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Mutagenic Activity of Thiocarbamate Herbicides in *Salmonella typhimurium*

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Three thiocarbamate herbicides, diallate (*S*-(2,3-dichloroallyl)diisopropylthiocarbamate), triallate (*S*-(2,3,3-trichloroallyl)diisopropylthiocarbamate), and CDEC (2-chloroallyl-*N*,*N*-diethyldithiocarbamate), were evaluated for their ability to induce mutations in four histidine-requiring strains of *Salmonella typhimurium* (TA 100, TA 1535, TA 98, and TA 1538) with and without a rat liver microsomal activation system (Ames test). These herbicides were mutagenic in TA 100 and TA 1535 (base-pair substitution mutants) only in the presence of the liver microsomal preparation, indicating that the chemicals require metabolic activation for their conversion into active mutagens. None of the herbicides caused mutations in strains TA 98 and TA 1538 (frameshift mutants). Diallate was considerably more potent than triallate or CDEC, showing mutagenic activity at 1 μg/plate.

Diallate (*S*-(2,3-dichloroallyl)diisopropylthiocarbamate), triallate (*S*-(2,3,3-trichloroallyl)diisopropylthiocarbamate), and CDEC (2-chloroallyl-*N*,*N*-diethyldithiocarbamate) are used as preemergence herbicides for controlling weeds in certain vegetable and field crops (Weed Science Society of America, 1974). The annual consumption of diallate, triallate, and CDEC in 1975 was reported to be 0.3, 1.3, and 0.1 million pounds, respectively (Stanford Research Institute, 1976). Man may be exposed to these chemicals through occupational exposure or by consuming food containing residues of the chemicals. Although the toxicity and biochemical effects of these herbicides in plants have been reported (Ashton and Crafts, 1973; Fang, 1975), an extremely limited amount of information is available on their carcinogenicity or mutagenicity. Innes et al. (1969) reported that diallate was carcinogenic in mice. In view of the fact that most carcinogens are also mutagens (McCann et al., 1975a; McCann and Ames, 1976), it was of interest to determine whether or not the carcinogenic diallate and the structurally related triallate and CDEC were capable of inducing mutations.

A simple, rapid, sensitive, and economical method for detecting potential mutagens is the microbial mutagenicity assay developed by Ames et al. (1973). Anderson et al. (1972) evaluated 110 herbicides including diallate, triallate, and CDEC for their ability to induce point mutations in eight histidine-requiring strains of *Salmonella typhimurium*. They reported that none of the herbicides induced mutations in the test system. In their studies the bacterial cells were treated with the herbicides in the absence of a liver microsomal activation system. It is now well established that many chemicals are not mutagenic per se but require metabolic activation of the expression of their mutagenic activity (Ames et al., 1973; Ong and Malling, 1975; Malling, 1971). Such chemicals will not be detected by mutagenic screening techniques which use microorganisms to detect genetic damage unless mammalian metabolism is first allowed to act on the chemicals. It is quite possible that the lack of mutagenicity observed in *Salmonella* in the studies by Anderson et al. (1972) may have been due to the fact that the herbicides were not metabolized to the active form by bacterial enzymes. In order to test this possibility, we have investigated the mutagenicity of diallate, triallate, and CDEC by incubating them with *Salmonella* strains in the presence of rat liver microsomes. We show here that these herbicides which are nonmutagenic in *Salmonella* without metabolic activation can be converted to mutagens by a liver microsomal enzyme system.

MATERIALS AND METHODS

Bacterial Strains. The *Salmonella typhimurium* strains (TA 1535, TA 1538, TA 98, TA 100) used for the detection of mutagens were obtained from Dr. Bruce Ames, University of California at Berkeley. All the strains are histidine-dependent and are induced to mutate back to histidine-independence by particular mutagens. These

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Table I. Mutagenic Activity of Diallate, Triallate, and CDEC in *Salmonella typhimurium*

Chemical	Amount, μg/plate	Mutants per plate							
		TA 1535		TA 100		TA 1538		TA 98	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Diallate									
	0	28	25	175	194	15	31	45	52
	1	25	61[a]	189	334	19	27	51	55
	5	22	264[b]	201	658[b]	21	35	47	59
	10	27	556[b]	217	972[b]	15	33	41	68
	50	24	1016[b]	196	1354[b]	23	32	48	67
	100	25	1459[b]	283	2250[b]	20	29	42	87
	500	15	785[b]	146	1952[b]	14	12	11	64
Triallate									
	0	28	25	175	194	15	31	45	52
	10	24	33	190	265	11	21	51	68
	50	33	59[a]	196	480[b]	14	28	47	53
	100	31	89[b]	177	576[b]	14	26	51	47
	500	35	87[b]	196	614[b]	14	19	40	42
	1000	22	72[b]	183	488[b]	15	12	41	46
CDEC									
	0	24	35	211	242	22	31	52	47
	5	26	49	281	278	18	32	36	41
	10	20	69[a]	297	332	16	27	31	47
	50	27	124[b]	272	664[b]	18	33	43	53
	100	20	93[b]	222	545[b]	11	25	28	35
MNNG[c]	2	>1000	>1000	>1000	>1000				
2-AAF[d]	10					21	>1000	29	>1000

[a] Increase of mutation frequency over control is statistically significant at $P \leq 0.05$. [b] Increase of mutation frequency over control is statistically significant at $P \leq 0.01$. [c] *N*-Methyl-*N*-nitro-*N'*-nitrosoguanidine. [d] 2-Acetylaminofluorene.

strains detect different classes of mutagens: TA 1535 and TA 100 revert as a result of base-pair substitution mutations while TA 1538 and TA 98 do so in response to frameshift mutagens. Strains TA 100 and TA 98 are derived from TA 1535 and TA 1538 in which an R factor plasmid has been introduced. The presence of the plasmid enhances the sensitivity of these strains to certain mutagens (McCann et al., 1975b).

Preparation of Liver Microsomal Fraction ("S-9"): The "S-9" fraction was prepared from rat livers as described by Ames et al. (1975). In order to induce microsomal enzymes, 200-g Sprague-Dawley male rats were injected intraperitoneally (500 mg/kg) with Aroclor 1254 dissolved in corn oil. On the fifth day after injection the rats were sacrificed, and the "S-9" liver microsomal fraction was prepared according to Ames et al. (1975) and stored at −80 °C.

Chemicals. Analytical grade diallate, triallate, and CDEC were obtained from the Analytical Chemistry Branch, U.S. Environmental Protection Agency, Research Triangle Park, N.C. The herbicides were dissolved in dimethyl sulfoxide (Me_2SO) in concentrations varying from 0.01–10 mg/mL.

Mutagenesis Assays. Tests for mutagenicity were carried out according to the procedures developed by Ames et al. (1975). To 2 mL of molten top agar at 45 °C were added 0.1 mL of the bacterial tester strain culture ($\sim 10^9$ cells/mL), 0.1 mL of the herbicide solution, and 0.5 mL of "S-9" mix (if required) which was prepared in the prescribed manner (Ames et al., 1975). The control tubes contained 0.1 mL of Me_2SO; this amount of the chemical had no toxic or mutagenic effect on the tester strains. The contents of the tubes containing top agar were mixed and poured on agar plates. The plates were incubated for 48 h at 37 °C and the number of colonies, which represent reverse mutations of the original histidine-dependent to the histidine-independent form, were counted. *N*-Methyl-*N*-nitro-*N'*-nitrosoguanidine and 2-acetylaminofluorene were used as positive controls in each assay. Appropriate controls were included to check the sterility of the microsomal preparation and the test chemicals.

RESULTS AND DISCUSSION

The mutagenic activity of diallate, triallate, and CDEC in four strains of *S. typhimurium*, both in the presence and absence of the liver microsomal "S-9" fraction, is shown in Table I. All the herbicides showed a significant mutagenic activity in strains TA 1535 and TA 100 in the presence of the "S-9" fraction but were found to be inactive in these strains when assayed in the absence of the microsomal preparation. The herbicides failed to induce mutations in strains TA 98 and TA 1538, regardless of the presence of the "S-9" fraction. The mutagenic specificity of diallate, triallate, and CDEC in strains TA 100 and TA 1535 suggests that the herbicides induce mutations of the base-pair substitution type but not of the frameshift type.

Among the three herbicides, diallate was the the most effective in inducing mutations in *S. typhimurium*. The mutagenic activity of diallate in TA 1535 and TA 100 was detectable at concentrations as low as 1 μg/plate. An increase in diallate concentration up to 100 μg/plate increased the reversion frequency. The herbicide at a dose of 100 μg/plate increased the number of revertant colonies 59- and 12-fold in TA 1535 and TA 100, respectively, without a growth inhibition of the background lawn. An approximate linear dose–response was obtained in TA 1535 in the dose range 0–10 μg/plate (dillate or CDEC) and 0–100 μg/plate (triallate). In this strain, diallate, triallate, and CDEC induced 45.6, 0.7, and 3.2 revertants/microgram of the herbicide, respectively. The lower activity of triallate and CDEC compared to diallate may be due to a number of factors, including: (i) a lesser degree of metabolic transformation of the chemicals to active forms; (ii) poor solubility of the herbicides; and/or their metabolites in the agar medium, or (iii) their low permeability with respect to the bacterial cells. In all three herbicides, an increase in herbicide concentration up to a certain level (100, 500, and 50 μg/plate for diallate, triallate, and CDEC, respectively) increased the reversion frequency. At higher concentrations, the number of revertants decreased,

presumably due to toxicity resulting from inactivation of many essential genes on the chromosomes caused by the action of the herbicides (Ames et al., 1973).

The mechanism by which diallate, triallate, and CDEC produce mutagenic effects is not known. Our findings indicate that these herbicides are metabolized by a mammalian enzyme system to a product(s) which can induce mutations in *S. typhimurium*. It has been proposed that one of the metabolites of certain thiocarbamate herbicides in rats is an alkylmercaptan which is formed following hydrolysis of thiocarbamate molecules at the ester linkage (Fang, 1975; Fang et al., 1964; Ong and Fang, 1970). It is possible that the chloroalkylmercaptan moiety formed from the three herbicides may react with the pyrimidine bases in the DNA molecule, thereby inducing mutagenic effects. 5-Bromouracil derivatives have been reported to react with sulfur nucleophiles to give 5,6-addition products (Szabo et al., 1970).

The ability of diallate, Triallate, and CDEC to cause mutations only in the presence of the liver homogenate suggests that these chemicals are promutagens being converted by rat liver metabolic action to ultimate mutagens. A similar activation to ultimate mutagens has been demonstrated for a variety of other chemicals (Ames et al., 1973; Ong and Malling, 1975; Malling, 1971). Since metabolic activation of diallate, triallate, and CDEC appears to be prerequisite for their inducing mutations, our results may explain why these herbicides were found to be nonmutagenic in the studies of Anderson et al. (1972) which were carried out in the absence of a mammalian activation system. Our findings strongly suggest that other herbicides, particularly thiocarbamates that were previously reported to be nonmutagenic in *Salmonella* when tested without metabolic activation (Anderson et al., 1972), should be tested for mutagenicity in the presence of a liver microsomal activation system.

Our findings that diallate has a mutagenic activity of 27 revertants/nanomole in TA 100 makes it comparable in activity to many aromatic amines and polycyclic hydrocarbons (McCann et al., 1975). Such activity may be reflected in the results of the cancer study (Innes et al., 1969) where 13 of 16 mice exposed to diallate developed tumors at the dose (1000 mg/kg) tested, indicating that diallate is quite an active carcinogen and exposure to it could be hazardous.

It has been shown that the ability of a chemical to induce mutations in bacteria in the presence of liver microsomes correlates well with its carcinogenic potential. Our finding that diallate, a known carcinogen, is also a mutagen provides further support to the theory that chemical carcinogens cause cancer through damage to DNA (Miller and Miller, 1971). Because of a high correlation between mutagenicity in bacterial systems and carcinogenicity in animals, it is suggested that triallate and CDEC may also be potentially carcinogenic. However, caution should be used in extrapolating the mutagenic effects observed in bacterial systems to mammals since some mutagens may not be carcinogens (McCann et al., 1975; McCann and Ames, 1976; Rosenkranz and Speck, 1976). A thorough testing of triallate and CDEC in mammalian systems would be required before a definitive evaluation of their carcinogenic potential can be made.

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