show (Table II) marked differences in the uptake for minerals of the different inbreds of pearl millet. Phosphorus and potassium were the predominant elements in all of them, along with substantial quantities of calcium, iron, zinc, copper, magnesium, and manganese, all of which are necessary for good nutrition in man and animals (Jones, 1977; Tuman and Doisy, 1975). The phosphorus to calcium ratio, however, is poor since it is of the order of 20:1, whereas comparable quantities would be preferable.

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A Novel Antagonistic Effect to the Toxicity in the Rat of *O,O,S*-Trimethyl Phosphorothioate by Its Phosphorothionate Isomer

O,O,S-Trimethyl phosphorothioate (thiolate isomer) has a rat oral LD_{50} value of approximately 15 mg/kg, with death occurring up to 22 days following administration. However, coadministration of the isomeric *O,O,O*-trimethyl phosphorothioate prevents this intoxication. Poisoning by the thiolate isomer, when given at either the 60 or 200 mg/kg level, is completely blocked by 1.0% (w/w) of the antagonist (thionate isomer). At the 60 mg/kg dose, 0.5% of the antagonist gave partial protection, and ~50% of the treated animals recovered. Administration of the antagonist 24 and 48 h after treatment by the thiolate did not affect any reversal of the poisoning symptomology or prevent mortality.

In a previous communication (Mallipudi et al., 1979), we described the delayed toxicity in rats that ensued following single doses of *O,O,S*-trimethyl and *O,O,S*-triethyl phosphorothioates (thiolate isomers). Both compounds are potential impurities in technical grade organophosphorus pesticides and they represent a far greater hazard, particularly for the methyl derivative, than suggested by their 24–48-h acute toxicity mortality date. Subsequent work has revealed an unusual specific antagonism effect to the action of the trimethyl compound, elicited by coadministration of minor amounts of *O,O,O*-trimethyl phosphorothioate (thionate isomer), and we now report our preliminary results defining this observation.

EXPERIMENTAL SECTION

Rat oral LD_{50} data were determined as previously described (Mallipudi et al., 1979) using 95–130-g female albino rats (Sprague-Dawley derived) from Simonsen Laboratories, Gilroy, CA. *O,O,S*-Trimethyl phosphorothioate was synthesized from trimethyl phosphite and methylsulfenyl chloride (Morrison, 1955). Initial product purification was by vacuum distillation, with further cleanup by either preparative TLC [solvent system, hexane–ether (1:1)] or column silica gel chromatography (solvent system, hexane–ether gradient). Structural assignments were verified by NMR and mass spectroscopy and product purity was determined by TLC [solvent system, benzene–ethyl acetate, 1:1; spray reagent, 2,6-dibromoquinone-4-chloroimide (Menn et al., 1957)] and GLC using 2.5% EGSP-Z (Applied Science Laboratories, State College, PA) on Chromosorb 750, 80/100 mesh, surface modified (Aue et al., 1973), and alkali flame ionization detection.

RESULTS AND DISCUSSION

In a continuing investigation of the toxicity of *O,O,S*-trimethyl phosphorothioate to rats, we observed that a sample of the compound which was purified by vacuum distillation could be administered at doses up to the 200 mg/kg level without mortality and symptoms of intoxication (diarrhea, excessive urination, and bleeding), except

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Table I. Toxicological Symptoms in Rats Treated with *O,O,S*-Trimethyl Phosphorothioate Purified by Column Chromatography (A) and Vacuum Distillation (B), and with *O,O,O*-Trimethyl Phosphorothioate Added to Column Purified *O,O,S*-Trimethyl Phosphorothioate

sample and dose	amount of $(CH_3O)_3$-P=S added, %	rat no.	symptoms (*a*, *b*, and *c*) after treatment (days)																									
			1			2			3			4			5			6			7			8–25					
			a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*			
A (60 mg/kg)		1	16		+	24	±	–	31	+	–	34	±	–	37	–	–	40	–	–	42	(died)								
		2	13	+	–	22	–	–	27	+	–	32	±	–	35	–	–	39	(died)										
		3	9	–	+	20	–	+	23	±	–	32	+	–	35	–	–	39	(died)										
		4	13	–	–	26	–	+	31	±	–	34	(died)																
		5	15	–	–	22	±	–	27	–	–	35	–	–	38	±	–	44	±	–	46	(died)								
		6	18	–	+	26	–	±	31	–	–	35	±	–	34	–	–	40	–	–	47	(died)								
B (60 mg/kg)		1	1	–	–	4	–	–	4	–	–	0	–	–	+4	–	–							+37[d]	–	–			
		2	5	–	–	10	–	–	10	–	–	7	–	–	2	–	–							+40[d]	–	–			
		3	2	–	–	10	–	–	2	–	–	+4	–	–				+13	–	–	+17	–	–	+44[e]	–	–			
		4	3	–	–	5	–	–	+3	–	–	+7	–	–				+22	–	–	+27	–	–	+48[e]	–	–			
		5	9	–	–	12	–	–	10	–	–	2	–	–	+1	–	–	5	–	–	4	–	–	+9[e]	–	–			
		6	9	–	–	10	–	–	8	–	–	3	–	–	1	–	–	6	–	–	5	–	–	+3[e]	–	–			
B (200 mg/kg)		1	8	–	–	11	–	–	7	–	–	4	–	–										+19[e]	–	–			
		2	10	–	–	15	–	–	7	–	–	2	–	–										+19[e]	–	–			
A (60 mg/kg)		1	0	–	–	2	–	–	+5	–	–	+10	–	–	+9	–	–	+3	–	–	1	–	–	+10[f]	–	–			
		2	4	–	–	9	–	–	2	–	–	+3	–	–	+6	–	–	+4	–	–	+7	–	–	+21[f]	–	–			
	1.0	3	6	–	–	12	–	–	3	–	–	+4	–	–	+8	–	–	+4	–	–	+8	–	–	+13[g]	–	–			
		4	4	–	–	12	–	–	8	–	–	6	–	–	3	–	–	4	–	–	0	–	–	+11[g]	–	–			
A (200 mg/kg)		1	15	–	±	10	–	–	6	–	–	1	–	–	+3	–	–	+9	–	–	+12	–	–	+30[f]	–	–			
		2	8	–	–	10	–	–	7	–	–	3	–	–	+3	–	–	+9	–	–	+14	–	–	+30[f]	–	–			
	1.0	3	11	–	+	12	–	–	7	–	–	0	–	–	+7	–	–	+3	–	–	1	–	–	8[g]	–	–			
		4	14	–	–	19	–	–	27	–	–	26	–	–	14	–	–	12	–	–	10	–	–	0[g]	–	–			
control (corn oil)		1	+4	–	–	+10	–	–	+16	–	–	+16	–	–	+21	–	–	+25	–	–				+37[h]	–	–			
		2	+9	–	–	+16	–	–	+21	–	–	+22	–	–	+30	–	–	+35	–	–				+55[h]	–	–			

[a] Percent weight loss from initial weight. [b] Diarrhea or urination: ++, heavy; +, observed; –, not observed. [c] Bleeding from mouth, nose or ear: ++, heavy; +, observed; –, not observed. [d] Datum on 21st day. [e] Datum on 12th day. [f] Datum on 11th day. [g] Datum on 13th day. [h] Datum on 9th day.

Table II. Effect of *O,O,O*-Trimethyl Phosphorothioate (II) and *O,O,O*-Trimethyl Phosphate (III) on the Toxicity of *O,O,S*-Trimethyl Phosphorothioate (I) to Rats

dose of I, mg/kg	amount of II or III added, %		no. of rats treated	time (days) of occurrence of death								total rats killed	% killed
				1	2	3	4	5	6	7	8-25		
200			5		2	3						5	100
200	II	0.1	4	1	3							4	100
200	II	0.5	4		4							4	100
200	II	1.0	6									0	0
200	II	3.0	4									0	0
60			6			1		4	1			6	100
60	II	0.1	4				1	1	1			3	75
60	II	0.5	7				2		2			4	57
60	II	1.0	6									0	0
60	II	3.0	8									0	0
60	II	3.0[a]	4		1				2	1		4	100
60	II	3.0[b]	2				1	1				2	100
60	III	1.0	2					2				2	100
60	III	3.0	4				1	2			1[c]	4	100

[a] Dosed orally 24 h after treatment with I. [b] Dosed orally 48 h after treatment with I. [c] Died on the ninth day.

for weight loss (Table I). Subsequent analysis of this sample showed the presence of ~3% of the isomeric *O,O,O*-trimethyl phosphorothioate, the structure being confirmed, after its isolation by column chromatography, from cochromatography with authentic material using TLC and GC methods and by mass spectroscopy.

Following this observation, samples of the thiolate isomer were purified by either column or preparative TLC, varying percentages of the *O,O,O*-trimethyl phosphorothioate added, and the rat toxicity of the mixtures were evaluated. The results of this study are presented in Tables I and II. These date show that the presence of the antagonist blocks the mortality and poisoning symptomology otherwise associated with administration of the thiolate alone. The external appearance and behavior of the treated animals was not visibly different to those of the controls. At doses of either 60 or 200 mg/kg of the toxicant, 1% (w/w) of the antagonist was sufficient to protect the animals from poisoning. Higher doses of the thiolate were not utilized in this study since the distinction between delayed and acute toxicity becomes tenuous. (In the latter case, the poisoning symptoms are also characteristically cholinergic.)

For animals treated with the thiolate at 60 mg/kg, decreasing the level of antagonist below 1% appeared to give partial protection and at 0.5%, half of the treated animals recovered. Administration of 3% of the antagonist 24 and 48 h after treatment by the thiolate did not protect the animals from poisoning symptomology or mortality. Coadministration of up to 3% trimethyl phosphate, the oxon of *O,O,O*-trimethyl phosphorothioate, to the thiolate did not cause any antagonism of the toxicity of the thiolate.

As stated previously (Mallipudi et al., 1979), *O,O,S*-trimethyl phosphorothioate causes significant and prolonged inhibition of rat serum cholinesterase and carboxylesterase enzymes following administration of a single oral dose. While the significance of this extended inhibition is not known, it is interesting to note that when the isomeric phosphorothionate is coadministered (1%) with the phosphorothiolate total recovery of both enzymes is observed over 6 days.

At this time, the antagonism exerted by the *O,O,O*-trimethyl phosphorothioate, as with the delayed toxic effects of the *O,O,S*-trimethyl phosphorothioate, appears to be a highly specific phenomenon. Particularly striking is the low percentage of the antagonist necessary compared to the toxicant. The investigation directed toward a refined definition of both of these delayed toxicity and antagonism effects is continuing.

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