Dyrene Toxicity and Lack of Carboxylesterase Inhibition in Mice and Rats

The intraperitoneal LD_{50} for Dyrene [2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine] in both rats and mice was <100 mg/kg. Neither single nor repeated injections of Dyrene caused a dose-dependent inhibition of liver carboxylesterase activity. Feeding rats 1000 and 5000 ppm of Dyrene in the diet for 3 weeks resulted in approximately a 20% decrease in liver carboxylesterase activity. This decrease did not seem to be toxicologically important because it did not affect the susceptibility of rats to malathion poisoning. Doses of 25 mg/kg or greater of Dyrene caused a rapid hypothermia in mice but not in rats. Dimethoxydyrene was much less toxic to mice than Dyrene and did not inhibit liver carboxylesterase activity *in vivo* or *in vitro*.

Mendoza and Hatina (1970) reported that methoxy derivatives of the fungicide Dyrene [2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine], formed by heating with methanol, inhibited liver carboxylesterases differentiated by starch-gel electrophoresis. Dyrene reacts with other alkyl alcohols in the presence of NaOH or HCl to form products which have been shown, by a spot test on thin-layer chromatographic plates, to inhibit liver carboxylesterase activity (Mendoza *et al.*, 1971). Dyrene itself did not inhibit carboxylesterases in the *in vitro* tlc test system; however, no tests for the possibility of formation of a carboxylesterase inhibitor in intact animals were reported. On the basis of their *in vitro* studies, Mendoza *et al.* (1971) indicated a need for further evaluation of the biologic significance of enzyme inhibition by Dyrene derivatives.

Many drugs, pesticides, and other toxic chemicals contain carboxylester groups, and some of these compounds are detoxified by carboxylesterase enzymes. Inhibition of carboxylesterases *in vivo* greatly increases the toxicity of the carboxylester-containing insecticide malathion [*O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate] (DuBois, 1969). This study was undertaken to determine if dyrene or possible metabolites inhibited carboxylesterases *in vivo* or potentiated the toxicity of malathion.

EXPERIMENTAL SECTION

Male mice (Charles River, CD-1) and male rats (Holtzmann) weighing 25–30 g and 175–200 g, respectively, were used. They were housed in air-conditioned rooms (75–80°) and were supplied with food and water *ad libitum*. Dyrene (93% technical grade) and malathion (98.5% analytical standard) were injected intraperitoneally as corn oil solutions. Concentrations of chemicals were adjusted to provide the desired dose in an injection volume of 5 ml/kg for mice and 2 ml/kg for rats.

For the feeding study, Purina Rat Chow was ground to a coarse powder. The appropriate amount of Dyrene was dissolved or suspended in 50 ml of acetone and this was thoroughly mixed into 100 g of ground chow. The acetone was allowed to evaporate and the Dyrene-chow concentrate was diluted to the proper weight with additional ground chow. Control rats were fed a diet prepared similarly with acetone but without dyrene. Rats were caged separately for the duration of the feeding study and body weights and food consumption were recorded at 2–3-day intervals throughout the experiment.

Animals were sacrificed by decapitation and exsanguination, and liver homogenates were prepared in 0.026 *M* sodium bicarbonate buffer, pH 7.6. For carboxylesterase determinations, hydrolysis of 0.0067 *M* diethyl succinate (DES) by 2.5 mg and 0.027 *M* triacetin (TA) by 5.0 mg of homogenized liver was determined manometrically as described previously (Cohen and Murphy, 1971a,b). Brain cholinesterase activity was determined manometrically using acetylcholine chloride (0.01 *M*) as the substrate (DuBois and Mangun, 1947).

RESULTS AND DISCUSSION

Acute Toxicity. The intraperitoneal LD_{50}'s for rats determined in this study were approximately 25–40 mg/kg in 24 hr and 16–25 mg/kg in 7 days. Fifty rats were used in estimating the intraperitoneal LD_{50}'s. The difference between the reported oral LD_{50} of 2700 mg/kg (Lehman, 1965) and the observed intraperitoneal LD_{50}'s suggests that orally administered Dyrene is poorly absorbed in rats. Fifty mice were used and the intraperitoneal LD_{50}'s for dyrene were estimated to be 50–70 mg/kg in 24 hr and 30–50 mg/kg in 7 days.

Carboxylesterase Activity. During the acute toxicity studies, livers were removed from several Dyrene-treated mice at the time of death for carboxylesterase determination. Liver DES and TA esterase activities were the same in dyrene- and corn oil-treated mice.

To determine if repeated injections of sublethal doses of dyrene might inhibit liver carboxylesterase activity, groups of five mice were given daily injections of corn oil or 5, 15, or 25 mg of Dyrene per kg for 3 days and were sacrificed on the fourth day for liver carboxylesterase determinations. Mean ±S.E. activities of five control livers were 60.8 ± 4.4 and 45.3 ± 5.3 μl CO_2/mg/15 min for DES and TA esterase, respectively. Dyrene treatment did not significantly ($p > 0.05$) alter liver TA esterase activity at any dosage tested. The group given daily injections of 15 mg of Dyrene per kg had 20.6 ± 4% lower liver DES esterase activity ($p < 0.05$) than controls; however, neither the 5 nor the 25 mg/kg treatments significantly lowered DES esterase activity.

Feeding Study. Since the preceding experiments did not demonstrate a dose-related effect of single or repeated Dyrene injections on liver carboxylesterase activity, the potential for a toxic interaction to occur after acute exposure to Dyrene and subsequent exposure to a carboxylester-containing chemical seemed slight. To test the effects of more prolonged exposure to low levels of Dyrene on carboxylesterase activity, a short-term feeding study was undertaken. Rats were provided with diets containing Dyrene at and below the reported "No Effect" level (5000 ppm; Lehman, 1965) for 1 or 3 weeks.

Twelve control rats consumed an average of 25.3 ± 0.4 g of diet/rat/day and gained an average of 7.1 ± 0.2 g of body weight/rat/day. Weight gain and food consumption were the same for rats on the control and dyrene diets. Feeding dyrene in the diet for 1 week had no detectable effect on rat liver carboxylesterase activity.

Results of the 3-week study are summarized in Table I. Although the inhibition of DES esterase activity by 1000 ppm and TA esterase activity by 1000 and 5000 ppm of Dyrene was statistically significant ($p < 0.05$), it did not seem of toxicologic importance in that it did not increase the susceptibility of rats to malathion poisoning. Thus, groups of four rats from each diet group were injected with the cholinesterase-inhibiting insecticide malathion (200 mg/kg) after 3 weeks on the diets. The mean ±S.E.

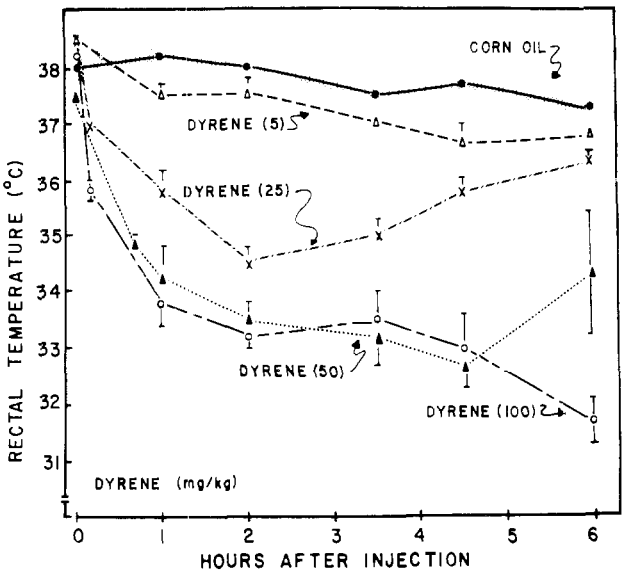

Figure 1. Effect of Dyrene (i.p.) on rectal temperature of male mice. Each point represents the mean ±S.E. of three or more animals.

brain cholinesterase activity of four untreated control rats was 101.0 ± 1.6 μl of CO_2/50 mg/10 min. Malathion treatment reduced brain cholinesterase activity to 49.5 ± 10.1% of control activity in rats fed the control diet. Brain cholinesterase activity after malathion in the Dyrene-fed rats ranged from 37.2 ± 3.6 to 61.6 ± 15.2% of control, and did not differ significantly ($p > 0.05$) from the activity in the malathion-challenged controls.

Dyrene Hypothermia. During the acute toxicity studies it was observed that shortly after injection of Dyrene, mice but not rats became hypothermic. To study this effect, groups of three to five mice were injected with selected doses of Dyrene and rectal temperature was measured at several intervals up to 6 hr after injection. Results are presented in Figure 1. Significant decreases in rectal temperature were detected within 10 min after injection. The hypothermia was dose dependent and preceded a generalized depression of spontaneous activity which was not observed until 90–120 min after injection. Depression of spontaneous activity was not observed in the control mice nor in those which received 5 mg of Dyrene per kg. The unexpected hypothermic effect of dyrene in mice merits further investigation.

Dimethoxydyrene. Dimethoxydyrene [2,4-dimethoxy-6-(*o*-chloroanilino)-*s*-triazine] was prepared from Dyrene and NaOH (0.02 *M*) in methanol, and identity of the product was confirmed by melting point determination and by cochromatography with Dyrene in benzene and also in acetone–hexane (20:80) (Mendoza *et al.*, 1971). Three mice were given 500 mg of dimethoxydyrene per kg intraperitoneally and were sacrificed 18 hr later. Carboxylesterase determinations did not indicate any inhibition of DES esterase activity. At the time of sacrifice the mice appeared normal.

To test the action of dimethoxydyrene as an *in vitro* inhibitor of mouse liver DES esterase activity, dimethoxydyrene was solubilized with 0.08% *N,N*-dimethylformamide and 0.5% Triton X-100 and was added to 2.5 mg of homogenized liver in bicarbonate buffer. After 24 min the DES substrate was added and carboxylesterase activity was determined. Concentrations of dimethoxydyrene up to 7.5×10^{-4} *M* were tested and did not inhibit liver hydrolysis of DES *in vitro*.

Table I. Effect of Feeding Dyrene for 3 Weeks on Rat Liver Carboxylesterase Activity

Dyrene, ppm	% of control[a]	
	DES	TA
0	100 ± 4	100 ± 4
500	106 ± 2	104 ± 9
1000	86 ± 4[b]	79 ± 6[b]
5000	93 ± 3	80 ±4[b]

[a]Values are mean ±S.E. of four or more animals. Mean ±S.E. activities of four to six control rat livers were 43.2 ± 1.6 and 14.2 ± 0.6 μl CO_2/mg/15 min for diethyl succinate (DES) and triacetin (TA), respectively. [b]Significantly different from control ($p < 0.05$).

The results of this investigation indicate that neither dyrene nor any potential metabolites that might be formed in intact mice or rats are strong inhibitors of liver carboxylesterase *in vivo*. Dimethoxydyrene in concentrations as high as 7.5×10^{-4} *M* did not inhibit mouse liver carboxylesterase activity when tested in an *in vitro* assay system, which had been shown in previous studies (Cohen and Murphy, 1971a,b) to be sensitive to organophosphate esterase inhibitors. Limited *in vivo* tests with dimethoxydyrene supported the *in vitro* tests, as 500 mg/kg of the compound did not inhibit mouse liver hydrolysis of diethylsuccinate.

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