ppm and no β-naphthol residues were detected at any sampling period.

The use of β-naphthoxyacetic acid as a hormone-type fruit set on tomato plants appears safe considering that there were no significant residues (<0.01 ppm) of both BNOA and β-naphthol as shown by the data currently presented.

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Received for review July 20, 1979. Accepted February 4, 1980.

Mechanism for the Mutagenic Activation of the Herbicide Sulfallate

The carcinogenic herbicide *S*-(2-chloroallyl) diethyldithiocarbamate (sulfallate) is metabolized in vitro to 2-chloroacrolein, a potent and direct-acting mutagen in the *Salmonella typhimurium* assay, using strain TA 100. Although not identified, the proximate mutagen may be formed by hydroxylation of sulfallate or one of its dithio- or thiocarbamate metabolites at the methylene group adjacent to the sulfur.

The *S*-chloroallyl thio- and dithiocarbamate herbicides diallate and sulfallate are converted by liver enzymes to potent mutagens (Carere et al., 1978; De Lorenzo et al., 1978; Schuphan et al., 1979; Sikka and Florczyk, 1978). These herbicides are also carcinogens (Innes et al., 1969; National Cancer Institute, 1978) as is often the case with mutagens (McCann et al., 1975). The ultimate mutagen formed from diallate is probably 2-chloroacrolein, resulting from a sequence of sulfoxidation, rearrangement and elimination reactions (Schuphan and Casida, 1979a,b; Schuphan et al., 1979) (Figure 1). We now provide evidence that 2-chloroacrolein is also likely to be the ultimate mutagen formed on sulfallate metabolism.

MATERIALS AND METHODS

Mutagenesis assays were made with *Salmonella typhimurium* strain TA 100 in the presence or absence of the S-9 mix for microsomal activation (Ames et al., 1975).

Sulfallate was obtained from Chem Service (Westchester, PA) and compound 4 (Figure 2) was obtained from K & K Labs (Plainview, NY). The syntheses of compounds **1–3** were described by Schuphan and Casida (1979a); the synthesis of 2-chloroacrolein may be found in Schuphan and Casida (1979b). Compound **5** was prepared by refluxing 0.1 mol of 2,3-dichloro-1-propene (Aldrich, Milwaukee, WI) with 10% excess sodium sulfite in 100 mL of H_2O for 1 h and following the work-up procedure given by Schuphan et al. (1977) for the preparation of the sodium salt of (2,3,3-trichloro-2-propene)sulfonic acid. NMR (D_2O, DSS) δ 5.56 (s, $=CH_2$), 3.93 (s, $-CH_2$). [*allyl*-^{14}C]Sulfallate (a gift from Monsanto Co., St. Louis, MO) was used as the substrate for the rat liver microsomal mixed-function oxidase system. Isolation and identification of [^{14}C]-2-chloroacrolein was as previously described (Schuphan and Casida, 1979b); this trapping system was probably of relatively low efficiency so the findings are more qualitative than quantitative in nature.

RESULTS

Figure 2 compares the mutagenic potencies of sulfallate and 2-chloroacrolein with those of three sulfallate derivatives (1–3) formed easily on oxidation (Schuphan and Casida, 1979a) and two other potential metabolites (4 and **5**). Except for 2-chloroacrolein, each compound is inactive or requires the S-9 mix for detection or enhanced activity. The mutagenic potency with S-9 mix decreases in the order 2-chloroacrolein ≫ sulfallate ≫ **1** ≫ **2** = **3** > **4** > **5**.

2-Chloroacrolein was established as a microsomal oxidase metabolite of sulfallate by trapping this aldehyde as its 2,4-dinitrophenylhydrazone derivative. The amount recovered was 0.4 and 0.8% (two experiments) with the oxidase cofactor, NADPH, and 0.01% for the control without this cofactor.

DISCUSSION

Two types of evidence strongly indicate that 2-chloroacrolein is the ultimate mutagen formed during sulfallate metabolism. First, it is the only likely metabolite of the chloroallyl moiety with sufficiently high mutagenic activity (Rosen et al., 1980) to be a candidate for the ultimate mutagen. Second, 2-chloroacrolein is liberated from sulfallate in the microsomal oxidase system. It is therefore of interest to consider the nature of the proximate mutagen.

2-Chloroacrolein is easily formed from diallate by sulfoxidation followed by [2,3] sigmatropic rearrangement and 1,2-elimination reactions. However, this sequence is not applicable to sulfallate because it lacks the 3-chloro substituent necessary for the final elimination reaction to form an aldehyde (Schuphan and Casida, 1979a). Sulfallate oxidation and rearrangement forming **1**, **2**, and **3** yields no direct-acting mutagen. Each of these compounds requires metabolic activation for mutagenic activity.

2-Chloroallyl alcohol, a possible intermediate to 2-chloroacrolein, may be formed by several pathways (Figure 2), including hydrolysis of **3** and metabolism of 2-chloroallyl mercaptan. It is difficult to accept metabolic routes via 2-chloroallyl alcohol as major contributors to sulfallate activation since the herbicide is 145 times more potent as a mutagen than this alcohol.

Metabolic activation of sulfallate must occur by a pathway(s) of relatively high efficiency in forming 2-chloroacrolein. This would be the case for α-hydroxylation of sulfallate followed by immediate decomposition of the α-hydroxy compound to 2-chloroacrolein (Figure 2). An α-hydroxy intermediate may also be involved with a variety of other compounds including **1**, **3**, and 2-chloroallyl mercaptan. Previous studies on thiocarbamates have established the importance of α-hydroxylation in aldehyde liberation from the thiol moiety. About 7–17% of the

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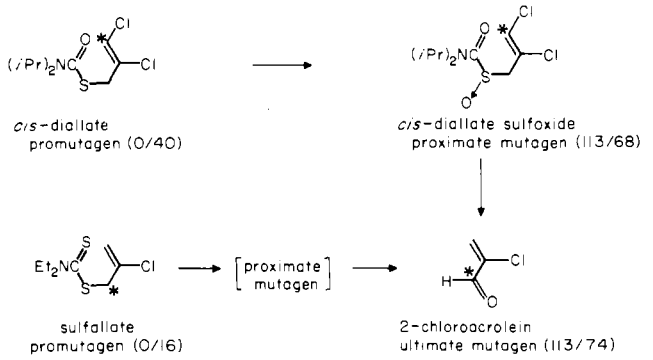

Figure 1. Conversion of the promutagens sulfallate and diallate to the ultimate mutagen 2-chloroacrolein indicating mutagenic activities in the *S. typhimurium* TA 100 assay (revertants/nanomole; without activation/with activation). Asterisks designate carbon atom of herbicide allyl group ending up as carboxaldehyde substituent of 2-chloroacrolein based on mechanistic considerations rather than experimental evidence. Some or all of the diallate sulfoxide decomposes to 2-chloroacrolein under the assay conditions.

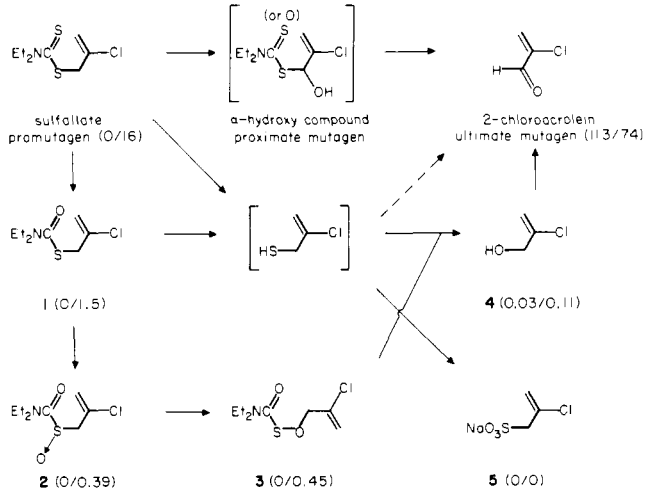

Figure 2. Oxidation and other reactions of sulfallate indicating mutagenic activities of the products in the *S. typhimurium* TA 100 assay (revertants/nanomole; without activation/with activation). Some or all of compound **2** decomposes to **3** under the assay conditions.

overall microsomal oxidase metabolism of *S*-ethyl dipropylthiocarbamate (EPTC herbicide) involves an α-hydroxy intermediate that decomposes to acetaldehyde and carbonyl sulfide (Chen and Casida, 1978). Diallate also probably undergoes α-hydroxylation since carbonyl sulfide is liberated in an amount equivalent to 2–10% of the overall metabolism (Chen et al., 1979).

α-Hydroxylation provides a convenient mechanism to rationalize mutagen formation during sulfallate metabolism. It may also be applicable to the metabolic activation of **1**, diallate, and *S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate (triallate) (revertants/nanomole; 0 without activation/13 with activation), forming 2-chloro-, 2,3-dichloro-, and 2,3,3-trichloroacrolein, respectively, each a highly potent mutagen [>100 revertants/nmol; Rosen et al. (1980)]. The release of 2-chloroacrolein is the only obvious explanation for the mutagenic behavior of sulfallate and **1**, yet on activation sulfallate is 10-fold more mutagenic than compound **1**. The relative importance of α-hydroxylation to sulfoxidation may be less with **1** than with sulfallate based on their ease of peracid oxidation at sulfur; thus, compound **1** is completely converted to its sulfoxide **2** on reaction with equimolar peracid, whereas sulfallate is only partially degraded to other products unless 3 mol of oxidant is used (Schuphan et al., 1980). Diallate yields the most potent mutagen(s), possibly because it gives highly active mono- and dichloroacroleins both on the preferred sulfoxidation–rearrangement–elimination sequence and on α-hydroxylation. The sulfoxidation pathway for triallate gives a weak mutagen, 2-chloroacrylyl chloride, suggesting that α-hydroxylation and trichloroacrolein release may be more significant in mutagen formation. 2-Haloacroleins are highly mutagenic compounds (Rosen et al., 1980) whether formed by sulfoxidation–rearrangement reactions involving dechlorination or by α-hydroxylation without dechlorination.

ACKNOWLEDGMENT

We thank B. N. Ames for generously providing tester strains and E. C. Kimmel for technical assistance.

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Received for review September 17, 1979. Accepted January 21, 1980. Supported in part by the National Institutes of Health (Grant No. 5 P01 ES00049 to J.E.C.), the Deutsche Forschungsgemeinschaft (grant to I.S.), and the Embassy of Israel in Washington (grant to Y.S.).