Effect of Casein Diets on the Toxicity of Malathion and Parathion and Their Oxygen Analogues[1]

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Various exogenous and endogenous factors are known to influence the toxicity of the esterase-inhibiting pesticides. The nutritional status of an animal is an example of an exogenous factor that has been shown to alter the lethal action of malathion (BOYD and TANIKELLA 1969) and parathion (CASTERLINE and WILLIAMS 1969) in rats.

The present paper describes the effects of protein-deficient diets on the toxicity of malathion and parathion and their oxygen analogues, malaoxon and paraoxon. Studies were also conducted to determine the effects of low protein diets on the urinary excretion of p-nitrophenol in animals treated with parathion and paraoxon.

Materials and Methods

Materials. Malathion (>95%), malaoxon (>95%), parathion (>98.5%), and paraoxon (99%) were supplied by the American Cyanamid Company, Princeton, New Jersey.

Toxicity Studies. For each pesticide tested, three groups of 50 male weanling albino rats were used. Two of the groups were fed 5% and 20% casein diets ad libitum while the third group received a 20% casein diet pair-fed to the 5% casein diet group.

The 20% casein diet consisted of 69.6% sucrose, 20% vitamin-free casein, 4% Jones-Foster Salt Mixture, 4% corn oil, 2.2% vitamin mix, and 0.2% DL-methionine. In preparing the 5% casein diet, casein was replaced by additional sucrose. Since magnesium was found to be low in all prepared diets, magnesium oxide was added at the rate of 0.0332 g/100 g diet. The vitamin mix (Vitamin Diet Fortification Mixture), vitamin-free casein, and salt mixture were obtained from Nutritional Biochemicals Corporation.

On the tenth day of the feeding period, the rats were injected i.p. with the pesticide using five levels with 10 animals per treatment level. The vehicle for pesticide injection was a mixture of ethanol:propylene glycol (20:80). The dosages used were determined from pilot studies. After injection, the animals were

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observed for symptoms of intoxication and deaths were recorded at 24, 48, and 72 hours. The LD_{50} values were calculated by probit analysis.

Studies on the urinary excretion of p-nitrophenol. Three groups of rats were fed according to the protocol used in the toxicity studies. On the tenth day, five rats from each dietary regimen were injected i.p. with parathion (0.3429 mg/ml), paraoxon (0.0177 mg/ml) or p-nitrophenol (0.09 mg/ml) solution, each rat receiving 0.5 ml/100 g body weight. Twelve-hour urine samples from the five treated rats on each dietary regimen were pooled. The urine samples were acidified by the addition of concentrated HCl (1:1) before assaying for total p-nitrophenol by the method of ELLIOT et al. (1960).

Results

The results of the LD_{50} determination are summarized in Tables 1 and 2.

TABLE 1

Comparative LD_{50} values (mg/kg ± S.E.) for malathion and malaoxon

Dietary Casein (%)	No. of rats	Malathion	No. of rats	Malaoxon
5[1]	59	151.1 ± 15.70[2]	57	7.75 ± 0.35[2]
20	60	273.6 ± 17.07[3]	58	14.33 ± 0.73
20	59	418.6 ± 11.09	50	15.23 ± 1.03

[1] Pair-fed to the 5% casein group.

[2] Significantly different from 20% pair-fed ($p<0.01$) and 20% ad libitum ($p<0.01$).

[3] Significantly different from 20% ad libitum ($p<0.01$).

Dietary protein levels affected not only the toxicity of the parent compounds, malathion and parathion, but also their oxygen analogues, malaoxon and paraoxon. The LD_{50} values of the four compounds were significantly lower ($p<0.01$ and $p<0.05$) in rats fed 5% casein as compared to rats fed 20% casein diets (either pair-fed or fed ad libitum).

All the pesticides that were injected produced common clinical signs of intoxication such as lacrymation, salivation, twitching, and convulsions followed by death. Most deaths occurred quickly, especially after the administration of parathion and paraoxon (Table 3). Mortality was observed as early as 10 to 15 minutes after injection. No consistent effect of level of dietary protein was observed on the rate of mortality.

TABLE 2

Comparative LD_{50} values (mg/kg ± S.E.) for parathion and paraoxon

Percent Dietary Casein (%)	No. of rats	Parathion	No. of rats	Paraoxon
5[1]	50	1.24 ± 0.06[2]	50	0.39 ± 0.021[2]
20[1]	48	1.65 ± 0.14	47	0.54 ± 0.035[3]
20	50	2.04 ± 0.15	48	0.67 ± 0.020

[1]Pair-fed to the 5% casein group.

[2]Significantly different from 20% pair-fed ($p<0.05$) and 20% *ad libitum* ($p<0.01$).

[3]Significantly different from 20% *ad libitum* ($p<0.05$).

TABLE 3

Mortality expressed as a percent of total deaths[1]

	Malathion			Malaoxon		
Dietary Casein (%)	1st 24 Hours	2nd 24 Hours	3rd 24 Hours	1st 24 Hours	2nd 24 Hours	3rd 24 Hours
5[2]	79.1	20.9	0.0	66.7	23.3	10.0
20[2]	79.4	11.8	8.8	76.7	13.3	10.0
20	59.5	35.1	5.4	76.7	10.0	13.3
	Parathion			Paraoxon		
5[2]	89.7	6.9	3.4	81.0	14.3	4.8
20[2]	94.1	2.9	2.9	79.4	20.6	0.0
20	100.0	0.0	0.0	80.8	19.2	0.0

[1]Deaths were produced by injecting i.p. the pesticide at any of the 5 levels which were used in the LD_{50} determinations.

[2]Pair-fed to the 5% casein group.

The urinary excretion of total p-nitrophenol from rats injected with parathion, paraoxon and p-nitrophenol is presented in Table 4. The treatment of the urine samples with acid before assay allowed the determination of p-nitrophenol which existed in the free and bound forms. The bound forms include parathion, paraoxon, p-nitrophenol sulfate, or other metabolites which contained p-nitrophenol groups.

During the first 12 hours after the injection of the pesticides, parathion and paraoxon, the 20% casein diet excreted the greatest amount of p-nitrophenol as compared with the other groups.

During the first 24 hours after the injection of the same pesticides, the rats fed 20% casein ad libitum appeared to excrete less urinary p-nitrophenol than those fed the 5% casein diet or the 20% casein diet (pair-fed). The urinary excretion of p-nitrophenol from rats injected with p-nitrophenol did not seem to be affected by dietary protein.

TABLE 4

Urinary excretion of p-nitrophenol expressed as a percent of injected compound

Compound injected	Dietary Protein (%)	Time after injection 0-12 hr.	0-24 hr.
Parathion	5[1]	45.91	62.39
	20	60.45	65.34
	20	39.83	49.14
Paraoxon	5[1]	76.76	96.79
	20	91.95	91.95
	20	57.97	72.70
p-Nitrophenol	5[1]	70.07	74.89
	20	75.54	82.56
	20	69.48	73.68

[1]Pair-fed to the 5% casein group.

Discussion

The LD_{50} values for malathion and parathion obtained in this study were lower than those reported by other workers. For malathion, BOYD and TANIKELLA (1969) reported a LD_{50} value (oral) of 1090 mg/kg in male rats fed 3.5% casein diet and of 1401 mg/kg in male rats fed 20% casein diet. GAINES (1960) found an acute oral LD_{50} value of 1375 mg/kg for malathion and 13 mg/kg for parathion, both in male rats.

Although variation in LD_{50} values of malathion existed between those reported in this study and those by BOYD and TANIKELLA (1969), the effect of dietary protein was consistent in both studies. The toxicity of malathion is definitely enhanced by the feeding of low protein diets. Its oxygen analogue, malaoxon, as well as parathion or paraoxon, was similarly found to be more toxic to rats fed the protein deficient diet.

The effect of dietary variables other than protein on the toxicity of the pesticides has been ruled out by restricting the food intake of one group of rats given a 20% casein diet to that of the group fed 5% casein diet. Such pair-feeding insured the uniform intake of calories, vitamins and minerals of these two groups of rats.

The observation that the toxicities of malathion and paraoxon were significantly greater ($p<0.01$ and $p<0.05$, respectively) for rats pair-fed the 20% casein diet than for the animals fed a similar diet ad libitum is difficult to explain. The animals fed ad libitum were ingesting much more food than the pair-fed animals, hence, the variation in LD_{50} between the two groups did not only arise from differences in protein intake but also from total intake of other nutrients. The stress of food restriction imposed on the pair-fed animals could be another factor contributing to their greater susceptibility to pesticide toxicity. Further studies are needed to confirm this possibility.

Since dietary protein level altered the lethal action of both the inactive parent compounds and their oxygen analogues, the effect of the protein is possibly manifested primarily in the detoxication rather than the toxication of the compounds. In contrast, WEATHERHOLTZ et al. (1969) found an effect of dietary protein on the toxication of heptachlor but not on the detoxication of the active metabolite, heptachlor epoxide. However, heptachlor epoxide, unlike the active metabolites of malathion and parathion, is not known to undergo further degradative metabolism.

The results of the studies on the urinary excretion of p-nitrophenol are difficult to reconcile with those obtained in the toxicity studies. For example, the toxicities of parathion and paraoxon were low in the animals fed 20% casein ad libitum but a group similarly fed eliminated less urinary p-nitrophenol as compared to the other groups. These findings are inconsistent since it was expected that a greater, not a smaller, rate of urinary excretion of p-nitrophenol should reflect an increased metabolism and consequently a lowered toxicity of the compounds. Apparently, total urinary p-nitrophenol excretion does not bear any direct relationship with the degree of toxicity of the pesticides. The possibility also exists that metabolic responses to lethal and sublethal doses of the toxicant may be different. Large doses were used in the toxicity studies as compared with sublethal doses used in the urinary excretion studies.

Summary

The toxicities of malathion and parathion and their oxygen analogues were investigated in rats fed different levels of dietary protein. Male weanling rats were fed either a 5 or 20% casein diet for 10 days.

Rats fed 5% casein diets were more susceptible to malathion and malaoxon acute toxicity when compared with rats pair-fed 20% casein diet or with rats fed 20% casein diet ad libitum. Parathion and paraoxon were likewise more toxic to rats fed the 5% casein diet than to those fed the 20% casein diet (pair-fed or fed ad libitum). The urinary excretion of p-nitrophenol as affected by dietary protein was not indicative of the toxicity of parathion and paraoxon.

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