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Mutagenicity of Pesticides Containing 1,3-Dichloropropene[1]

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SUMMARY

In a systematic study of the mutagenic effect of chemical compounds used as pesticides, we found that D. D. soil fumigant and Telone are mutagenic. The test was performed using the bacterial tester strains following the procedure developed by Ames. The active principle of D. D. soil fumigant and Telone is a mixture of the *cis* and *trans* isomers of 1,3-dichloropropene. Both isomers are mutagenic in *Salmonella* strains TA 1535 and TA 100. 2,3-Dichloro-1-propene, a minor component (5%) of the commercial preparation Telone, was also found to be mutagenic in strains TA 1535 and TA 100. Mutagenesis of these tester strains is an indication of a base-pair substitution event causing a missense mutation.

1,3-Dichloropropene is widely used in agriculture all over the world. In Italy 2,187,100 kg were produced in 1972. In California over 1,000,000 kg of 1,3-dichloropropene-containing pesticides were used in 1971.

INTRODUCTION

The papers of Ames (1) and McCann *et al.* (7) have described a simple and sensitive bacterial test for the detection of mutagens. This test measures the number of back mutations induced by mutagens in strains of *Salmonella typhimurium* auxotrophic for histidine. The compound to be tested, the bacterial tester strain, and, when required, a liver microsomal extract are combined directly on a histidine-free agar plate. The number of bacteria reverted back to prototrophy are counted after a 48-hr incubation.

The tester strains have been selected for their sensitivity and specificity in being reverted to prototrophy by a variety of mutagens. Strain TA 1535 detects mutagens causing missense mutations, while TA 1537 and TA 1538 detect frame-shift mutagens. All these strains lack the DNA-excision repair system, and this lack greatly increases their sensitivity to mutagens. Strain TA 1978 has the same mutation as does TA 1538, but it has a normal DNA-excision repair system. More recently (7), McCann et al. have constructed 2 more tester strains, TA 100 and TA 98, which show increased sensitivity to reversion with a variety of potent carcinogens such as the fungal toxin aflatoxin, 7,12-dimethylbenzanthracene, and benzopyrene and which can also be used to detect a variety of carcinogens and mutagens not detected by the other tester strains (7). This mutagenic bacterial test system can be used for screening artificial chemicals present in the environment, e.g., pesticides, food additives, dyes (2), and others.

This bacterial tester system allows the detection of mutagenic events even when their probability is low, since it is performed on a population of 100,000,000 independent genomes. The diffusion of artificial chemicals in our environment is such that millions of individuals are continuously exposed to them. We have used the bacterial tester strains to screen the potential mutagenic property of pesticides used in Italy that were obtained from Ministero della Sanità, Rome, Italy. In this paper we describe the mutagenic properties of 2 widely used pesticides, D. D. soil fumigant and Telone, the active principle of which is a mixture of *cis* and *trans* isomers of 1,3-dichloropropene.

MATERIALS AND METHODS

The bacterial strains and the experimental procedure are those previously described (1, 7). Pesticides were diluted in dimethyl sulfoxide (Carlo Erba Chemical Company, Milan, Italy) as specified under "Results." Allyl chloride and 1,3-dichloro-2-butene were purchased from Aldrich Chemical Company, Milwaukee, Wis. *cis* and *trans* isomers of 1,3-dichloropropene, 1,2-dichloropropane, and 2,3-dichloro-1-propene, as well as the pesticides in their commercial formulas, were given to us by Ministero della Sanità. The data reported in Tables 1 and 2 are obtained from an experiment that was repeated twice and that gave similar results.

RESULTS

Table 1 shows the results of a standard mutagenicity test of the commercial preparation of D. D. soil fumigant and Telone. The mutagenicity in strains TA 1535 and TA 100 is probably a result of a covalent interaction with DNA (1, 7). Mutagenicity in strain TA 1978 increased significantly following treatment with liver microsomal fraction. This shows that probably a metabolite with new mutagenic properties was formed following treatment with liver microsomal fraction.

D. D. soil fumigant is composed of 40% 1,3-dichloropropene, 40% 1,2-dichloropropane, and 20% other unknown chemicals. Telone is composed of 30% *cis*-1,3-dichloropropene, 30% trans-1,3-dichloropropene, 20% 1,2-dichloropropane, 5% 2,3-dichloro-1-propene, 2% allyl chloride, and about 15% unknown compound. We tested the mutagenicity of these compounds separately. The results are shown in Table 2. *cis* and *trans* isomers of 1,3-dichloropropene were strongly mutagenic in strains TA 1535 and TA 100, therefore

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Table 1

Mutagenicity of Telone and D. D. soil fumigant with and without liver microsomal fraction

D. D. soil fumigant or Telone were diluted with dimethyl sulfoxide, and aliquots containing the different amount of the compounds were added to the plates. For the experimental procedure and amount of liver microsomal fraction used see Refs. 1 and 6.

Compound	Amount/plate	No. of mutant colonies/plate with *Salmonella* strains									
		TA 1978		TA 1535		TA 100		TA 1537		TA 98	
		Without	With	Without	With	Without	With	Without	With	Without	With
Telone	0	8	8	17	17	71	71	15	15	22	22
	100 μg	24	115	12	15	178	151	25	23	28	32
	250 μg	36	225	48	59	225	191	31	27	35	31
	1 mg	45	249	75	90	263	242	18	19	27	36
	2.5 mg	53	270	115	135	425	385	34	32	33	39
	5 mg	61	365	150	220	282	500	27	25	24	30
	10 mg	15	150	78	61	192	212	12	35	31	41
D. D. soil fumigant	0	8	8	17	17	71	71	15	15	22	22
	500 μg	11	123	35	42	125	112	28	33	33	30
	5 mg	38	181	45	61	198	250	23	26	28	33
	15 mg	80	300	151	151	350	450	32	24	36	23
	25 mg	75	446	145	150	470	512	19	31	25	27

Table 2

Mutagenicity of dichloropropene and dichloropropane with and without liver microsomal fraction

For experimental procedure see Table 1.

Compound	Amount/plate	No. of mutant colonies/plate with *Salmonella* strains					
		TA 1535		TA 1978		TA 100	
		Without	With	Without	With	Without	With
No compound (control)		19	21	25	28	87	89
cis-1,3-Dichloropropene	20 μg	243	77	19	21	594	731
	50 μg	680	490	90	71	1800	2100
	100 μg	1210	990	119	131	1750	1551
trans-1,3-Dichloropropene	20 μg	235	109	27	31	362	650
	50 μg	430	381	68	75	1750	2200
	100 μg	925	828	115	91	1820	1550
2,3-Dichloro-1-propene	20 μg	190	212	31	51	531	450
	50 μg	650	451	85	97	1520	1091
	100 μg	1080	875	98	81	1900	1355
1,2-Dichloropropane	10 mg	75	81	27	38	220	185
	20 mg	210	185	38	21	480	450
	50 mg	411	312	48	15	850	920

causing base-pair substitutions (missense mutations), and only weakly mutagenic in strain TA 1978. 2,3-Dichloro-1-propene showed similar behavior. 1,2-Dichloropropane was mutagenic in strains TA 1535 and TA 100 but only at concentrations at least 500-fold higher than those of dichloropropene. We tested allyl chloride and 1,3-dichloropropene-2-butene, which showed structural similarity with 1,3-dichloropropene, but they did not show any mutagenic effect (see Chart 1). The mutagenic properties of available purified components do not fully account for the mutagenicity of the commercial preparations of Telone and D. D. soil fumigant; the difference might be due to the presence of the 20% unknown compounds in the latter.

DISCUSSION

1,3-Dichloropropene is the active principle of many widely used pesticides. In 1972 in Italy 2,187,100 kg were produced (Annuario Italiano di Statistica); in the Netherlands it has been used on more than 300 sq km/year to combat potato cyst nematodes (11). The California State Department of Agriculture reported (State Pesticide Use Report 1971) that in 1971 approximately 1,284,841 kg of pesticides containing 1,3-dichloropropene were used in the state and that it could be estimated that 8% (72,576 kg) was lost in the atmosphere (8). The residues of 1,3-dichloropropene in crops are below the limit of determination (10). 1,3-Dichloropropene disappears from the soil at the rate of 2 to 20% per day depending on the composition of the soil (5).

It is difficult to evaluate quantitatively the danger of the contamination of the environment resulting from the use of 1,3-dichloropropene. We must emphasize, however, that due to the nature of the danger (mutagenic effect) even trace amounts in the environment must be considered

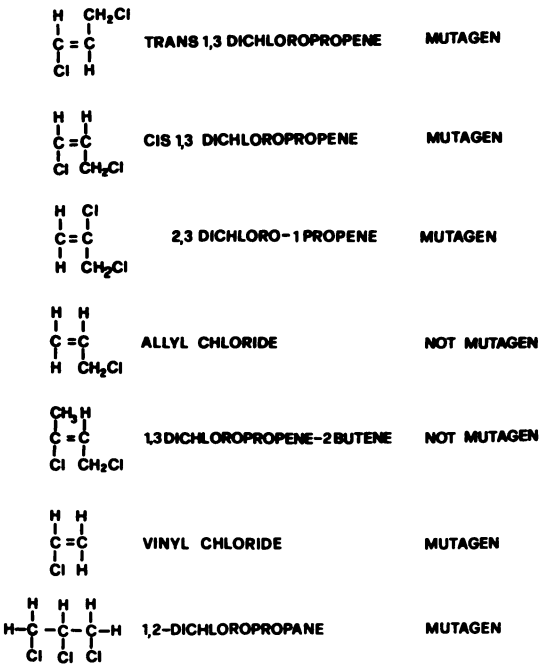

Chart 1.

harmful. These considerations intensify when applied to the safety provisions of the factories producing this compound.

The relationship between mutagenicity and carcinogenicity of chemical compounds has been well documented in recent years. Ames et al. (3) have shown that a great number of well-known chemical carcinogens are mutagens on the bacterial tester strains. The results of our studies on the mutagenic effect of 1,3-dichloropropene and of some related compounds can be compared to the mutagenic and carcinogenic effect of vinyl chloride (see Chart 1 and Refs. 4, 6, 9, and 12). The structural similarity between these compounds suggests a similar mechanism of action. Studies on the carcinogenicity of dichloropropene are now being carried out in this laboratory.

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