# Diallate, Triallate, and Sulfallate Herbicides: Identification of Thiocarbamate Sulfoxides, Chloroacroleins, and Chloroallylthiols as Mouse Microsomal Oxidase and Glutathione *S*-Transferase Metabolites

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The major (57–83% yields) mouse microsomal mixed-function oxidase (MFO) metabolites of (*E*)- and (*Z*)-diallate and triallate are identified by HPLC cochromatography as the corresponding sulfoxides. Other MFO metabolites identified by headspace GLC/MS and quantitated by GLC/ECD are 2-chloroacrolein from diallate (9.2% and 1.6% from the *Z* and *E* isomers, respectively) and sulfallate (0.3% yield) and 2,3,3-trichloroacrolein from triallate. Addition of glutathione (GSH) and GSH *S*-transferase (GST) to the MFO system reduces the amounts of sulfoxides and yields new products identified by headspace GLC/ECD and GLC/MS as 2,3-dichloroallylthiol from (*E*)- and (*Z*)-diallate and 2,3,3-trichloroallylthiol from triallate; they also increase by 9-fold the liberation of 2-chloroallylthiol from sulfallate. Thus, formation of mutagenic chloroacroleins involves primarily sulfoxidation of (*E*)- and (*Z*)-diallate followed by [2,3] sigmatropic rearrangement–1,2-elimination reactions and *S*-methylene hydroxylation of triallate and sulfallate and then decomposition of their α-hydroxy intermediates. Competing GST-catalyzed conjugations with GSH divert the sulfoxidized intermediates from activation involving chloroacrolein formation to detoxification on chloroallylthiol liberation.

## INTRODUCTION

*S*-(2,3-Dichloroallyl) diisopropylthiocarbamate or diallate (**1**, an *E*/*Z* mixture) and *S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate or triallate (**2**) are selective herbicides for control of wild oats and other weeds in several crops (Weed Science Society of America, 1989; Worthing, 1987). The related *S*-(2-chloroallyl) diethyldithiocarbamate or sulfallate (**3**) was formerly used as a herbicide. These chloroallyl thiocarbamates are also promutagens in bacterial assays (Carere et al., 1978; De Lorenzo et al., 1978; Sikka and Florczyk, 1978). They undergo metabolic activation on oxidation by cytochrome $P_{450}$ dependent microsomal mixed-function oxidases (MFOs) (microsomes and NADPH) and detoxification by conjugation reactions involving glutathione (GSH) (Chen et al., 1979; Rosen et al., 1980a; Schuphan and Casida, 1979b; Schuphan et al., 1979). Partial proposed metabolic pathways are shown for **1** and **2** in Figure 1 and for **3** in Figure 2.

Peracid oxidation of thiocarbamates **1** and **2** in aprotic solvents yields sulfoxides **4** and **5** which undergo [2,3] sigmatropic rearrangements and 1,2-elimination reactions to form **6** from **1** and **9** from **2** plus the carbamoylsulfenyl chloride in both cases (Schuphan and Casida, 1979a,b; Schuphan et al., 1979). Under comparable conditions dithiocarbamate **3** gives its sulfine derivative with a small amount of desulfuration to the thiolcarbamate (Segall and Casida, 1983). Compounds **4**–**8** are potent bacterial mutagens (Rosen et al., 1980b; Schuphan and Casida, 1979a,b; Schuphan et al., 1979; Segall et al., 1985). It appears that peracid oxidation reactions serve as biomimetic models for mutagen formation from **1** and possibly **2** but not from **3** (Schuphan and Casida, 1979a,b).

The metabolic chemistry of **1**–**3** is partially established from in vitro and in vivo studies. Conversion of **1a** and **1b** to their respective sulfoxides **4a** and **4b** is implied by their efficient conversion in MFO plus GSH systems to *S*-(diisopropylcarbamoyl)GSH and in rats to *S*-(diisopropylcarbamoyl)mercapturic acid (Chen et al., 1979). This conjugation presumably liberates chloroallylsulfenic acids which decompose to **10a** and **10b**, on the basis of analogy with studies on *S*-alkyl thiocarbamates (Casida et al., 1975), but these volatile products were not identified. One volatile mutagenic metabolite of **1**, i.e., 2-chloroacrolein (**6**), is identified by TLC as the 2,4-dinitrophenylhydrazone in 1.6% yield (Schuphan and Casida, 1979a,b). Metabolism of **2** in a microsome plus NADPH system alone or with GSH yields metabolites derived from **8** as an intermediate, indicating activation by *S*-methylene hydroxylation (Hackett et al., 1990; Marsden and Casida, 1982). Dithiocarbamate **3** is metabolized in an MFO system to **6** analyzed as above in 0.6% yield (Rosen et al., 1980a), again suggesting allylic hydroxylation as the source of the mutagen (Rosen et al., 1980a; Schuphan et al., 1979).

The proposed metabolites of **1**–**3** in Figures 1 and 2 are consistent with the findings on mutagenicity. However, rigorous chemical proof requires suitable analytical methods for the reactive intermediates. In the present study they are identified and quantitated by HPLC (sulfoxides **4** and **5**) and GLC headspace analysis (chloroacroleins **6** and **8** and chloroallylthiols **10**–**12**). The quantitative relationships indicate that thiocarbamates **1**–**3** yield chloroacroleins **6** and **8** on metabolic activation with the MFO system alone, whereas the intermediate S-oxidation products are detoxified on diversion to chloroallylthiols **10**–**12** when the GSH/GST system is also present.

## MATERIALS AND METHODS

**Chemicals.** Thiocarbamates **1**–**3** from Chem Services (West Chester, PA) had a stated purity of 98%. Sulfoxides **4a**, **4b**, and **5** were prepared by oxidation of **1a**, **1b**, and **2**, respectively, with *m*-chloroperoxybenzoic acid according to the method of Schuphan and Casida (1979a,b). 2-Chloroacrolein (**6**) was synthesized according to the method of Shostakovskii et al. (1967). 2-Chlo-

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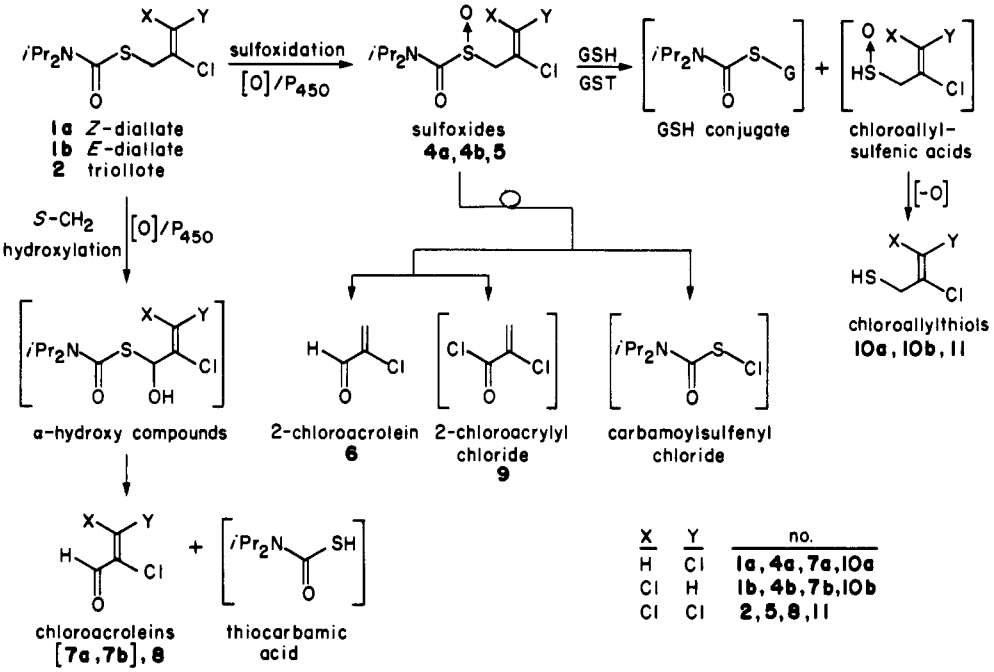


**Figure 1.** Structures and designations for diallate isomers and triallate and their proposed metabolites including sulfoxides, chloroacroleins, and chloroallylthiols. Compounds in brackets are possible intermediates (α-hydroxy compounds and chloroallylsulfenic acids), unstable products (chloroacrylyl and carbamoylsulfenyl chlorides), not observed as metabolites (dichloroacrolein), or lack a chloro substituent and are not examined here (GSH conjugate and thiocarbamic acid).

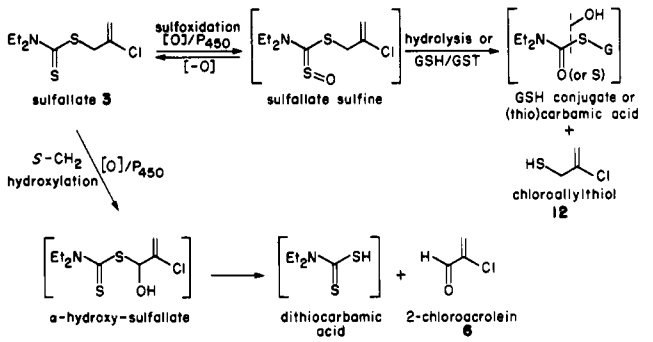


**Figure 2.** Structures and designations for sulfallate and its proposed metabolites including chloroacrolein and chloroallylthiol. Compounds in brackets are possible intermediates (sulfallate sulfine and α-hydroxysulfallate) or lack a chloro substituent and are not examined here (GSH conjugate and dithiocarbamic acid).

roallylthiol (**12**), prepared as described by Schuphan et al. (1977) for the trichloro analogue, gave $^1$H NMR ($CDCl_3$) δ 3.38 (m, 2 H), 5.25 (m, 1 H), and 5.45 (m, 1 H) and MS negative chemical ionization (MS-NCI) $m/z$ 107 (M − 1, 1 Cl) (100%). The dichloro compound **10**, prepared by an analogous procedure as a **10a**/**10b** mixture (separable on the DB-5 GLC column below), gave for each isomer MS-NCI $m/z$ 141 (M − 1, 2 Cl) (100%). All solvents used were of a purity suitable for trace analysis.

**HPLC.** HPLC was performed on a dual pump gradient system monitoring the eluate at 218 nm. **1a** and **1b** were separated in 1-mg aliquots of 1 on a 5-$NO_2$ column (250 mm × 10 mm) (Macherey & Nagel, Düren, Germany) with 0.5% dioxane in hexane. The purities of **1a** and **1b** based on GLC were 96% and 99%, respectively. Sulfoxides **4a**, **4b**, and **5** were analyzed on a RP-18 LiChroCART column (125 mm × 4 mm, particle size 5 μm) with a RP-18 precolumn (4 mm × 4 mm) (Merck, Darmstadt, Germany) and a flow rate of 0.85 mL/min. Sulfoxides **4a** and **4b** and 2-chloroacrolein (**6**) were eluted with 45% acetonitrile in water (Rt = 6.6, 5.4, and 4.0 min, respectively), and sulfoxide **5** was eluted with 55% acetonitrile in water (Rt = 5.9 min) followed by a gradient to 80% acetonitrile in water to separate and elute **1a** and **1b**. The sulfoxides were quantitated by calibration with external standards.

**GLC.** Analysis of the thiocarbamates involved a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm) (J&W Scientific, Folsom, CA) with 1-min splitless injection at 240 °C, hydrogen at 51 cm/s linear velocity (at 300 °C oven temperature) as the carrier gas, and the following temperature program: 150 °C for 3 min and then 6 °C/min to 200 °C. A FID was used at 300 °C with helium as the makeup gas. Rt values for **1a**, **1b**, **2**, and **3** were 7.1, 6.9, 8.6, and 6.9 min, respectively.

Headspace GLC for detection or quantitation of volatile metabolites used a DB-1 fused silica column (15 m × 0.53 mm, film thickness 3.0 μm) with 0.5-min splitless injection at 150 °C, helium at 33 cm/s (at 140 °C) as the carrier gas, and the following temperature program: 20 °C for 0.5 min, 20 °C/min to 30 °C, 15 °C/min to 75 °C, 20 °C/min to 140 °C, and then hold for 2 min. An ECD was operated at 300 °C with argon–methane (95:5) as the makeup gas. The concentrations of **6** and **12** were determined from calibration curves involving equilibration of authentic standards for 1 min in 1 mL of pH 7.4 100 mM phosphate buffer in 5-mL reaction vials described below.

Headspace GLC for identification of volatile metabolites employed a DB-1 fused silica column (30 m × 0.32 mm, film thickness 3.0 μm) with 1-min splitless injection at 150 °C, helium as the carrier gas at 31 cm/s (at 200 °C), and the following temperature program: 35 °C for 1 min, 10 °C/min to 80 °C, 20 °C/min to 200 °C, and then hold for 5 min. Methane at 1.0 Torr was used as the reagent gas for NCI. The transfer line to the Hewlett-Packard 5985 MS system was set at 100 °C and the source at 130 °C. Authentic standards and metabolites were examined in the full-scan mode and the metabolites also with single ion monitoring (SIM) to confirm the diagnostic ions and isotope ratios.

**Microsomal MFO and GSH *S*-Transferase (GST) Assays.** Liver microsomes from male albino Swiss-Webster mice were prepared according to the method of Johnston et al. (1989) and washed with 100 mM, pH 7.4, phosphate buffer; their protein content was determined (Bradford, 1976), and the microsomes were stored frozen at −80 °C. Each substrate (50 nmol of **1a**, **1b**, or **3**; 25 nmol of **2**; 10 nmol of **6**) added in ethanol (1–3 μL) was incubated with microsomal protein (0 or 1 mg), NADPH (0 or 1.3 μmol), GSH (0 or 1 μmol), and GST (equine liver lyophilized powder, Sigma Chemical Co., St. Louis, MO) (0 or 75 μg of protein) in 1 mL of 100 mM, pH 7.4, phosphate buffer for 30 min at 37 °C. Each reaction was carried out in a 5.0-mL Reacti-Vial (Pierce, Rockford, IL) sealed with a Teflon/silicone septum. At 1, 15, and 30 min, 1.0 mL of air was introduced, and 1.0 mL of headspace gas and 20 μL of solution were withdrawn through the

**Table I. Effects of NADPH, GSH, and GST on the Mouse Microsomal Conversion of Thiocarbamates to Thiocarbamate Sulfoxides and 2-Chloroacrolein**

| additions to microsomes[a] | min | substrate:[b] product: | sulfoxide,[c] % 1a 4a | sulfoxide,[c] % 1b 4b | sulfoxide,[c] % 2 5 | chloroacrolein,[d] % 1a 6 | chloroacrolein,[d] % 1b 6 |
|---|---|---|---|---|---|---|---|
| none | 1 | | <2, - | <2, - | <2, - | 1.0, - | <0.02, - |
| | 15 | | <2, - | <2, - | <2, - | 0.9, - | 0.06, - |
| | 30 | | <2, - | <2, - | <2, - | 0.8, - | 0.02, - |
| NADPH | 1 | | 42, - | 28, - | 40, 24[e] | 2.0, - | 0.1, 0.5[e] |
| | 15 | | 94, - | 76, 92[e] | 44, 72 | 6.4, - | 0.7, 0.8 |
| | 30 | | 68, 86 | 66, 84 | 36, 76 | 7.4, 11 | 2.0, 1.1 |
| NADPH + GSH | 1 | | 30, 30 | 30, 32 | 32, 40 | 0.9, 1.2 | 0.2, 0.4 |
| | 15 | | 60, 72 | 102, 98 | 24, 32 | 0.9, - | 0.2, 0.4 |
| | 30 | | 20, 86 | 60, 52 | 4, 4 | 1.7, 1.3 | 0.6, 0.4 |
| NADPH + GSH + GST | 1 | | 24, 26 | 46, - | 20, 24 | 0.8, 1.1 | 0.06, 0.3 |
| | 15 | | 56, 64 | 64, 78 | 20, 40 | 3.0, 1.5 | 1.6, 1.4 |
| | 30 | | 10, 20 | 62, 38 | 4, 12 | 1.0, 1.1 | 1.2, 0.5 |

[a] Mouse liver microsomes (1 mg of protein) in pH 7.4 100 mM phosphate buffer (1.0 mL) alone or with NADPH (1.3 μmol), GSH (1 μmol), or GST (75 μg of protein). [b] Substrates **1a** and **1b** at 50 nmol/mL and **2** at 25 nmol/mL. These substrate levels are completely metabolized in 30 min with microsomes and NADPH, microsomes and NADPH + GSH or microsomes and NADPH + GSH + GST. [c] HPLC analysis. [d] GLC/ECD headspace analysis. Corrected for blank values of 0.6–1.4%. [e] Results of duplicate incubation mixtures.

septum for GLC analysis of chloroacroleins and chloroallylthiols and HPLC analysis of sulfoxides, respectively. In studies with **6** only the headspace was analyzed. At the end of the 30-min incubation period, the reaction mixtures were treated sequentially with ice-cold ethyl acetate (1 mL), internal standard (50 nmol in 10 μL of ethanol) and NaCl (100 mg) followed by mixing with a vortex for 30 s, phase separation by centrifugation, and GLC analysis for content of unmetabolized substrate.

## RESULTS

**Analytical Methods.** Sulfoxides **4a**, **4b**, and **5** were readily analyzed with high sensitivity on a reversed-phase HPLC column with an acetonitrile–water gradient and UV monitoring at 218 nm. Although **6** was resolved from **4a** and **4b**, this method was not useful for analysis of this compound when formed in the microsomal reactions because its peak was overlapped by that of interfering endogenous compounds. With suitable modifications, this HPLC method could also be applied to analysis of **1a**, **1b**, **2**, and **3**. Chloroacroleins **6** and **8** and chloroallylthiols **10–12** were conveniently determined with little or no interference by headspace GLC/ECD analysis.

**Sulfoxides as Metabolites of Thiocarbamates (Table I).** Thiocarbamates **1a**, **1b**, and **2** were not metabolized by microsomes alone but were completely degraded within 30 min by microsomes and NADPH. Sulfoxides **4a**, **4b**, and **5** were identified by HPLC cochromatography as major metabolities in incubation mixtures of **1a**, **1b**, and **2**, respectively, with microsomes and NADPH. These sulfoxides were evident even after only 1 min of incubation, and their yields averaged 83%, 80%, and 57% at 15–30 min from **1a**, **1b**, and **2**, respectively. The combination of GSH and GST decreased the amount of sulfoxide recovery with the greatest effect on **5** and **4a** and the least on **4b**.

**Kinetics of Decomposition of Thiocarbamate Sulfoxides (Table II).** The levels of sulfoxides noted above for the MFO system reflect the balance between their rates of formation and decomposition. Accordingly, HPLC analysis was used to establish the rates at which **4a**, **4b**, and **5** decomposed in pH 7.4 buffer or buffer containing GSH, GST, GSH plus GST, or GSH plus mouse liver cytosol. The relative stability of the sulfoxides in pH 7.4 buffer was **4a** < **5** < **4b** with half-life values of 1.0, 3.5, and 6.2 h, respectively. The decomposition rate in each case was not greatly affected by addition of either GSH or GST. However, addition of the combination of GSH with GST or mouse liver cytosol greatly accelerated the decomposition, with **4b** always reacting more slowly than **4a** or **5**.

**Table II. Effects of GSH, GST, and Mouse Liver Cytosol on the Rates of Decomposition of Thiocarbamate Sulfoxides**

| additions[a] | half-life, h ± SE[b] 4a | 4b | 5 |
|---|---|---|---|
| none | 1.0 ± 0.02 | 6.2 ± 0.3 | 3.5 ± 0.3 |
| GSH | 0.9 ± 0.01 | 4.4 ± 0.1 | 2.2 ± 0.1 |
| GST | 0.7 ± 0.05 | 6.3 ± 0.6 | 3.4 ± 0.3 |
| GSH + GST | 0.4 ± 0.01 | 1.1 ± 0.1 | 1.0 ± 0.1 |
| GSH + cytosol 1 mg | 0.8 ± 0.06 | 1.8 ± 0.1 | 0.9 ± 0.1 |
| GSH + cytosol 10 mg | 0.3 ± 0.01 | 0.8 ± 0.2 | 0.3 ± 0.06 |

[a] pH 7.4 100 mM phosphate buffer alone (1.0 mL) or with GSH (1 μmol), GST (75 μg of protein), or cytosol (1 or 10 mg of protein). [b] Linear regression from four or more data points between 0.5 and 8 h.

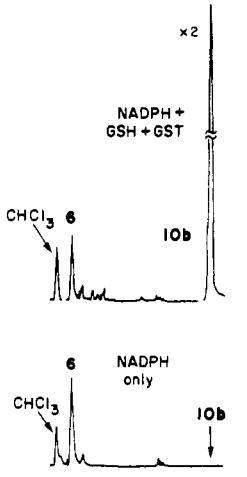


**Figure 3.** Partial GC/ECD headspace chromatograms showing metabolites of (*E*)-diallate including chloroacrolein **6** with mouse microsomes and NADPH only and chloroallylthiol **10b** with microsomes and NADPH plus GSH and GST. Rt values are 2.84 min for **6** and 6.39 min for **10b** compared with 2.45 min for chloroform as a reference.

**Chloroacroleins as Metabolites of Thiocarbamates.** 2-Chloroacrolein (**6**) was identified as a metabolite of **1a**, **1b** (Figure 3), and **3**, and trichloroacrolein **8** as a metabolite of **2** by headspace analyses involving GLC Rt values and GC/MS (Table III). No GLC peak was evident by NCI-SIM in the region appropriate for **7** with **1a** or **1b** as the substrate or for **9** with **2** as the starting material. The

**Table III. GLC and MS Characterization of Chloroacroleins and Chloroallylthiols as Metabolites of Thiocarbamates**

| | chloroacroleins[a] | | chloroallylthiols | | |
|---|---|---|---|---|---|
| parameter | 6 ($Cl_1$) | 8 ($Cl_3$) | 12 ($Cl_1$) | 10 ($Cl_2$) | 11 ($Cl_3$) |
| substrate | **1a**, **1b**, **3** | **2** | **3** | **1a**, **1b** | **2** |
| GLC Rt, min[b] | 2.84 | 6.49 | 4.56 | 6.39[c] | 7.90 |
| MS, NCI-SIM[d] | | | | | |
| $m/z$ (100%) | 90 (1 Cl) | 122 (1 Cl) | 107 (1 Cl) | 141 (2 Cl) | 104 (1 Cl) |
| fragment | $M^-$ | M − HCl | M − 1 | M − 1 | M − 2HCl |

[a] Dichloroacrolein **7** is not detected from **1a** or **1b**. [b] Chloroform as the reference gives Rt = 2.45 min. [c] **10a** and **10b** not resolved. [d] Other diagnostic ions are as follows: for **8** $m/z$ 158 ($M^-$) (0.2%); for **11** $m/z$ 140 (M − HCl) (2%), and for **12** $m/z$ 108 ($M^-$) (8%).

yield of **6** at 30 min was 9.2% from **1a** and 1.6% from **1b** with NADPH compared with 0.8% and 0.02%, respectively, without this cofactor (Table I). The presence of GSH or GSH plus GST substantially reduced the amount of **6** from **1a** or **1b**. The yield of **6** from **3** at 30 min was up to 0.3% for microsomes with NADPH and <0.02% without NADPH.

Chloroacrolein (**6**) quickly decomposed with a half-life of ~15 min in pH 7.4 buffer or microsomal assays independent of NADPH fortification. When GSH or GSH plus GST was also present, **6** quickly fell to background levels and quantitation could not be achieved after 15 min. Thus, the yield of **6** reflected a balance between rapid rates of both formation and decomposition.

**Chloroallylthiols as Metabolites of Thiocarbamates.** Chloroallylthiols **10a** or **10b** and **12** were identified as metabolites of **1a** or **1b** (Figure 3) and **3**, respectively, by headspace analysis involving GLC Rt values and GLC/MS in comparison with standard compounds (Table III). The trichloro analogue **11** was tentatively characterized as a metabolite of **2** by headspace GLC/MS (Table III). The MFO system must be fortified with GSH or GSH plus GST for any detectable conversion of **1a** and **1b** to **10** and **2** to **11**; the higher yields were always obtained with GSH plus GST. Although not quantitated, the yield of **10** at 1, 15, and 30 min was severalfold higher from **1b** than from **1a**. With **3** as the substrate, the yield of **12** was maximal after 15 min at 6% with the MFO system, 36% on addition of GSH, and 54% with GSH plus GST. No other compounds derived from **3** were detected in incubations fortified with GSH or GSH plus GST.

## DISCUSSION

The adverse toxicological properties of the *S*-chloroallyl thiocarbamates are probably due in the most part to their chloroacrolein metabolites. It is therefore important to understand the pathways and processes controlling the formation and levels of these chloroacroleins. Their formation is initiated by MFO oxidations at sulfur or the *S*-methylene carbon. Detoxification involves GSH-mediated reactions of the sulfoxides and possibly the sulfine (Figures 1 and 2) or of the chloroacroleins.

**1a** and **1b** are converted in the MFO system to **4a** and **4b** in yields of 83% and 80%, respectively, at 15–30 min based on HPLC analysis and to **6** in yields of 9.2% and 1.6%, respectively, at 30 min based on headspace GLC analysis (Figure 1). The latter procedure underestimates the yields of **6** from **1a** and **1b** compared with mutagenicity as a bioassay for the conversion efficiency, i.e., 35% from **1a** and 22% from **1b** (Schuphan et al., 1979). It appears that a higher proportion of the **6** formed reaches the *Salmonella* within the time span of the mutagenicity assay than ultimately arrives at the GLC detector or, less likely, that other metabolites also contribute to the mutagenicity. Sulfoxide **4a** is generally more reactive than **4b** with respect to formation of **6** in nonbiological media (Schuphan and Casida, 1979a,b), hydrolysis in pH 7.4 buffer, and decomposition in the GSH plus GST system. On the other hand, **4b** yields more **10** than **4a** possibly because of its greater stability to the rearrangement–elimination reactions. Coupling of **6** and **8** with GSH involves Michael addition (Hackett et al., 1990; Segall et al., 1985).

**2** is converted to **5** in 57% yield at 15–30 min by the MFO system, and **5** in turn presumably yields **9** (Figure 1) which, although not detected, would hydrolyze to chloroacrylic acid, a known metabolite of **2** (Marsden and Casida, 1982). The primary route for mutagen formation appears to be the conversion of **2** to **8** by *S*-methylene hydroxylation (Hackett et al., 1990; Marsden and Casida, 1982) rather than a reaction sequence initiated by sulfoxidation. The detoxifying activity of GSH is therefore primarily via direct reaction with **8** (Hackett et al., 1990) rather than with **5**.

**3** as a dithiocarbamate is a special case (Figure 2). The thiono sulfur is more sensitive than the thiolo sulfur to peracid oxidation, forming the sulfine (Segall and Casida, 1983). Sulfoxidation of sulfallate or the corresponding thiolcarbamate would not lead to **6** because this requires a 3-chloro substituent (Schuphan and Casida, 1979a). NADPH-dependent formation of **6** must therefore be via the $\alpha$-hydroxy compound. Liberation of **12** in high yield requires fortification of the MFO system with GSH and GST, suggesting that the sulfine serves as a carbamoylating agent for GSH.

The standard mouse liver system examined here has a particular balance of oxidative activation (microsomes and NADPH) and conjugative detoxification (GST and GSH) which may differ from that of other in vitro or in vivo systems. However, the findings in the present in vitro studies are consistent with those established for **1a** and **1b** in rats (Chen et al., 1979) and demonstrate for the first time the formation of thiocarbamate sulfoxides and chloroallylthiols as reactive intermediates on in vitro metabolism of **1–3** and the preferred reaction pathways for liberation of chloroacroleins.

## ABBREVIATIONS USED

GSH, glutathione; GST, glutathione *S*-transferase; MFO, mixed-function oxidase; NCI, negative chemical ionization; SIM, single ion monitoring; Et, ethyl; *i*Pr, isopropyl.

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