Table I. Comparative Study of the Copper Method (Hill et al., 1967) and the Present Method

sample no.	malathion content: 50% e.c. (w/w)	95% technical grade (w/w)	50% e.c. (w/w)	95% technical grade (w/w)
1	48.7	93.9	48.8	94.0
2	49.3	94.5	49.3	94.4
3	48.6	94.5	48.8	94.5
4	50.5	95.2	50.2	95.1
av	49.3	94.5	49.3	94.5

Table II. Determination of Malathion with Percentage of Relative Error

sample no.	amount of malathion: taken W,[a] g	found, g: 50%	found, g: 95%	relative error, %: 50% sample	relative error, %: 95% sample
1	0.2000	0.1874	0.1934	6.3	3.3
2	0.3000	0.2844	0.2934	5.2	2.2
3	0.4000	0.3824	0.3952	4.4	1.2
4	0.5000	0.4815	0.4973	3.7	0.53
5	0.6000	0.5850	0.5968	2.5	0.52
6	0.7000	0.6888		1.6	
7	0.8000	0.7887	0.7958	1.41	0.52
8	0.9000	0.8874		1.40	
9	1.0000	0.9860		1.40	

[a] W is the weight in gram of malathion taken for analysis.

are needed: (ii) reducing substances do not interfere with the determination; (iii) isomalathion does not interfere. Hence, the bismuth method could be used in the place of the copper method for the determination of malathion in formulations.

The method is applicable only for the determination of malathion in malathion formulations. The results of the present investigation (Table II) also point out that the method fails to yield accurate results when the expected amount of malathion is less than 0.8 g in the case of 50% formulations and 0.5 g in the case of 95% formulations.

Finally, the method may be used to determine other DMDTP-containing compounds.

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Registry No. Malathion, 121-75-5; NaDMDTP, 26377-29-7; dimethyl dithiophosphate·$^1/_3$bismuth(III), 30903-97-0.

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Mutagenicity Assays with (2,4-Dichlorophenoxy)acetic Acid–Amino Acid Conjugates

The mutagenic effects of five amino acid conjugates of (2,4-dichlorophenoxy)acetic acid (alanine, aspartic acid, leucine, methionine, and tryptophan) on five *Salmonella typhimurium* strains (TA97, TA98, TA100, TA1535, and TA1538) were investigated. The tested compounds did not increase the reversions of any of the tester strains' 2-fold over the spontaneous controls nor did they show a dose–response effect and, therefore, were considered to be not direct-acting mutagens under these conditions.

The aspartic acid conjugate of indoleacetic acid (IAA) has been reported in plants for many years (Andreae and Good, 1955). Amino acid conjugates are now recognized as major metabolites of (2,4-dichlorophenoxy)acetic acid (2,4-D) (Feung et al., 1972, 1975), (2,4-dichlorophenoxy)-butyric acid (2,4-DB) (Smith, 1979), and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) (Arjmand et al., 1978). Seven amino acid conjugates were isolated and identified from soybean callus tissue (Feung et al., 1975). The glutamic and aspartic acid conjugates predominated; however, the alanine, valine, leucine, phenylalanine, and tryptophan conjugates were also identified. Feung et al. (1976) have also isolated the glycine, alanine, and valine conjugates of IAA from Boston ivy (*Parthenocissus tricuspidata*) crown gall tissue culture. Glutamic acid and aspartic acid conjugates were isolated as metabolites of 2,4,5-T in soybean callus tissue. In residue studies of greenhouse grown soybean plants sprayed with 0.45 kg/a.i. (active ingredient) propylene glycol butyl ether esters of 2,4-D per 0.4 ha (hectare), the glutamic and aspartic acid conjugates of 2,4-D were found to be present after 4 days at a level of 125 ppm (Zama and Mumma, 1983). These data suggest that amino acid conjugates may be more prevalent than once thought and that we should examine the toxicological

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Table I. Effects of 2,4-D-Amino Acid Conjugates on the Reversion of Various *S. typhimurium* Strains

amino acid conjugates	concn, μg/plate	average number of revertant colonies[a]				
		TA1535	TA100	TA1538	TA97	TA98
spontaneous (control)	0	65 ± 2	188 ± 6	16 ± 1	124 ± 12	41 ± 1
2,4-D-L-alanine	10	89 ± 4	205 ± 5	11 ± 1	100 ± 4	37 ± 1
	100	109 ± 5	200 ± 5	13 ± 1	104 ± 9	32 ± 1
	1000	107 ± 4	190 ± 11	18 ± 2	97 ± 5	37 ± 4
2,4-D-L-aspartic acid	10	95 ± 5	202 ± 9	14 ± 1	97 ± 9	30 ± 3
	100	102 ± 4	195 ± 1	14 ± 1	104 ± 14	33 ± 8
	1000	93 ± 3	179 ± 5	18 ± 0	96 ± 2	28 ± 6
2,4-D-L-leucine	10	85 ± 5	192 ± 11	19 ± 1	97 ± 11	26 ± 6
	100	86 ± 5	181 ± 3	17 ± 4	94 ± 4	32 ± 8
	1000	63 ± 4	200 ± 13	15 ± 1	98 ± 5	26 ± 2
2,4-D-L-methionine	10	106 ± 4	173 ± 5	21 ± 2	103 ± 7	31 ± 3
	100	88 ± 6	192 ± 9	13 ± 1	102 ± 3	35 ± 4
	1000	84 ± 3	184 ± 8	16 ± 2	108 ± 7	27 ± 2
2,4-D-L-tryptophan	10	77 ± 6	199 ± 6	13 ± 1	114 ± 18	26 ± 1
	100	90 ± 4	189 ± 8	14 ± 1	107 ± 5	29 ± 3
	1000	99 ± 3	184 ± 9	16 ± 9	101 ± 9	28 ± 7
standard mutagen[b]		>1000	>2000	257	431	651

[a] Averages of three plates. [b] Methylmethanesulfonate (TA1535, TA100) and 4-nitro-*o*-phenylenediamine (TA1538, TA97, TA98) were used for confirming the reversion properties of the tester strains.

significance of these metabolites.

Since 2,4-D has been widely used to control broad-leaved plants, we have undertaken an investigation of the mutagenic effects of selected amino acid conjugates of 2,4-D on five *Salmonella typhimurium* strains (TA97, TA98, TA100, TA1535, and TA1538). Five amino acid conjugates of 2,4-D (alanine, aspartic acid, leucine, methionine, and tryptophan) were chosen for testing as typical examples, four of which (alanine, aspartic acid, leucine, and tryptophan) have been isolated from plant tissues, and the methionine conjugate is an example of a potential metabolite that has demonstrated high biological activity (Feung et al., 1974, 1977).

EXPERIMENTAL SECTION

The five amino acid conjugates used in this study (2,4-D-L-alanine, 2,4-D-L-aspartic acid, 2,4-D-L-leucine, 2,4-D-L-methionine, and 2,4-D-L-tryptophan) were sythesized (Feung et al., 1974). Prior to testing, all chemicals were dissolved in dimethyl sulfoxide. Five tester strains of *S. typhimurium* were utilized in this study: TA98, TA100, TA1535, and TA1538 (Ames et al., 1975) and the newly developed strain TA97 (Levin et al., 1982). The strains were provided kindly by Bruce N. Ames, university of California, Berkeley, CA. The mutation induction assays were conducted using the standard plate incorporation assay (Ames et al., 1975). Oxoid nutrient broth was used for growing the tester strains (Maron and Ames, 1983). All chemicals were tested at the concentrations of 10, 100, and 1000 μg/plate. Negative controls, as well as positive controls, were included with each assay. The mutagen 4-nitro-*o*-phenylenediamine was used in the spot tests for confirming the reversion properties of the strains TA97, TA98, and TA1538. Methylmethanesulfonate was used for confirming the reversion properties of the strains TA1535 and TA100.

RESULTS AND DISCUSSION

The results of the mutagenicity tests are presented in Table I. None of the five tested amino acid conjugates of 2,4-D increased the reversion of any of the five strains of *Salmonella* 2-fold over the spontaneous controls nor did any of them show any toxic effects. There were no dose–response effects by any of these chemicals. Therefore, it was concluded that the amino acid conjugates of 2,4-D tested in this study, four of which have been shown to be formed by plants, were not direct-acting mutagens in the *Salmonella* strains under the conditions of this test. 2,4-D was also not mutagenic when tested in the *Salmonella* assay (Rashid, 1978).

Registry No. 2,4-D-L-Alanine, 50648-96-9; 2,4-D-L-aspartic acid, 35144-55-9; 2,4-D-L-leucine, 2752-54-7; 2,4-D-L-methionine, 50834-39-4; 2,4-D-L-tryptophan, 50649-06-4.

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