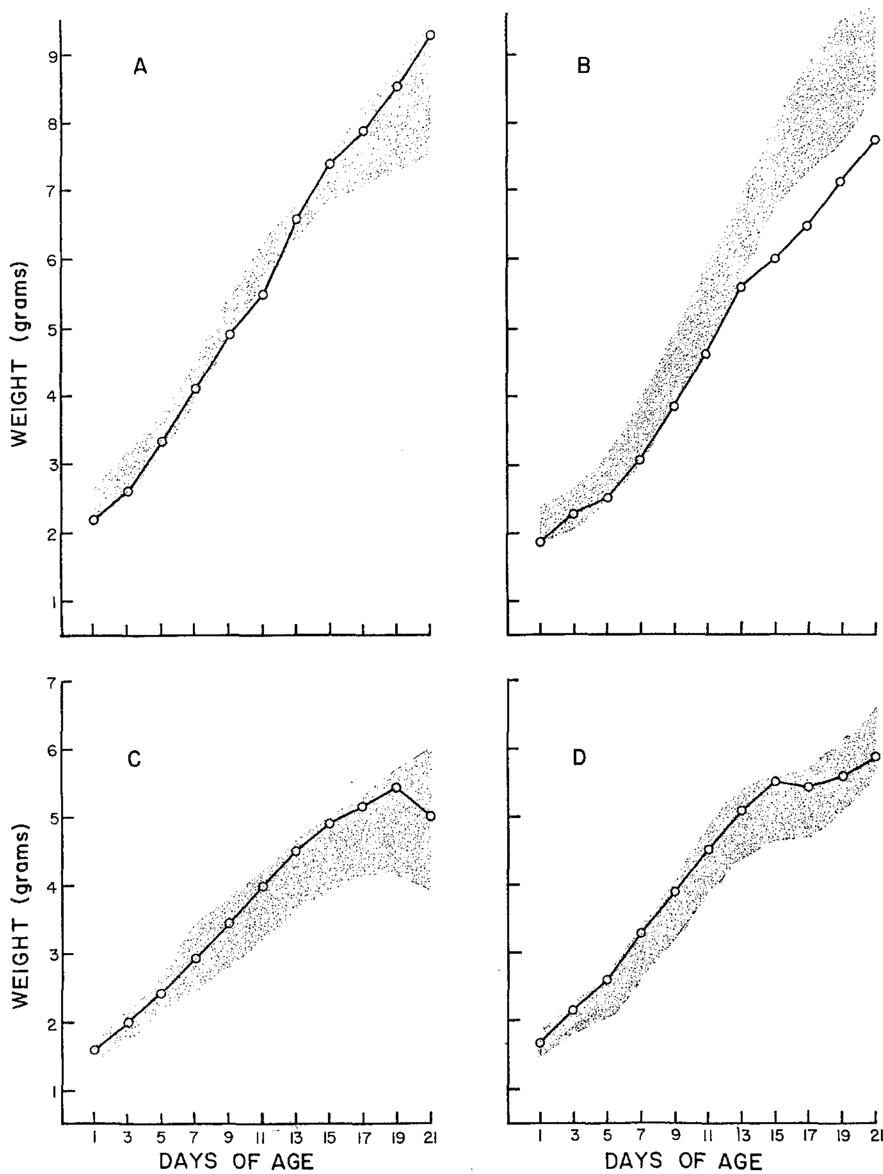

Fig. 1. Postnatal growth of _P. gossypinus_ (A & B) and _P. polionotus_ (C & D). The lines on A and C represent offspring of lindane-treated parents. B and D were from carbofuran-treated parents. Shaded area = controls ± 2 SE.

Chronic tests. Food consumption for the first 3 weeks of treatment was measured and found to be normal (2.7 g/day for *P. polionotus*, and 3.6 g/day for *P. gossypinus*). There was no difference in the consumption of the control and experimental diets [$P > 0.21$, two way ANOV with repeated measures (DIXON 1975)].

There was no significant difference in mortality between animals on the control and the experimental diets ($P > 0.05$; Chi-square), except for 44% mortality which occurred in *P. polionotus* on carbofuran ($P < 0.01$; Chi-square). Most deaths occurred late in the regime, and had little effect on our reproductive data.

Reproduction. Neither experimental diet had an adverse effect on reproduction (Table 1). The only significant result in the number of litters produced was a higher production ($P < 0.05$; Chi-square) by lindane-treated animals. The apparent decrease in litter size in lindane-treated animals is not significant ($P > 0.05$, Student's t-test). There was no significant difference between groups in survival of young to 14 days of age.

Growth and development of young. Young were removed when discovered, weighed, checked for the appearance of characters listed in Table 2, and then returned to the parent's cage. This process was repeated at two-day intervals through weaning at 3-weeks of age.

There was no significant effect of either treatment on growth rate (Figure 1) or the rate of appearance of any of the developmental characteristics measured (Table 2).

Behavior. In the first testing regime (Figure 2), there was no difference in the slopes or intercepts of individuals on either treatment ($P > 0.05$). Results of testing after 8-mo chronic treatment are given in Table 3. Here also there were no significant differences between either pesticide-fed group and its control ($P > 0.11$).

As a test for possible disruption of diel activity patterns, we examined the percent of activity (entries into all arms) that occurred during daylight or darkness for *P. polionotus*. This species is characteristically nocturnal, and activity patterns during these tests did not deviate from that. A Chi-square test comparing activity in hours of darkness versus hours of day did not indicate a significant difference between either treatment and its control ($P > 0.05$).

DISCUSSION

Although the insecticides studied have high (Carbofuran) or moderate (lindane) acute toxicity to mammals, we found no indication of cumulative or delayed effects of eight months of chronic treatment at the levels tested, except for some delayed mortality of *P. polionotus* on carbofuran.

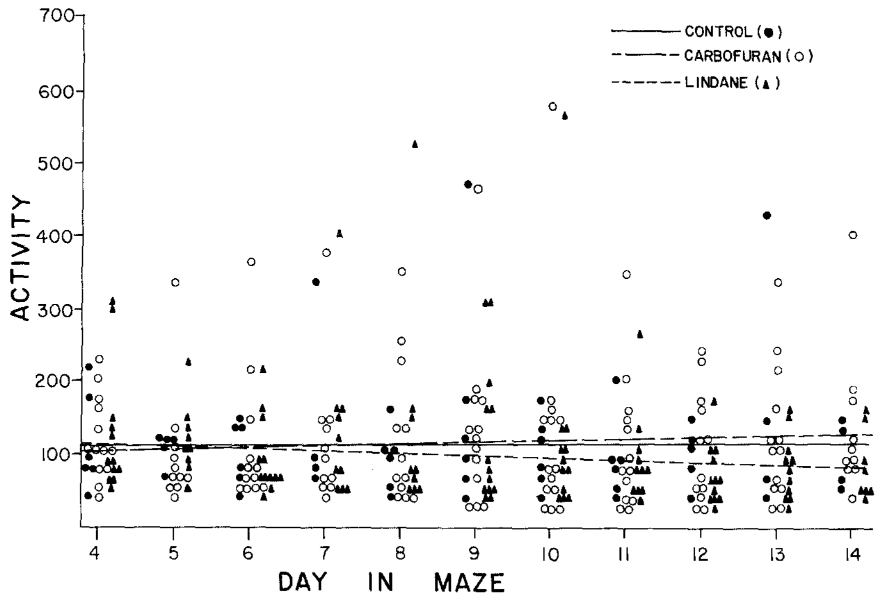

Figure 2. Activity (entries into maze arms) of P. polionotus expressed as percent of activity on two pre-treatment days.

TABLE 3

Entries into arms of a residential maze by mice following 8-month treatment. Data from days one and four of a four-day test are presented. Mean ± SE.

Diet	N	Entries, Day 1	Entries, Day 4
Hexane	5	1600 ± 595	1038 ± 712
Lindane	5	1132 ± 619	310 ± 92
Ethanol	5	739 ± 440	500 ± 107
Carbofuran	5	641 ± 370	225 ± 117

Physiological and cytological effects of carbofuran previously demonstrated include acetylcholinesterase inhibition and alteration of prostate gland function (YU et al. 1972, SCHEIN et al. 1976, SCHAIN et al. 1977). Effects of lindane include changes in microsomal enzyme activity (PELISSIER & ALBRECHT 1976, LOWY et al. 1977) and in nerve cell conductivity (WHITCOMB & SANTOLUCITO 1976).

These effects were not translated into measurable changes in reproduction, growth and development of young, or behavior in our tests.

In an earlier study on laboratory rats, MCCARTHY et al. (1971) found some loss of weight in parents and reduced survival of young on 0.01% carbofuran. Our results (at the same feeding level) indicate the possibility of reduced survival of young *P. polionotus*, but the differences were not statistically significant. High control mortality in *P. gossypinus* precluded a meaningful comparison there (see Table 1).

Apparently lindane had a positive effect on reproduction. The mechanism may possibly be due to an estrogenic effect as has been reported for other organochlorine insecticides (WELCH et al. 1969).

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