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INHIBITION OF RAT LIVER LOW K_m ALDEHYDE DEHYDROGENASE BY THIOCARBAMATE HERBICIDES

OCCUPATIONAL IMPLICATIONS

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Abstract—*S*-Methyl *N*,*N*-diethylthiolcarbamate (DETC-Me) is a metabolite formed during the bioactivation of disulfiram. The formation of its corresponding sulfoxide, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide (DETC-MeSO), from DETC-Me is required for low K_m mitochondrial aldehyde dehydrogenase ($ALDH_2$, EC 1.2.1.3) inhibition. DETC-Me is similar in structure to thiocarbamate herbicides with the general structure R1R2NC(O)SR3. Representative herbicides studied were *n*-propyl, *n*-propylthiocarbamate ethyl ester (EPTC), molinate, vernolate, ethiolate and butylate. All of these thiocarbamate herbicides inhibited rat liver $ALDH_2$ *in vivo*. The dose of these thiocarbamates that inhibited rat liver $ALDH_2$ by 50% (ID_{50}) when administered 8 hr before determination of $ALDH_2$, was found to be 5.2, 3.1, 1.6, 12, and 174 mg/kg, respectively. These thiocarbamates were ineffective rat liver $ALDH_2$ inhibitors *in vitro*, unless rat liver microsomes and an NADPH-generating system were added to the incubation. The respective thiocarbamate sulfoxides were formed when the thiocarbamates were incubated with liver microsomes and an NADPH-generating system. The thiocarbamate sulfoxides all inhibited rat liver $ALDH_2$ *in vitro*. An equimolar dose of molinate and molinate sulfoxide inhibited rat liver $ALDH_2$ *in vivo* to the same degree. Molinate-treated rats challenged with ethanol exhibited a disulfiram-like ethanol reaction. In conclusion, thiocarbamate herbicides inhibit $ALDH_2$, probably due to the formation of their sulfoxide, and therefore have the potential to produce a disulfiram-like ethanol reaction in an unsuspecting population.

Key words: aldehyde dehydrogenase; thiocarbamate herbicides; sulfoxide; disulfiram; ethanol; bioactivation

Disulfiram is used in the treatment of alcohol abuse, with inhibition of liver $ALDH_2$‡ (EC 1.2.1.3) by disulfiram being the proposed mechanism for its deterrent action [1]. It has been recognized only recently that disulfiram must be bioactivated in order for rat liver $ALDH_2$ to be inhibited *in vivo* [2–7]. The putative metabolite of disulfiram responsible for rat liver $ALDH_2$ inhibition has been identified as DETC-MeSO [8]. The immediate precursor to DETC-MeSO in rats is DETC-Me, and it, too, is a potent *in vivo* liver $ALDH_2$ inhibitor [9, 10], but is ineffective *in vitro* [11].

DETC-Me is structurally similar to thiocarbamate herbicides, having the general structure R1R2NC(O)SR3. Examples include EPTC, molinate, vernolate, butylate and ethiolate (Fig. 1). These herbicides are used for weed control in a number of crops including corn, rice and tomatoes [12], and their metabolism has been studied previously [13, 14]. A common metabolite of the thiocarbamate herbicides in both mammals and plants is their respective sulfoxide [15]. The sulfoxides are more reactive than their parent thiocarbamates, and are thought to be the chemical entity responsible for herbicidal activity [16, 17].

Because of the similarity in chemical structure between DETC-Me and these thiocarbamate herbicides, preliminary studies were carried out in rats to investigate their $ALDH_2$ inhibitory properties. In these studies, molinate was found to be a potent *in vivo* rat liver $ALDH_2$ inhibitor [18], as were EPTC, ethiolate, butylate, and vernolate. Furthermore, molinate-treated rats exhibited a disulfiram-like ethanol reaction when challenged with ethanol [19]. In the present studies, the inhibition of rat liver $ALDH_2$ by the herbicides molinate, EPTC, ethiolate, vernolate and butylate was examined in greater detail. In addition, the metabolism of these herbicides to their respective sulfoxides, employing a microsomal activating system, was investigated, and

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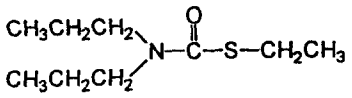
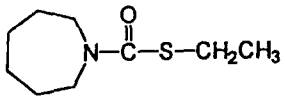
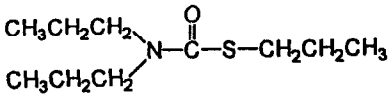
‡ Abbreviations: $ALDH_2$, mitochondrial low K_m aldehyde dehydrogenase; DETC-Me, *S*-methyl *N*,*N*-diethylthiolcarbamate; DETC-MeSO, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide; MCPBA, *m*-chloroperoxybenzoic acid; MPLC, medium pressure liquid chromatography; MAP, mean arterial pressure; EPTC, *n*-propyl, *n*-propylthiocarbamate ethyl ester; DDTC, diethyldithiocarbamate; and DDTC-Me, diethyldithiocarbamate methyl ester.



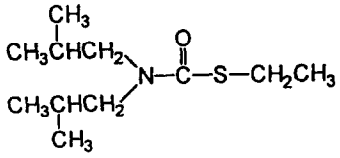
EPTC



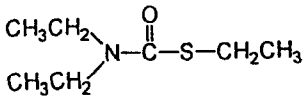
molinate



vernolate



butylate



ethiolate

Fig. 1. Thiocarbamate herbicides used in this study were: *n*-propyl, *n*-propylthiocarbamate ethyl ester (EPTC); homopiperidylthiocarbamate ethyl ester (molinate); *n*-propyl, *n*-propylthiocarbamate *n*-propyl ester (vernolate); iso-butyl, isobutylthiocarbamate ethyl ester (butylate); and diethylthiocarbamate ethyl ester (ethiolate).

the rat liver $ALDH_2$ inhibitory property of the respective sulfoxides was examined.

MATERIALS AND METHODS

Animals

Sprague–Dawley derived male rats (250–350 g) were maintained on a 12-hr light–dark cycle in the Animal Care Unit of The University of Kansas. Rats had access to food and water *ad lib* until the night before an experiment, at which time food was removed.

Chemicals

Technical grade (purity > 96%) molinate, EPTC, butylate and vernolate were provided by Dr. Thomas Castles of the Stauffer Chemical Co., Richmond, CA. Ethiolate (99% purity) was purchased from the Crescent Chemical Co., Hauppauge, NY. The thiocarbamate sulfoxides of EPTC, molinate, vernolate and butylate were synthesized by MCPBA oxidation as described by Casida *et al.* [17]. The crude product was purified by reverse-phase MPLC on a solid support of 40 μm Bondesil™ C18-bonded phase silica and eluted with a mobile phase of 50:50 acetonitrile:water at a flow rate of 8 mL/min. The mobile phase was changed to 60:40 acetonitrile:water for butylate sulfoxide. The MPLC fractions containing the various thiocarbamate sulfoxides were extracted with methylene chloride and dried with sodium sulfate, and the solvent was removed under reduced pressure. Ethiolate sulfoxide was synthesized by an alternative method using sodium metaperiodate (Sigma Chemical Co., St. Louis, MO). Ethiolate was dissolved in methanol, and sodium metaperiodate (1.05 molar equivalent) was added in an equal volume of water. The reaction mixture was stirred overnight, and then extracted with methylene chloride. The organic phase was dried with sodium sulfate and concentrated under reduced pressure. Purification of ethiolate sulfoxide was carried out as described above, with an MPLC mobile phase of 40:60 acetonitrile:water. The purity of the sulfoxides was greater than 98% as determined by HPLC, and the structures were confirmed by ^{1}H-NMR and chemical-ionization mass spectrometry.

Drug administration

For all *in vivo* rat liver $ALDH_2$ inhibition studies, the various doses of the thiocarbamate herbicides studied were administered i.p. in corn oil (1 mL/kg). Control rats were treated with 1 mL/kg corn oil, i.p. Molinate sulfoxide was administered i.p. at a dose of 3.2 mg/kg (17.1 μmol/kg) in polyethylene glycol (PEG) 200 (1 mL/kg). Control rats in the molinate sulfoxide studies were treated with vehicle only (1 mL/kg PEG 200), i.p.

Rat liver $ALDH_2$ dose–response and time-course studies

In vivo. In the dose–response studies, rats were treated i.p. with various doses of the thiocarbamate herbicides. Eight hours after treatment with the various thiocarbamates, the rats were killed and liver $ALDH_2$ activity was determined. In the time-course studies, the rats were treated i.p. with a thiocarbamate dose that approximated its ID_{50}. The doses used were 4.5 mg/kg (24.0 μmol/kg) for EPTC, 3.0 mg/kg (16.0 μmol/kg) for molinate, 1.6 mg/kg (7.9 μmol/kg) for vernolate, 165 mg/kg (761 μmol/kg) for butylate and 10.3 mg/kg (64.0 μmol/kg) for ethiolate. At various times thereafter, the rats were killed, and liver $ALDH_2$ activity was determined. In all instances, the rats were first anesthetized with CO_2 and then killed by decapitation. After the rats were killed, the liver was removed rapidly, the liver mitochondria was isolated by differential centrifugation, and $ALDH_2$ activity was determined by measuring the rate of NADH formation, using acetaldehyde as the substrate as described by Tottmar *et al.* [20].

In vitro. Studies investigating the effect of the

respective thiocarbamate sulfoxides on rat liver $ALDH_2$ inhibition were carried out in the following manner. Mitochondria from rat liver were isolated by differential centrifugation, and 2 mg of mitochondrial protein was placed in a tube and diluted with 0.1 M sodium phosphate buffer (pH 7.4) containing 1 mM EDTA. Various concentrations of the respective thiocarbamate sulfoxides were added in 100 μL ethanol to the mitochondria, and incubated for 60 min at 37°. Control incubations contained 100 μL of ethanol. The use of ethanol as a solvent had no effect on $ALDH_2$ activity. After incubation for 60 min, the mitochondria were isolated by centrifugation at 4300 *g* for 10 min, and $ALDH_2$ activity was determined [20]. The time-course for $ALDH_2$ inhibition also was investigated. Mitochondria from rat liver were isolated by differential centrifugation, and 2 mg of mitochondrial protein was placed in a tube and diluted with 0.1 M sodium phosphate buffer (pH 7.4) with 1 mM EDTA. The respective thiocarbamate sulfoxides were added in 100 μL ethanol, and the individual tubes were incubated at 37°. Control incubations contained 100 μL of ethanol. At various times, the mitochondria were isolated by centrifugation at 4300 *g* for 10 min, and $ALDH_2$ activity was determined [20].

Determination of MAP and heart rate

MAP was determined directly using a femoral artery catheter in conscious, unrestrained rats as described by Yourick and Faiman [21].

Microsomal metabolism studies

Rat liver microsomes were isolated and incubated with mitochondria in the presence of an NADPH-generating system as described previously [2]. The respective thiocarbamates were added in 10 μL of acetonitrile (final concentration = 2 mM). The tubes were incubated at 37° and, after 60 min, 0.5 mL of the incubation mixture was transferred to a microcentrifuge tube containing 0.5 mL of ice-cold acetonitrile. The acetonitrile contained an internal standard for quantitation of the sulfoxides by HPLC. HPLC analysis was carried out on a C18 reverse-phase column (Beckman/Altex 2 mm i.d. × 25 cm, 5 μm) with a specific mobile phase and internal standard for each thiocarbamate. The flow rate of the mobile phase was 0.2 mL/min. For the analysis of: molinate sulfoxide, the mobile phase was 40:60 acetonitrile:water and the internal standard was ethiolate; EPTC sulfoxide, the mobile phase was 50:50 acetonitrile:water and the internal standard was molinate; vernolate sulfoxide and butylate sulfoxide, the mobile phase was 60:40 acetonitrile:water and the internal standard was EPTC; ethiolate sulfoxide, the mobile phase was 25:75 acetonitrile:water and the internal standard was DETC-Me. Detection was carried out at 215 nm for all compounds.

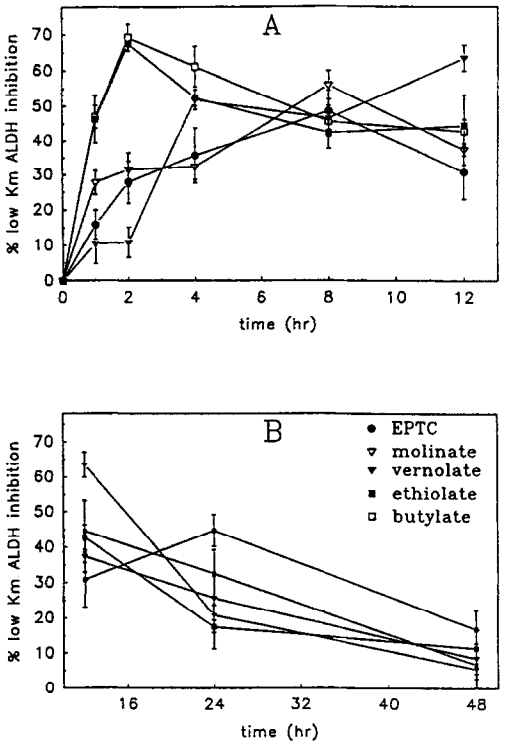


Fig. 3. Onset (A) and recovery (B) of rat liver $ALDH_2$ inhibition after treatment with thiocarbamate herbicides. Rats were treated i.p. with EPTC (4.5 mg/kg), molinate (3.0 mg/kg), vernolate (1.6 mg/kg), butylate (165 mg/kg) and ethiolate (10.3 mg/kg). At the times indicated, liver $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four rats.

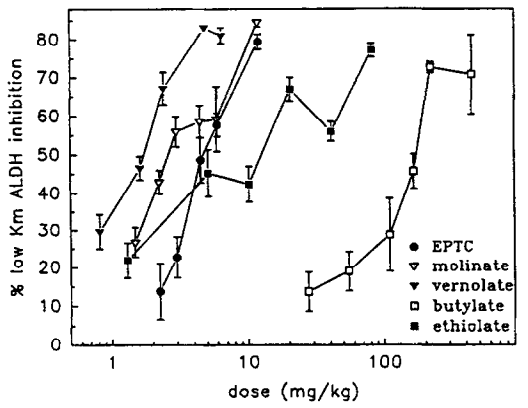


Fig. 2. Effect of thiocarbamate herbicides on rat liver $ALDH_2$. Rats were treated i.p. with various doses of the thiocarbamates, and 8 hr later, liver $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four rats.

Statistical analysis

Means were compared by Student's two-tailed *t*-test or by one-way ANOVA followed by Bonferroni *a posteriori post hoc* test.

RESULTS

The various thiocarbamate herbicides studied all

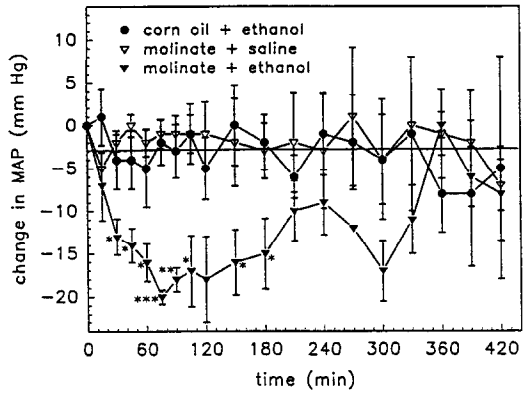


Fig. 4. Disulfiram-like ethanol reaction produced by the administration of ethanol to molinate-treated rats. Rats were treated with corn oil or molinate (11.84 mg/kg) through an i.p. catheter. After 8 hr, the rats were dosed with saline or 1 g/kg ethanol (20%, v/v, in saline) through the i.p. catheter, and blood pressure was measured for 7 hr. Results are presented as means ± SEM of observations from each of four rats. Key: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ compared with corn oil + ethanol.

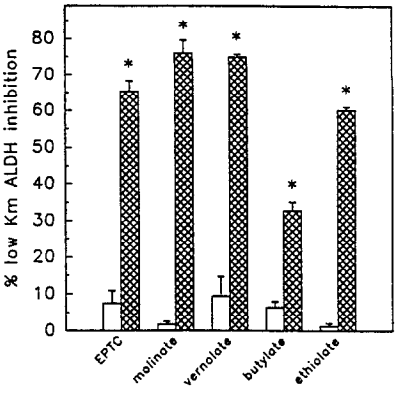


Fig. 5. Effect of microsomal activation on thiocarbamate-induced rat liver $ALDH_2$ inhibition in isolated mitochondria. EPTC, molinate, vernolate, butylate and ethiolate (200 μM) were incubated with mitochondria and microsomes in the absence (□) or presence (⊠) of an NADPH-generating system. After 60 min, the mitochondria were isolated, and $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four incubations. Key: (*) $P < 0.0001$ when compared with control (□).

inhibited rat liver $ALDH_2$ *in vivo*. The respective dose–response data for each thiocarbamate were calculated from a probit plot and are shown in Fig. 2. Employing an 8-hr pretreatment time, vernolate was found to be the most potent of the thiocarbamates, with the dose that inhibited $ALDH_2$ by 50% (ID_{50}) calculated to be 1.6 mg/kg (7.9 μmol/kg). Following, in order of potency, were molinate (ID_{50} = 3.1 mg/kg, 16.6 μmol/kg), EPTC (5.2 mg/kg; 27.5 μmol/kg), ethiolate (12 mg/kg; 74.5 μmol/kg), and butylate (174 mg/kg; 818.4 μmol/kg).

The time-course for rat liver $ALDH_2$ inhibition by the respective thiocarbamates was investigated (Fig. 3). The dose of the thiocarbamate used in these studies was that which produced approximately 50% $ALDH_2$ inhibition 8 hr after drug treatment. Ethiolate (10.3 mg/kg; 64.0 μmol/kg) and butylate (165 mg/kg; 761 μmol/kg) exhibited peak $ALDH_2$ inhibition 2 hr after administration. Molinate (3.0 mg/kg; 16.0 μmol/kg) and EPTC (4.5 mg/kg; 24.0 μmol/kg) exhibited peak $ALDH_2$ inhibition 8 hr after thiocarbamate administration, and inhibition of $ALDH_2$ after vernolate (1.6 mg/kg; 7.9 μmol/kg) peaked 12 hr after thiocarbamate treatment (Fig. 3A). For all of the thiocarbamates investigated, rat liver $ALDH_2$ activity returned to near normal within 48 hr (Fig. 3B).

The potential of these thiocarbamates in producing a disulfiram-like ethanol reaction was also investigated. Molinate was selected as the prototype thiocarbamate because of the rat liver $ALDH_2$ inhibition data previously generated in the laboratory for molinate [18, 19]. Rats were treated with 11.8 mg/kg (63.1 μmol/kg) molinate 8 hr prior to an ethanol challenge. This dose was selected to ensure that sufficient liver $ALDH_2$ was inhibited. After an ethanol challenge to molinate-treated rats, a disulfiram-like ethanol reaction occurred, characterized by a marked decrease in MAP, with maximum hypotension occurring 75 min after ethanol administration (Fig. 4). The hypotension persisted for approximately 3 hr.

Table 1. Formation of thiocarbamate sulfoxides by isolated rat liver microsomes in the presence of NADPH

Thiocarbamate	Sulfoxide formed (nmol/60 min/mg microsomal protein)
EPTC	124 ± 12.1
Molinate	152 ± 6.5
Vernolate	186 ± 8.9
Butylate	158 ± 20.6
Ethiolate	257 ± 5.8

Thiocarbamates (2 mM) were incubated with isolated rat liver microsomes and NADPH for 60 min, and the respective sulfoxide formed was determined by HPLC (see Materials and Methods). Results are presented as means ± SEM of single observations from each of four incubations.

In vitro $ALDH_2$ inhibition studies by the respective thiocarbamates also were carried out. Employing a concentration of 200 μM and an incubation period of 60 min, no inhibition of $ALDH_2$ was observed by any of the thiocarbamates. However, when rat liver microsomes and an NADPH-generating system were added to the incubation, $ALDH_2$ was inhibited markedly (Fig. 5). Analysis of the incubation, which contained the respective thiocarbamates and rat liver microsomes, showed that the sulfoxide of the respective thiocarbamates was formed when the NADPH-generating system was added to the

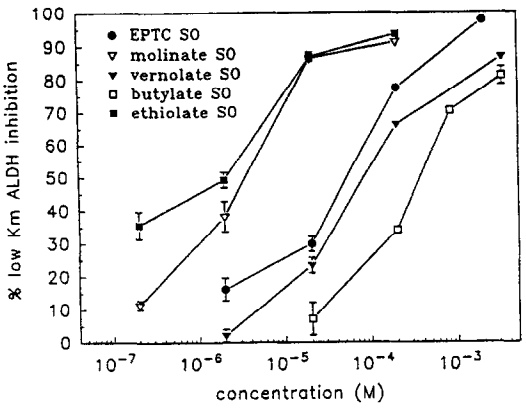


Fig. 6. Effect of thiocarbamate sulfoxide (SO) on rat liver $ALDH_2$ activity in isolated mitochondria. Various concentrations of EPTC SO, molinate SO, vernolate SO, butylate SO and ethiolate SO were incubated with rat liver mitochondria for 60 min. The mitochondria were then isolated, and $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four incubations.

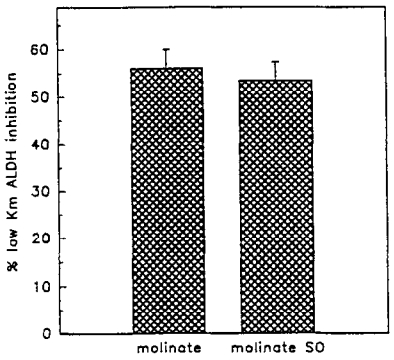


Fig. 7. Effects of molinate and molinate sulfoxide (SO) on rat liver $ALDH_2$ *in vivo*. Molinate (3.0 mg/kg) and an equimolar dose of molinate SO (3.2 mg/kg) were administered to rats i.p. After 8 hr, rat liver $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four rats.

incubation (Table 1). The respective thiocarbamate sulfoxides were synthesized, and their effects on rat liver $ALDH_2$ *in vitro* were determined. The various thiocarbamate sulfoxides all inhibited rat liver $ALDH_2$ (Fig. 6). In these studies, ethiolate sulfoxide and molinate sulfoxide were found to be very potent *in vitro* $ALDH_2$ inhibitors with IC_{50} values of 0.9 and 1.0 μM, respectively. Both exhibited maximal inhibition at approximately 20 μM. EPTC sulfoxide, vernolate sulfoxide, and butylate sulfoxide were less potent with IC_{50} values of 38, 113 and 438 μM, respectively. These three sulfoxides exhibited maximal inhibition at approximately 2 mM.

Equimolar doses of molinate and molinate sulfoxide were administered to rats, and the animals were killed 8 hr later. The same degree of rat liver $ALDH_2$ inhibition was observed with both molinate and its sulfoxide (Fig. 7). Although similar studies were not carried out with ethiolate, EPTC, vernolate, butylate, and their respective sulfoxides, similar results would be expected.

DISCUSSION

In both rats and humans, disulfiram is reduced to DDTC [22], and then methylated to DDTC-Me [23, 24]. Subsequent oxidative desulfuration of DDTC-Me results in the formation of DETC-Me [6, 9–11], which is then oxidized to DETC-MeSO, the putative metabolite responsible for rat liver $ALDH_2$ inhibition [8]. Although DETC-MeSO can be oxidized to the sulfone, at the present time it is not known whether this occurs *in vivo*. Furthermore, if formed *in vivo*, its formation in a concentration sufficient to inhibit liver $ALDH_2$ is questionable [25]. The structural similarity between DETC-Me and the thiocarbamate herbicides (Fig. 1) suggested that these thiocarbamates also may be inhibitors of rat liver $ALDH_2$, since DETC-Me is an intermediate in the formation of DETC-MeSO. As hypothesized, the five representative thiocarbamate herbicides studied all inhibited rat liver $ALDH_2$ *in vivo*. Employing a pretreatment time that resulted in maximal $ALDH_2$ inhibition, the rank order of potency for rat liver $ALDH_2$ inhibition was vernolate >molinate > EPTC > ethiolate >> butylate (Fig. 2). This rank order for rat liver $ALDH_2$ inhibition was valid when rats were treated with the thiocarbamate herbicides 8 hr before the determination of rat liver $ALDH_2$. However, it is possible that a different pretreatment time could produce a different rank order in potency. Absorption, distribution and metabolism, all of which can contribute to the potency of rat liver $ALDH_2$ inhibition by these thiocarbamates, probably account for these differences. For example, in studies with [^{14}C]EPTC and [^{14}C]butylate, Hubbell and Casida [14] showed that differences in the N-substituents can affect the extent of thiocarbamate metabolism.

Hypotension has been used as an indicator in rats to study the disulfiram–ethanol [2] and disulfiram-like ethanol reaction [19] in rats. Molinate-treated rats challenged with ethanol exhibited a hypotensive response indicative of a disulfiram-like ethanol reaction (Fig. 4). This is similar to that seen with disulfiram and the metabolites DDTC, DDTC-Me [2], and DETC-Me [11]. An increase in blood acetaldehyde also was found in the molinate-treated rats given ethanol (unpublished results), which again is consistent with that observed for disulfiram and its metabolites. Thus, molinate can produce a disulfiram-like ethanol reaction in rats, and more than likely so do the other thiocarbamates.

In studies with the respective thiocarbamates, in all instances only minimal inhibition of rat liver $ALDH_2$ was found approximately 48 hr after dosing (Fig. 3B). This is similar to that observed with disulfiram, as well as several disulfiram metabolites [2] including DETC-Me [11]. Since inhibition of rat liver $ALDH_2$ is responsible for the increase in blood

acetaldehyde, which is associated with the disulfiram–ethanol reaction [2], these data (Fig. 3B) suggest that no disulfiram-like ethanol reaction should occur 48 hr after thiocarbamate administration. This again would be consistent with the findings for disulfiram, DDTC, or DDTC-Me [2], in which no disulfiram–ethanol reaction was observed 48 hr after dosing. Of significance is that similar findings were seen in the studies by Iber and Chowdhury [26], in which patients given disulfiram did not exhibit a disulfiram–ethanol reaction when challenged with ethanol 24 hr after disulfiram dosing.

DETC-Me is the immediate precursor of DETC-MeSO, the putative metabolite of disulfiram responsible for rat liver $ALDH_2$ inhibition [8]. Formation of DETC-MeSO from DETC-Me is catalyzed by cytochrome P450 [4, 5, 27], and it is the formation of DETC-MeSO which has been suggested as being responsible for inhibition of rat liver $ALDH_2$ [4, 8, 27]. This requirement for bioactivation would also be expected for the formation of the respective sulfoxides from EPTC, molinate, ethiolate, vernolate, and butylate, and subsequent inhibition of rat liver $ALDH_2$. This was indeed found to be the case. These thiocarbamates did not inhibit rat liver $ALDH_2$ *in vitro*, but inhibited rat liver $ALDH_2$ when a microsomal enzyme system was added to the incubation (Fig. 5). The importance of the formation of the sulfoxide was supported by the finding that when these respective thiocarbamates were incubated with a microsomal enzyme system, the respective sulfoxides could be isolated and identified (Table 1). Thus, formation of the sulfoxide is dependent upon microsomal monooxygenases. To be considered the chemical species responsible for rat liver $ALDH_2$ inhibition, the thiocarbamate sulfoxides should be active both *in vitro* and *in vivo*. Molinate sulfoxide was active *in vivo* (Fig. 7), and all the thiocarbamate sulfoxides studied inhibited rat liver $ALDH_2$ *in vitro* (Fig. 6). The rank order of potency for the thiocarbamate sulfoxides as rat liver $ALDH_2$ inhibitors after 1 hr of incubation was: ethiolate SO > molinate SO >> EPTC SO > vernolate SO > butylate SO. Furthermore, employing a concentration that estimated 1-hr IC_{50} values, all of the thiocarbamate sulfoxides showed time-dependent rat liver $ALDH_2$ inhibition when incubated with isolated mitochondria (data not shown). Of interest is that the more potent thiocarbamate sulfoxides contain smaller R substituents. This may suggest that the active site of rat liver $ALDH_2$ may be subject to steric hindrance. Although the rank order of potency between the thiocarbamate herbicides *in vivo* (Fig. 2) and their respective sulfoxides *in vitro* (Fig. 6) is not the same, a comparison between *in vivo* and *in vitro* inhibition is difficult since inhibition of rat liver $ALDH_2$ *in vivo* is dependent upon bioactivation and the formation of the corresponding sulfoxide.

Employing molinate and molinate sulfoxide as pharmacological tools, equimolar doses of each produced similar *in vivo* rat liver $ALDH_2$ inhibition (Fig. 7). This result, combined with the observation that molinate sulfoxide (as well as the other sulfoxides) inhibits rat liver $ALDH_2$ *in vitro*, suggests that it is the thiocarbamate sulfoxide that is responsible for rat liver $ALDH_2$ inhibition.

In conclusion, all the thiocarbamate herbicides studied inhibited rat liver $ALDH_2$ both *in vivo* and *in vitro*. Using molinate as a prototype, this thiocarbamate produced a disulfiram-like ethanol reaction in rats challenged with ethanol. The thiocarbamates were ineffective rat liver $ALDH_2$ inhibitors *in vitro*, but were potent rat liver $ALDH_2$ inhibitors when microsomes and an NADPH-generating system were added to the incubation. The thiocarbamates are oxidized to their respective sulfoxides, which inhibit rat liver $ALDH_2$ both *in vitro* and *in vivo*. The thiocarbamate sulfoxides therefore appear to be the chemical species responsible for the rat liver $ALDH_2$ inhibition. The common structural unit of these compounds is the N—C(O)—S(O) moiety, suggesting that this chemical structure is required for the inhibition of rat liver $ALDH_2$. The mechanism of rat liver $ALDH_2$ inhibition by the thiocarbamate sulfoxides is presently unknown, and is in the process of being investigated. Finally, these studies suggest that care must be taken in the use of thiocarbamate herbicides in agriculture, since consumption of ethanol after the use of these herbicides could lead to a severe disulfiram-like ethanol reaction.

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