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Mutagenicity of Diallate, Sulfallate, and Triallate and Relationship between Structure and Mutagenic Effects of Carbamates Used Widely in Agriculture[1]

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ABSTRACT

In an investigation of the mutagenic properties of 20 carbamate herbicides and fungicides by use of the *Salmonella*/microsome mutagenicity test as developed by Ames *et al.* (Mutation Res., *31:* 347-364, 1975), we have found that three thiocarbamate compounds, diallate, sulfallate and triallate, are mutagenic in the presence of a liver microsomal fraction on strains TA1535 and TA100. This indicates that the metabolic products of these thiocarbamates are causing base-pair substitutions. Since the 2-chloro-allyl group is common to the three mutagenic compounds but is not common to the 17 nonmutagenic compounds, a metabolic derivative of this group is probably responsible for the mutagenic activity.

INTRODUCTION

Carbamates are widely used in agriculture as herbicides and fungicides; they are useful for the control of wild oats in cereals and crop diseases, mainly because of their relatively low acute toxicity and rapid degradation in the soil. However, the presence of a number of herbicides and fungicides in the food chain and in the environment constitutes a potential source of hazard to mammalian species including humans. Because of their extensive use, we have investigated the mutagenic properties of 20 of the most important of these compounds, using the *Salmonella*/microsome mutagenicity test developed by Ames *et al.* (1-3). We find that diallate (*S*-2,3-dichloroallyl diisopropylthiocarbamate), sulfallate (2-chloroallyl diethyldithiocarbamate), and triallate (*S*-2,2,3-trichloroallyl diisopropylthiocarbamate) are positive on strains TA1535 and TA100. The mutagenic activity requires activation with the liver microsomal fraction.

Diallate, sulfallate, and triallate have been used for about 15 years (9), and current production (1971) in the United States of each of these is approximately 500,000 kg/year (7). The European production of diallate is 1,000,000 to 5,000,000 kg/year (9); triallate has been used since 1959 to treat about 750,000 acres of the Canadian prairies annually with doses of 600 to 700 g/acre (16).

The persistence of diallate and triallate depends on soil and environmental conditions. Loss of either chemical from the soil is attributed to microbial degradation, volatilization, and photodecomposition (5, 16, 17). Triallate persists for a much longer time than diallate because of a slightly slower rate of breakdown and a lower volatility. The vapor of diallate may be largely responsible for the control of wild oat seedlings (5, 16, 17), and a residual amount of this compound may occur on or in treated crops after harvesting (9).

In this report, by comparison of the chemical structures (see Chart 2) with the mutagenic effects of the carbamates tested, we present evidence that the 2-chloro-allyl group is responsible for the mutagenicity of diallate, sulfallate, and triallate. Diallate has been shown to be a carcinogen in mice (10), and we discuss the hazards of the use of this compound and of the related thiocarbamates, sulfallate and triallate.

MATERIALS AND METHODS

The *Salmonella typhimurium* strains (TA1535, TA1537, TA1538, TA98, and TA100) used for the detection of mutagens (3) were obtained from Dr. B. N. Ames. The microsomal S-9[3] fraction was prepared from liver homogenates of Sprague-Dawley rats induced with phenobarbital, as described by Ames *et al.* (1), and was stored at −80°.

Technical and commercial formulations of the carbamates as well as diallate, sulfallate, and triallate, purchased from Monsanto Co. (St. Louis, Mo.) and estimated to be at least 96 to 99% pure, were gifts of the Ministero della Sanità, Rome, Italy. The compounds were dissolved in dimethyl sulfoxide from Carlo Erba Chemical Company, Milan, Italy.

The mutagenesis assay on plates was carried out according to the procedures developed by Ames *et al.* (3). The compound to be tested, the bacterial tester strain and, when required, the liver homogenates are added to 2 ml soft agar. After a 48-hr incubation at 37°, the number of *his*$^+$ revertants are determined. The S-9 mix was prepared as reported (3) and contained 0.1 ml S-9 per ml. Appropriate controls were included to check the sterility of the microsomal preparation and the test chemicals. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine and 2-acetylaminofluorene were used as positive controls. The data reported are obtained from experiments that were repeated several times and that gave similar results.

RESULTS AND DISCUSSION

The carbamates listed in Table 1 were evaluated for mutagenic activity with the 5 strains of the *Salmonella* test, in the presence and absence of the S-9 fraction of rat liver.

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[3] The abbreviation used is: S-9, 9000 × *g* supernatant.

Table 1

Response of histidine-requiring mutants of S. typhimurium with and without liver microsomal fraction to carbamate herbicides and fungicides

The carbamates were tested with or without liver microsomal fraction (1, 3). The range of concentrations analyzed was between 10 and 1500 μg/plate on all of the tester strains. No revertant colonies significantly above the spontaneous were observed unless indicated.

		Mutagenicity ± S-9	
Common name	Chemical name	With	Without
Barban	4-Chloro-2-butynyl *m*-chlorocarbanilate	–	–
Carbaryl	1-Naphthyl *N*-methylcarbamate	–	–
Chlorbupham	1-Methylprop-2-ynyl 3-chlorophenylcarbamate	–	–
Chlorpropham	Isopropyl *N*-(3-chlorophenyl)carbamate	–	–
Diallate	*S*-2,3-Dichloroallyl diisopropylthiocarbamate	+	–
EPTC	*S*-Ethyl dipropylthiocarbamate	–	–
Ferbam	Ferric dimethyldithiocarbamate	–	–
Mancozeb	Manganous-zinc ethylenebis(dithiocarbamate)	–	–
Maneb	Manganous ethylenebis(dithiocarbamate)	–	–
Metham	Sodium *N*-methyldithiocarbamate	–	–
Metiram	Zinc ethylenebis(dithiocarbamate)	–	–
Molinate	*S*-Ethyl hexahydro-1*H*-azepine-1-carbothioate	–	–
Nabam	Disodium ethylenebis(dithiocarbamate)	–	–
Phenmedipham	Methyl *m*-hydroxycarbanilate *m*-methylcarbanilate	–	–
Propham	Isopropyl *N*-phenylcarbamate	–	–
Propoxur	2-Isopropoxyphenyl *N*-methylcarbamate	–	–
Sulfallate	2-Chloroallyl diethyldithiocarbamate	+	–
Triallate	*S*-2,2,3-Trichloroallyl diisopropylthiocarbamate	+	–
Zineb	Zinc ethylenebisdithiocarbamate	–	–
Ziram	Zinc dimethyldithiocarbamate	–	–

Only diallate, sulfallate, and triallate showed mutagenic activity.

The results of standard mutagenicity tests of diallate, sulfallate, and triallate in the presence and absence of the liver microsomal S-9 fraction are shown in Chart 1. A very high correlation of mutagenicity of the pure compounds to that of the assayed amount of the 3 herbicides in their commercial formulations was observed. The mutagenic effects are seen only with strains TA1535 and TA100 and require metabolic activation with S-9. The carbamates failed to induce mutations in frame-shift tester strains TA1537, TA1538, and TA98 with or without the S-9 fraction. The mutagenic specificity of diallate, sulfallate, and triallate for strains TA1535 and TA100 indicates that the metabolites of these compounds cause mutations of the base-pair substitution type (3). The lack of mutagenicity in the absence of S-9 agrees with earlier reports of Andersen *et al.* (4), in which 110 carbamates including diallate, sulfallate, and triallate, without activation, did not induce point mutations in 8 histidine-requiring strains of *S. typhimurium*.

Diallate (8 revertants/nmole) was the most effective of the 3 in inducing mutations in *Salmonella*, whereas sulfallate (2.2 revertants/nmole) and triallate (1.5 revertants/nmole) were less active. For all 3 compounds a linear dose-response was observed.

A feature common to diallate, sulfallate, and triallate is the presence of the 2-chloro-allyl group. We believe it is responsible for the mutagenicity of these compounds. It is probable that thiocarbamates are hydrolyzed at the thioester linkage, releasing the alkyl mercaptan as a first step in metabolism (11). It is reasonable to suppose that the 2-chloro-allyl group is further metabolized and could give a reactive intermediate analogous to the mutagenic and carcinogenic species postulated as an intermediate in the metabolism of vinyl chloride (6, 12, 14, 15, 18). In addition, the 2-chloro-allyl group has a chemical structure very similar to compounds related structurally to vinyl chloride, which are known to be mutagenic and carcinogenic, such as vinylidene chloride, which has the dichloroethenyl structure found in triallate (8). The involvement of the 2-chloro-allyl group is best illustrated by EPTC, which lacks this group and is not mutagenic (see Chart 2), although it still shows very strong herbicidal and fungicidal activity. Thus, the Ames test is extremely useful for the identification of the chemical groups that are responsible for the mutagenic activity of a compound and thus should aid in the design of new active compounds.

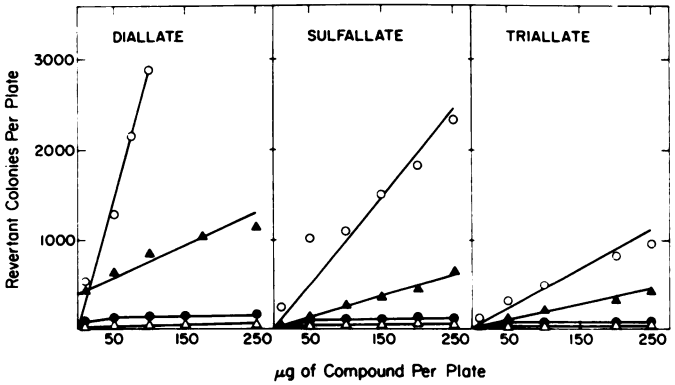

Chart 1. Dose-response curves for diallate, sulfallate, and triallate. Each compound was diluted with dimethyl sulfoxide, and aliquots containing the indicated amount were added directly to the plates or, when needed, were incubated with S-9 and the tester strains (see "Materials and Methods"). The spontaneous revertant colonies (20 to 30 in TA1535 and 150 to 170 in TA100) have been subtracted. At higher concentrations the number of revertants decreased, presumably due to the accumulation of lethal mutations in the bacteria. ○, strain TA100 with S-9; ●, strain TA100 without S-9; ▲, strain TA1535 with S-9; △, strain TA1535 without S-9.

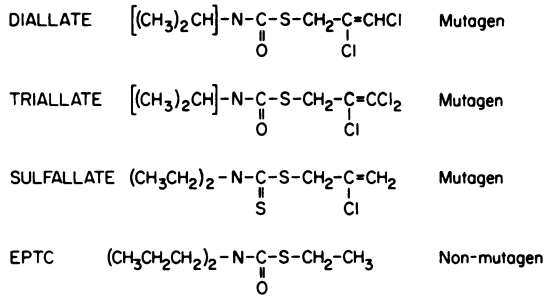

Chart 2. Chemical structures of diallate, sulfallate, triallate, and EPTC.

Pesticides (insecticides, herbicides, fungicides, etc.) are widely used in agriculture and are important in present methods of food production, but they may be toxic to humans and may cause alterations of the genetic material as a result of chronic exposure below toxic levels. This may occur in persons exposed daily to minute amounts of pesticides in their foods. Diallate is a clear example; we have shown that it is a potent mutagen (8 revertants/nmole), comparable to the activity of many carcinogenic aromatic amines and polycyclic hydrocarbons such as β-naphthylamine, bis(2-chloroethyl)amine, dibenz(a,h)anthracene, benz(a)anthracene, and 7,9-dimethylbenz(c)acridine (13). This activity may be reflected in the results of an earlier cancer experiment (10) in which 70% of the mice tested at a dose of 1000 μg/kg developed tumors, indicating that diallate is quite an active carcinogen. Occupational and alimentary exposures to it as well as to its structurally related compounds, sulfallate and triallate, could be hazardous. The finding that the carcinogenic diallate is also a mutagen adds further evidence to the well-known correlation between carcinogenicity and mutagenicity.

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