Chronic Ingestion of Lead and the Response of the Immature Rat to Parathion

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Introduction

The establishment of legal tolerances for pesticide residues in or on foodstuffs is based in many cases on the level of pesticide ingested that causes "no detrimental effect" in experimental animals. The experimental conditions are usually such that the test species is subjected to less stress than is man. Although a safety factor is applied which theoretically will protect against possible interactions that may potentiate pesticide toxicity, it would be beneficial to have precise data on possible interactions.

Pesticide metabolism and toxicity may vary widely depending on species (MURPHY et al 1968), nutritional status (CASTERLINE and WILLIAMS 1971), disease conditions (DEICHMANN et al 1968), drug interactions and interactions with other pesticides (BALL et al 1954). Man is subjected to many stress conditions of which environmental contaminants may form a significant part. Lead was chosen as a candidate contaminant for study with respect to pesticide toxicity due to the increasing concern and widespread occurrence of this heavy metal in the environment. CHISOLM (1971) stated that although there is no evidence that any groups in the general population have mean blood levels of lead that approach the dangerous levels, research is required to elucidate the effects of long term chronic exposure. There is a need to know whether or not the current level of lead absorption in the general population presents some subtle risk to health. In a previous study (PHILLIPS et al 1971) we have shown that the short term feeding (up to 90 days) of lead (200 ppm) had no effect on the disappearance of DDT residues and metabolites from adipose tissue of rats. Lead alone, and lead fed to DDT pre-treated animals had no effect on body weight gain, liver weights, liver vitamin A, liver protein, *in vitro* liver carboxylesterase activity and pentobarbital sleeping times. In the present study, progeny from rats raised through one generation on diets cont-

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aining various levels of inorganic lead were studied in regard to their response to intraperitoneal doses of parathion. This approach is considered more satisfactory for the purposes of this study than a model using massive levels of lead added to the maternal diet to intoxicate the suckling animal via the milk.

Materials and Method

Male and female weanling Wistar rats were raised on ground stock cubes (Maple Leaf Mills, Master Feeds Division, Toronto) containing 3% added corn oil and with additions of lead at levels of 0, 2, 20 or 200 ppm added as lead chloride. On reaching sexual maturity the animals were bred and the progeny, weaned at 21 days of age, were used for the interaction (lead vs parathion) studies. The weanlings were divided into five groups and dosed intraperitoneally with the vehicle or one of four dose levels of parathion. Male and female rats were studied separately, thirty animals of each sex at each level of dietary lead or a total of 48 animals per dose level of parathion. Parathion was dissolved in 20% ethanol and 80% propylene glycol. The dose was approximately 0.4% of body weight (i.e. 0.2 ml per 50 g body weight). The dosing levels were calculated from an assumed LD_{50} of 1.80 mg/kg body weight (ALARY and BRODEUR 1970a) to span a non-lethal to lethal range. The levels of parathion administered were 0, 0.45, 0.90, 1.80 and 3.60 mg/kg body weight. Animals were maintained for 24 hours after dosing with parathion. The survivors were killed, blood collected and the liver and brain excised. Serum was prepared and along with the other tissues, frozen pending analyses. Liver carboxylesterase activity was determined by preparing a 1 + 9 homogenate in water. Ten µl of the homogenate was added to 4 ml of 0.02 M o-nitrophenyl butyrate in 0.1 M phosphate buffer (filtered), pH 6.3 and the released o-nitrophenol was measured spectrophotometrically at 372 mu at a temperature of 25°C. The results were expressed as Δ O.D. per 3 min per g liver.

Plasma cholinesterase was determined spectrophotometrically by the method described by KLING and LONG (1969). The results were expressed as Δ O.D. per 3 min per ml of serum. Cholinesterase activity of brain homogenates (prepared as for liver) was determined by the same procedure as for serum, using 50 µl of homogenate. Results were expressed as Δ O.D./3 min/g brain.

TABLE 1

The effect of parathion on serum cholinesterase of weanling rats from dams raised on various levels of dietary lead

Dietary level of lead (ppm)		i.p. dose of parathion, mg/kg body weight			
		0	0.45	0.90	1.80
		Serum cholinesterase[c] Δ O.D./3 minutes/ml serum			
0	M[a]	2.10 ± .08	1.86 ± .09	1.80 ± .17	1.57 ± .07
	F[b]	1.72 ± .11	1.96 ± .12	1.63 ± .14	1.43 ± .12
2	M	2.24 ± .11	2.26 ± .20	1.73 ± .07	1.69 ± .06
	F	1.85 ± .13	1.83 ± .12	1.70 ± .10	1.50 ± .06
20	M	2.04 ± .21	2.06 ± .18	1.81 ± .09	1.71 ± .13
	F	1.87 ± .13	2.11 ± .19	1.64 ± .13	1.59 ± .17
200	M	2.04 ± .21	1.78 ± .08	1.69 ± .08	1.45 ± .16
	F	1.99 ± .08	1.63 ± .04	1.66 ± .06	1.47 ± .07

(a) M = male (b) F = female (c) 24 hours after parathion dose.

Results

The body weights of the weanling animals at the time of dosing with parathion (21 days of age) ranged from 32 to 54 g for females and 32 to 64 g for males. The levels of parathion administered spanned the anticipated range of toxicity. Twenty-four hours after dosing no deaths were recorded in the control group or at parathion dose levels of 0.45 and 0.90 mg per kg body weight. Doses of parathion at 1.80 mg/kg body weight caused 9 deaths while 3.60 mg/kg body weight caused 43 deaths from a total of 48 animals dosed at each level. Although the experiment was not designed to accurately determine an LD_{50} dose, it would not appear that the level of dietary lead influenced mortality. Similar rates of mortality to parathion were observed in the groups receiving 0 or 200 ppm lead.

The effect of graded doses of parathion on serum cholinesterase is shown in Table 1. Analyses were performed only on animals surviving twenty-four hours after dosing. Data are presented therefore for dose levels of 0 to 1.80 mg parathion/kg body weight since few animals survived the dose of 3.60 mg/kg. The serum cholinesterase in animals receiving no parathion were similar in all groups over the range of 0 to 200 ppm lead in the diet. Increasing rates of administration of parathion decreased the rates of serum cholinesterase activity. The enzyme activity was plotted against increasing doses of parathion for each dietary level of lead. Visual inspection of the data gave no indication of a lead by parathion interaction, thus detailed statistical analyses were not performed. The lack of a lead x parathion interaction can be clearly seen by the similarity of serum cholinesterase of animals receiving 1.80 mg parathion per kg body weight in animals raised on diets containing the two extremes in lead content, namely 0 and 200 ppm.

Dietary lead alone at the levels fed did not modify brain cholinesterase (Table 2). The enzyme activity 24 hours after the parathion dose was decreased by the higher doses of parathion but the enzyme depression was not altered in those animals consuming diets containing increasing levels of lead.

Liver carboxylesterase activity was not modified by dietary lead, however, parathion decreased activity. As with the other enzymes studied dietary lead did not influence the depression of enzyme activity evoked by parathion.

TABLE 2

The effect of parathion on brain cholinesterase of weanling rats from dams raised on various levels of dietary lead

Dietary level of lead (ppm)		i.p. dose of parathion, mg/kg body weight			
		0	0.45	0.90	1.80
		Brain cholinesterase Δ O.D./3 min/g brain wet weight			
0	M	57.8 ± 2.6	52.1 ± 2.8	44.3 ± 0.5	41.1 ± 1.0
	F	49.9 ± 4.1	52.5 ± 2.9	49.2 ± 2.9	41.2 ± 1.2
2	M	54.9 ± 3.0	53.8 ± 1.6	51.5 ± 1.7	41.9 ± 3.6
	F	54.7 ± 1.7	51.8 ± 3.0	50.8 ± 1.8	43.5 ± 1.5
20	M	51.6 ± 2.1	54.4 ± 3.4	47.9 ± 2.4	48.2 ± 3.9
	F	56.3 ± 1.9	51.8 ± 1.1	55.8 ± 2.0	41.2 ± 1.5
200	M	53.5 ± 1.3	55.1 ± 2.6	47.8 ± 2.6	44.8 ± 4.2
	F	51.1 ± 2.8	52.0 ± 1.9	46.8 ± 2.0	44.2 ± 1.5

TABLE 3.

The effect of parathion on liver carboxylesterase of weanling rats from dams raised on various levels of dietary lead

Dietary level of lead		i.p. dose of parathion mg/kg body weight			
		0	0.45	0.90	1.80
		Liver carboxylesterase Δ O.D./3 min/g liver			
0	M	345 ± 25	229 ± 20	235 ± 18	213 ± 19
	F	312 ± 14	231 ± 15	215 ± 14	160 ± 31
2	M	348 ± 20	243 ± 10	244 ± 19	227 ± 26
	F	330 ± 32	283 ± 18	247 ± 17	240 ± 14
20	M	361 ± 18	258 ± 7	253 ± 18	236 ± 19
	F	336 ± 22	295 ± 16	234 ± 15	218 ± 19
200	M	328 ± 26	250 ± 12	260 ± 11	232 ± 44
	F	354 ± 23	232 ± 17	226 ± 14	199 ± 31

The adult parent females were maintained on their respective diets until they were 347 days old. Twenty-four hours after a single i.p. dose of 2.5 mg of parathion, plasma and brain cholinesterase was determined. The data are not reproduced since as with the immature animal lead alone did not affect these esterases nor was there any lead by parathion interaction.

Discussion

Many studies have been undertaken to determine interaction effects with pesticides. In most cases, however, the designs were such that subacute levels of pesticides were administered for short periods of time. Although these studies provide valuable information on mechanisms of action, they may bear little relationship to interactions encountered under practical conditions in the environment. The results of a study by MACDONALD et al (1970) investigating the effects of parathion on liver microsomal enzyme activities induced by organochlorine pesticides and drugs led them to conclude that the net result of hepatic microsomal enzyme activity of two compounds cannot be predicted when one is a depressant and the other a stimulant. We consider that a similar situation may exist between parathion toxicity and other environmental contaminants, in that alterations in toxicity, if any, can only be determined by direct experimentation. The design of the experiment in the present study was such to overcome short term administration of high levels of the contaminant (lead) and to study the effect of the toxicant (parathion) on the weanling animal. Most toxicological testing of contaminants usually begins at the time of weaning of the animal or later. It is desirable that more information be obtained on stress conditions that may influence the neonate between the times of birth and weaning.

ALARY and BRODEUR (1970b) demonstrated that the rate of *in vitro* degradation of parathion by the liver was a satisfactory index of *in vivo* toxicity in adult rats but not immature rats. They state the susceptibility of the latter seems to involve factors other than the ability of the liver to detoxify the insecticide, these factors possibly being differences in absorption, distribution, non-specific binding or excretion of parathion, or an increased susceptibility of nervous tissue to the toxicant. Thus in the present study the resultant toxicant effect of parathion, namely, the depression of cholinesterase enzymes was determined.

CHISOLM (1971) reviewing the toxicology of lead considers that "the best known adverse effect of lead is its inhibition of the activity of enzymes that are dependent on the presence of free sulfhydryl groups for their activity". The clearest manifestation of the inhibitory effect of lead is the disturbance caused in the biosynthesis of heme, specifically in the metabolism of delta-aminolevulinic acid and in the final formation of heme from iron and protoporphyrin. Very recently it was demonstrated that levels of environmental contamination with lead can decrease the activity of erythrocyte aminolevulinic acid dehydratase in man (HERNBERG and NIKKANEN, 1970). The present study demonstrates however that the activities of serum and brain cholinesterase and liver carboxylesterase are unaffected in weanling rats born and nursed with dams having consumed diets containing up to 200 ppm lead during development, pregnancy and lactation. Increasing doses of parathion decreased the activity of the three enzymes studied as would be expected. However, the added stress of dietary lead up to 200 ppm in the diet of the dams did not modify the response of the enzymes to parathion.

Parent phosphorothionates (in this case parathion) are not capable of inhibiting esteratic enzymes in animals (NEAL, 1971); however, the oxygen analog does. Using a similar experimental protocol to that described in this paper, it is evident from longer term feeding studies now in progress that the effects of lead ingestion (0 to 200 ppm) on the liver mixed function oxidase would be minimal. Thus lead ingestion up to the time of weaning in the present study, would not alter the rate of paraoxon formation. This is supported by the finding that the response of the various enzymes to parathion was similar between all treatment levels of lead.

TRIOLO et al (1970) studied the interaction of a number of organochlorine insecticides on the toxicity of paraoxon and concluded that it was likely that factors other than the A-esterases of plasma are responsible for the protective action of these insecticides against paraoxon toxicity. The evidence indicated that these agents increase the plasma binding of paraoxon and thereby make less paraoxon available for the inhibition of brain cholinesterase. In the present study brain cholinesterase was depressed by parathion to a similar extent in groups receiving different levels of dietary lead. Thus it would appear that lead did not influence the plasma binding of paraoxon.

In summary the progeny from animals raised for one generation on extremely high levels of dietary lead had normal activity of serum and brain cholinesterase and liver carboxylesterase. The response of these enzymes in vivo to intraperitoneal injections of parathion were similar regardless of the level of dietary lead. The data indicate that lead does not potentiate parathion toxicity.

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