Investigations into the mechanism of triallate and difenzoquat resistance in wild oats
by Anthony John Kern

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
Wild oats (Avena fatua L.) are a serious weed competitor in small grain growing areas of North America and the world. One of the most effective herbicides used for wild oat control in these crops is triallate, and its continuous use in the Fairfield region of Montana has selected for populations of wild oats which are no longer controlled by the herbicide. Many of these triallate-resistant (R) wild oats are also resistant to the chemically unrelated herbicide difenzoquat. The objectives of these studies were to characterize resistance levels and investigate potential mechanisms of resistance in an inbred line.

An inbred R wild oat line selected through two generations of triallate treatment was shown in greenhouse and Petri dish dose response experiments to be 17-fold and 64-fold more resistant to triallate and difenzoquat, respectively, than susceptible (S) lines. The R line was also cross-resistant to diallate.

Uptake and translocation patterns of 14C triallate were not substantially different between R and S lines.

Reverse-phase high performance liquid chromatography (HPLC) was used to compare 14C-triallate metabolism patterns in R and S wild oat lines. Triallate was metabolized to one major product (2,3,3-trichloropropene sulfinic acid) and two minor products (unidentified) in R and S wild oats. However, the rate of triallate metabolism was more than 10-fold slower in R than in S lines Because triallate is thought to be activated in vivo through formation of the triallate sulfoxide, dose response tests were conducted to compare the toxicity of triallate sulfoxide in R and S lines. Triallate sulfoxide was equally phytotoxic to both lines, indicating that the mechanism of triallate resistance may be conferred by a decreased rate of sulfoxidation (activation) in R plants.

Difenzoquat uptake, translocation, and metabolism patterns were compared between R and S wild oats using 14C-IabeIed difenzoquat. Slightly increased rates of 14C-difenzoquat uptake and translocation were observed in the R line, which were considered unlikely to confer resistance. However, HPLC analysis indicated that difenzoquat quickly became unextractable in R plants, suggesting the presence of a novel difenzoquat metabolizing pathway and immobilization of the herbicide, possibly in cell wall material.

Because triallate was shown to inhibit lipid biosynthesis in susceptible plant species, the effects of triallate and triallate sulfoxide on lipid and wax biosynthesis in R and S wild oats were compared. Five days after application, wax deposition was dramatically inhibited in S wild oats but not in R. In addition, triallate reduced the amounts of long-chain free fatty acids in S but not R wild oats. However, treatment with triallate sulfoxide inhibited long-chain lipid biosynthesis equally in R and S wild oats. The results further support the idea that decreased synthesis of triallate sulfoxide confers resistance to the herbicide in R wild oats.

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APPROVAL

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Anthony John Kern

The thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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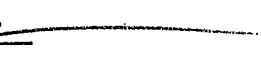
Signature 

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ABSTRACT

Wild oats (Avena fatua L.) are a serious weed competitor in small grain growing areas of North America and the world. One of the most effective herbicides used for wild oat control in these crops is triallate, and its continuous use in the Fairfield region of Montana has selected for populations of wild oats which are no longer controlled by the herbicide. Many of these triallate-resistant (R) wild oats are also resistant to the chemically unrelated herbicide difenzoquat. The objectives of these studies were to characterize resistance levels and investigate potential mechanisms of resistance in an inbred line.

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Reverse-phase high performance liquid chromatography (HPLC) was used to compare ^{14}C-triallate metabolism patterns in R and S wild oat lines. Triallate was metabolized to one major product (2,3,3-trichloropropene sulfinic acid) and two minor products (unidentified) in R and S wild oats. However, the rate of triallate metabolism was more than 10-fold slower in R than in S lines Because triallate is thought to be activated *in vivo* through formation of the triallate sulfoxide, dose response tests were conducted to compare the toxicity of triallate sulfoxide in R and S lines. Triallate sulfoxide was equally phytotoxic to both lines, indicating that the mechanism of triallate resistance may be conferred by a decreased rate of sulfoxidation (activation) in R plants.

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Because triallate was shown to inhibit lipid biosynthesis in susceptible plant species, the effects of triallate and triallate sulfoxide on lipid and wax biosynthesis in R and S wild oats were compared. Five days after application, wax deposition was dramatically inhibited in S wild oats but not in R. In addition, triallate reduced the amounts of long-chain free fatty acids in S but not R wild oats. However, treatment with triallate sulfoxide inhibited long-chain lipid biosynthesis equally in R and S wild oats. The results further support the idea that decreased synthesis of triallate sulfoxide confers resistance to the herbicide in R wild oats.

CHAPTER 1

INTRODUCTION

Since the introduction of 2,4-D (2,4-dichlorophenoxyacetic acid) in 1952, selective herbicides have been an important tool in modern agriculture. Herbicides are generally very effective in reducing competition from weeds, and have been an integral part of the so-called "Green Revolution." However, a current threat to the productivity of modern agriculture is the recent advent of weed populations that are resistant to previously effective herbicides, due to altered physiological or biochemical processes which enable the plant to escape injury. Under normal circumstances, wild type individuals of a plant species are capable of tolerating a certain amount of herbicide (herbicide tolerance). Maxwell and Mortimer (1994) defined herbicide resistance as "the inherited ability to not be controlled by a herbicide" when that herbicide is applied at rates substantially higher than rates that normally control the wild type individuals.

In 1970, Ryan reported the first case of herbicide resistance to the photosynthesis inhibitor simazine (6-chloro-N', N'-diethyl-1,3,5-triazine-2,4-diamine) in *Senecio vulgaris* (Ryan, 1970), and during the next twenty years several dozen instances of herbicide resistance have been documented,

involving many weedy plant species and nearly every herbicide class used in agriculture. For example, herbicide resistance has been reported for photosystem II inhibiting herbicides in over 60 weed species (LeBaron, 1991), and for the photosystem I inhibiting herbicides in over 12 species (Preston, 1994). Resistance to the acetolactate synthase inhibiting herbicides was detected less than five years after their introduction in 1982 in *Lactuca serriola* (Mallory-Smith et al., 1990), and since then over a dozen species have developed resistance (Saari et al., 1994). Resistance to the acetyl-coenzyme A carboxylase inhibiting herbicides used for grass control has been reported in nine weed species (Devine and Shimabukuro, 1994). Over 20 weedy species have been reported to be resistant to auxinic herbicides (Coupland, 1994), a class of herbicides with diverse chemical structures that mimic the natural plant growth regulator indoleacetic acid. Mitotic disrupting herbicides, such as the dinitroaniline herbicides, have been used for over two decades and nearly a dozen weeds are now reported to be resistant (Smeda and Vaughn, 1994; Morrison et al., 1989).

The great diversity of weeds developing resistance to many herbicide classes demonstrates the high levels of phenotypic variability present in weedy species. In addition, there is substantial variability in the mechanisms of resistance adopted by weeds to escape injury. These resistance mechanisms can be divided into two classes based on the type of physiological alteration present: non-target site resistance and target site resistance.

Non-target Site Resistance

Plants with non-target site resistance posess altered physiological processes that alter the fate of a herbicide before it can influence the normal biochemical processes that ultimately result in plant death. Generally, non-target site resistance confers levels of resistance that are about 10- to 20-fold higher than normal susceptible biotypes (Hall et al., 1994).

Reduced Uptake or Translocation.

Reduced herbicide uptake (absorption) through the cuticle, cell wall and plasmalemma may cause the applied herbicide to remain on the leaf surface, or slow the rate of absorption into the cell. Resistance due to decreased uptake in wheat (*Triticum aestivum)* has been documented for triasulfuron (2-(2-chloroethoxy)-n-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl] benzene sulfonamide) (Meyer and Muller, 1989) and several *Equisetum* species exhibit tolerance to the nonselective herbicide glyphosate (N-(phosphonomethyl) glycine) due to limited uptake (Marshall et al., 1987). However, there are very few documented examples of resistance due entirely to reduced herbicide uptake (Saari et al., 1994). In most cases where limited uptake plays a role in herbicide resistance, it is coupled with reduced translocation (movement) within the plant to the ultimate site of action. One example of reduced translocation conferring resistance is to the non-selective bipyridilium herbicides paraquat (1:1-dimethyl-4,4'-bipyridinium dichloride) and diquat (6,7-dihydroipyridol[1,2-a:2',1'-

c]pyrazidinium dibromide). Reduced uptake and translocation of paraquat (as measured by autoradiography) have been noted in certain biotypes of *Erigeron philadelphicus*, *Erigeron canadensis* (Tanaka et al., 1986), *Hordeum glaucum* (Bishop et al., 1987), *Conyza bonariensis* (Fuerst et al., 1985), and *Lolium perenne* (Faulkner, 1976). In all cases, paraquat and diquat were taken up and translocated more slowly in the resistant (R) than the susceptible (S) biotypes. Although the mechanism of reduced uptake and translocation appears to be sufficient to confer agronomically important levels of resistance, it has not been observed to be a widespread means of field-selected resistance.

Sequestration

Sequestration (or compartmentalization) of herbicides into cellular vacuoles has been documented for diclofop-methyl (methyl, 2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate) in *Avena fatua* (Devine et al., 1993), for paraquat in certain biotypes of *Hordeum vulgare* (Hart and DiTomaso, 1993), in a 2,4-D resistant biotype of soybean (Schmitt and Sandermann, 1982) and for other auxin-analog herbicides (Davis and Linscott, 1986; and Shaner et al., 1992). In addition, sequestration into cell walls has been proposed as a paraquat resistance mechanism for certain types of *Conyza bonariensis* (Fuerst et al., 1985, Vaughn et al., 1989) and *Hordeum glaucum* (Powles and Cornic, 1987). In *H. glaucum*, several polyamines inhibited paraquat uptake in S biotypes, and the polyamines were taken up more slowly in R biotypes than S biotypes (Preston et al., 1992). The authors proposed that a polyamine and/or paraquat transporter

had been altered in R biotypes, conferring reduced paraquat uptake into individual cells. Paraquat thus excluded may slowly accumulate in extracellular matrices and become bound to cell walls via non-enzymatic processes. Although sequestration has not been proposed as a mechanism of field-selected resistance, Hart et al. (1992) showed that more than 75% of applied paraquat became associated with maize root cell walls within 24 hours after application, indicating that sequestration of the bipyridilium herbicides could confer sufficient levels of resistance to allow plants to escape injury under normal field conditions.

Altered Metabolism

Although the metabolic fates of herbicides are quite diverse across plant species, an increase in the rate of any catabolic step of herbicide breakdown can reduce the concentration of its active form *in vivo*, thus effectively decreasing its phytotoxicity. There are several well-characterized examples of enzymatic steps common to herbicide metabolism, which have been shown to confer resistance in R populations.

Cytochrome P450-mediated aryl- and alkyl-hydroxylation, *O*-dealkylation, sulfoxidation, and deesterification reactions have been reported in R biotypes of several species as proposed mechanisms of metabolism-based detoxification (Brown, 1991; Fonne-Pfister et al., 1990; Frear et al., 1991; Omer et al., 1990). Increased cytochrome P450-mediated herbicide metabolism was shown to confer resistance to the acetolactate synthase-inhibiting herbicides such as chlorsulfuron (2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl)

benzenesulfonamide) in various species (Sweetser et al., 1982), and to the photosystem II inhibiting herbicides simazine and atrazine (Ritter, 1986). In addition, acetyl-coenzyme A carboxylase inhibiting herbicides such as diclofop-methyl undergo aryl hydroxylation in R species, yielding nontoxic metabolites (Jacobson and Shimabukuro, 1984; Zimmerlin and Durst, 1990). Two biotypes of *Lolium rigidum* have been shown to be resistant to a number of photosystem II inhibiting herbicides (including the triazines and phenylureas) due to enhanced N-dealkylation (Burnet et al., 1993; Burnet et al., 1993a), which is thought to be mediated by cytochrome P450-type enzymes. It is not known whether these increased oxidative capabilities are due to a mutant cytochrome P450 with broad substrate specificity, or due to increases in the activity of a number of cytochrome P450 isozymes. Inhibitors of cytochrome P450s such as 1-aminobenzotriazole and piperonyl butoxide not only decrease the rate of herbicide detoxification, but can also cause the plant to revert to susceptibility.

Subsequent reactions that lead to herbicide conjugation with glutathione (Dean et al., 1991), sugars (Gonneau et al., 1988), fatty acids, and other cellular components (Gronwald, 1994) can occur, all of which alter the chemical characteristics of a herbicide and can render it ineffective. For example, *Abutilon theophrasti* biotypes that are resistant to atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) have been shown to have a sixfold greater rate of atrazine conjugation to glutathione (GSH) than S biotypes (Gronwald et al., 1989). The enhanced rate of GSH conjugation was not due to an elevated GSH content, but rather to the overexpression of two glutathione-*S*-

transferase (GST) isozymes that exhibit activity with atrazine. The high natural levels of tolerance to atrazine shown by maize may be due to a similar rapid GST activity (Timmerman, 1989) and a high endogenous GSH content.

Herbicide resistance could also theoretically be conferred through a decreased rate of metabolism. For example, diclofop-methyl is considered a proherbicide because it must be demethylated *in vivo* to be activated, and thus a lack of metabolism would prevent formation of the herbicidally active metabolite of the parent molecule. Although this mechanism has been proposed for a number of herbicides, the phenomenon has not yet been documented in field- or laboratory-selected plants.

Target Site Resistance

Herbicide resistance may be caused by an alteration in the normal binding site of a herbicide (usually an enzyme) that results in decreased binding specificity or affinity. Resistance based on target site alterations has been best documented for photosystem II inhibitors such as the triazines and phenylureas. Tischer and Strotmann (1977) reported that many herbicides in these classes have a common binding site on thylakoid membranes known as the D1 protein or the 32-kDa protein (Barber and Andersson, 1992; Trebst, 1987). Herbicides of these families exert their effect by displacing plastoquinone at the Q_B binding site on the D1 protein, thereby blocking electron flow from Q_A to Q_B (Vermass et al., 1983; Vermass et al., 1984). Current models propose that plastoquinone binding

to the Q_B binding niche involves hydrogen bonding between the carbonyl oxygens of plastoquinone with His215 and Ser264 of D1 (Fuerst and Norman, 1991; Tietjen et al., 1991; Trebst, 1992). In all cases of target site resistance that have occurred in the field, a point mutation in the *psb*A gene (which encodes the D1 protein) (Morden and Golden, 1989) resulted in the substitution of a Gly residue for Ser264; this substitution has been identified as the alteration conferring resistance (Fuerst and Norman, 1991; Mets and Thiel, 1989; Trebst, 1991). This amino acid substitution results in an approximate 1000-fold reduction in the binding affinity for atrazine, and confers an approximate 100-fold increase in atrazine resistance in whole plants (Pfister and Arntzen, 1979). Such dramatic increases in herbicide resistance levels caused by target site mutations are common; similar R/S I_{50} (the herbicide concentration at which enzyme activity is inhibited by 50%) ratios have been documented for target site chlorsulfuron resistance in *Stellaria media* (Devine et al., 1991; Saari et al., 1992), *Lolium rigidum* (Saari et al., 1994), *Kochia scoparia* (Friesen, 1992), and sulfometuron-methyl (methyl 2-[[[[4,6-dimethyl-2-pyrimidinyl) amino] -carbonyl] amino]sulfonyl]benzoate) resistance in *Salsola iberica* (Saari et al., 1992). Herbicide resistance levels based on target site mutations are generally greater than resistance based on non-target site alterations.

Multiple- and Cross-resistance

Evolution of resistant populations under extensive herbicide selection pressure is not surprising (Maxwell and Mortimer, 1994), and illustrates that there is great diversity in resistance mechanisms and resistance levels among species and even within small populations. Among the many cases of herbicide resistance documented in recent years, an alarming and serious agricultural threat is the advent of populations that are resistant to more than one herbicide class. These populations are said to have multiple resistance, defined as the "expression (within individuals or populations) of more than one resistance mechanism, endowing the ability to withstand herbicides from different chemical classes" (Hall et al., 1994). Typically, plants exhibiting multiple resistance possess two or more distinct resistance mechanisms, which may include both non-target site and target-site based resistances. The most well-characterized example of multiple resistance is based on a set of *Lolium rigidum* populations from Australia (Hall et al., 1994). One particular population is resistant to 24 different herbicides, encompassing nine different chemical classes (the aryloxyphenocypropanoates and cyclohexanediones, which are acetyl-coenzyme A carboxylase inhibitors; the sulfonylureas and imadazolinones, which inhibit acetolactate synthase; the dinitroaniline herbicides, which appear to inhibit mitosis by preventing spindle microtuble formation (Hess, 1987; Morejohn et al., 1987); the carbamates, which are photosystem II disruptors; the thiocarbamates (see discussion below); and the chloracetamides and isoxalolidinones, which

may inhibit biosynthesis of fatty acids, proteins, isoprenoids, and flavonoids through conjugation with sulfhydryl-containing compounds such as acetyl coenzyme A (Fuerst, 1987).

The mechanisms that confer multiple resistance to such a wide range of herbicides include: 1) the "membrane recovery response" which, although poorly characterized, allows meristematic tissue to quickly recover trans-membrane proton gradients after application of the aryloxyphenocypropanoate and cyclohexanedione herbicides (Hausler et al., 1991); 2) enhanced herbicide metabolism which decreases the toxicity of diclofop-methyl, possibly by increased glutathione conjugation (Christopher et al., 1993); 3) increased chlorsulfuron metabolism, which appears to be cytochrome P450- mediated (Christopher et al., 1991; Cotterman and Saari, 1992); and 4) target site resistances due to mutations in the genes encoding acetyl-coenzyme A carboxylase and acetolactate synthase (Matthews et al., 1990).

A somewhat less complicated mechanism by which weedy species evolve resistance to more than one herbicide is known as cross resistance. In species exhibiting cross resistance, a single mutation confers resistance to more than one herbicide of the same class and mode of action. Populations of *Eleusine indica* which developed resistance to trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzenamine) were also resistant to seven other dinitroaniline herbicides, and exhibited R/S I_{50} ratios of over 1,000 (based on mitotic indices), even though the population had been selected in the field by trifluralin only (Mudge et al., 1984). Populations of *Setaria viridis* in Canada which also

developed resistance to trifluralin in the field were cross-resistant to five other dinitroaniline herbicides, although R/S I_{50} ratios were much lower (Smeda et al., 1992).

Herbicide Resistance in Wild Oats

Wild oats (*Avena fatua* L.) are a major weed problem causing significant yield reductions in small grain crops in the U.S. and Canada (Thurston and Phillipson, 1976). One of the most effective herbicides for controlling wild oats in spring wheat and barley is triallate (*S*-(2,3,3-trichloro-2-propenyl) bis(1-methylethyl)carbamothioate), a selective preemergence thiocarbamate herbicide. In certain cropping systems, the chemically unrelated bipyridilium postemergence herbicide difenzoquat (1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium) is also used for effective wild oat control. Both herbicides are widely used, and in certain areas triallate has been used annually for over 20 consecutive years since its introduction in 1972. Despite early reports of natural variation and increased levels of tolerance to triallate in wild oat populations repeatedly subjected to sublethal doses (Jana and Naylor, 1982, Thai et al. 1985), the herbicide has been used successfully for over two decades.

Field reports of poor triallate performance beginning in 1991 and 1992 led O'Donovan et al. (1994) to investigate the response of Canadian wild oat collections to triallate and several other herbicides. Seedlings from R collections exhibited about a 10-fold increase in resistance levels to triallate. In addition, the

R collections were about 40-fold more resistant to difenzoquat than S controls. At about the same time, wild oat field collections from near Fairfield, MT were tested due to complaints of non-performance. Malchow et al. (1993) reported that 61% of the fields sampled contained triallate-resistant wild oats. Many of these populations were tested at a later date and shown to be cross-resistant to difenzoquat (Kern et al., 1994). Although some wild oat lines with elevated tolerance to triallate were previously shown to be slightly cross-resistant to the aryloxyphenoxypropanoate herbicide diclofop-methyl and various cyclohexanedione herbicides (Thai et al., 1985), the collections from Canada (O'Donovan et al., 1994) and Montana (Kern et al., 1994) showed normal susceptibility to diclofop.

Triallate Use and Mode of Action

Triallate is used as a preplant incorporated herbicide for control of wild oats in spring wheat and barley. Although triallate is somewhat phytotoxic to most small grains, deep seeding (below the herbicide treatment zone) allows the seeds to germinate and grow through treated soil without injury. When applied at the recommended field rate (1.1 kg/ha), triallate normally provides greater than 80% control of wild oats. Wild oat seedlings germinate normally but usually do not emerge due to severe inhibition of shoot growth, most likely because of elongation of the first internode, which places the shoot meristem in contact with treated soil.

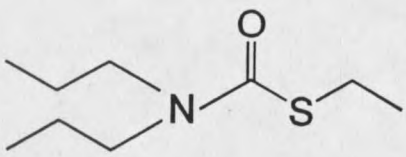
A definitive mode of action has not been established for triallate or the class of herbicides to which it belongs, the thiocarbamates. There is some evidence that other thiocarbamates such as EPTC (*S*-ethyl dipropylcarbamothioate) and diallate (*S*-2,3-dichloro-2-propenyl) bis(1-methylethyl) carbamothioate) inhibit gibberellin biosynthesis (Wilkinson, 1983; Wilkinson 1986), but this effect is more likely due to general cell damage following inital effects of the herbicide and not to specific enzyme inhibition. Other research has shown that thiocarbamates inhibit the biosynthesis of surface lipids such as suberin and waxes (Barrett and Harwood, 1993; Harwood et al., 1989; Harwood and Stumpf, 1971; Kolattukudy and Brown, 1974; Still et al., 1970). As early as 1966, Gentner noted that the total amount of wax deposition on developing cabbage leaves was reduced when treated with EPTC, and several reports confirming this have since been published (Leavitt et al., 1978; Wilkinson and Hardcastle, 1969). Gas chromatography was used to characterize wax biosynthesis inhibition by the thiocarbamates (Wilkinson, 1974; Still et al., 1970). In nearly all cases, species having a large primary alcohol component in their epicuticular waxes with carbon chain lengths greater than 20 were the most severely affected by this treatment. Epicuticular waxes are synthesized by condensation of 16- or 18-carbon fatty acids such as palmitate and stearate with malonyl-CoA by acyl-coenzyme A elongases in an NADPH-dependent reaction. Inhibition of acyl-coenzyme A elongases may thus be a primary site of action of the thiocarbamates (Bolton and Harwood, 1976; Harwood and Stumpf, 1971; Harwood, 1992; Harwood, 1994; Kolattukudy and Brown, 1974). However, total

fatty acid biosynthesis was also strongly affected by triallate treatment in some studies (Harwood et al., 1971; Hawke and Leech, 1987).

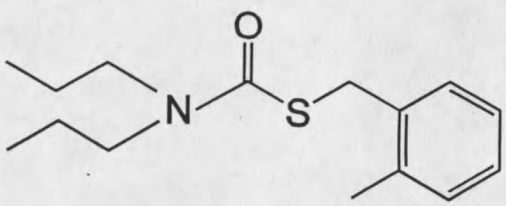
The sulfoxide derivatives of thiocarbamate herbicides have been shown to covalently bind sulfhydryl-containing molecules and consequently have been classified as carbamoylating agents (Lay and Casida, 1976; Leavitt and Penner, 1979). Nonenzymatic *in vitro* carbamoylation of EPTC sulfoxide to coenzyme A has been documented in several systems (Lay and Casida, 1976; Lay et al., 1975; Leavitt and Penner, 1979). Because of the extensive reactions in which coenzyme A is involved (including biosynthesis of lipids isoprenoids, and flavonoids), carbamoylation and subsequent depletion of coenzyme A could account for the wide variation in biochemical perturbations documented in susceptible species. It thus remains unclear whether triallate inhibits specific enzymes, or if the carbamoylating nature of triallate provides a more general enzyme inhibition.

Thiocarbamate Metabolism

Thiocarbamate herbicides have been shown to be metabolized extensively both in plants and mammals, and several metabolites have been identified. The *S*-alkyl (EPTC) and *S*-benzyl (orbencarb) thiocarbamates (Figure 1) are known to be proherbicides in that they require activation to exhibit herbicidal effects. These compounds undergo metabolic sulfoxidation to form the moderately stable sulfoxide derivatives as shown for EPTC in Figure 2.



EPTC

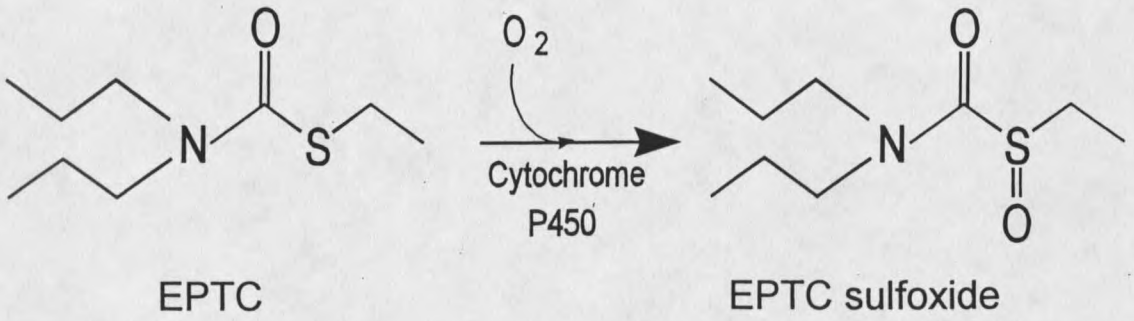


orbencarb

Figure 1. Chemical structures EPTC (*S*-alky) and orbencarb (*S*-benzyl) thiocarbamate herbicides.

After activation, degradation of the *S*-alkyl and *S*-benzyl thiocarbamates has been shown to occur via the sulfoxide intermediates in various systems including mice, rats, and corn (Casida et al., 1975; Hubbell and Casida, 1977; Wilkinson, 1983; Lay and Casida, 1979). Although oxygen is required for EPTC sulfoxidation in maize microsomal fractions (Casida et al., 1975; Jablonkai and Hatzios, 1994), which suggests a cytochrome P450-mediated reaction, subsequent metabolism was not shown to be enzyme dependent. Using ^{3}H-labeled GSH, Leavitt and Penner (1979) showed that EPTC sulfoxide is cleaved during conjugation with GSH, indicating a possible degradation pathway producing the mercapturic acid derivative of EPTC and the sulfinic acid (Figure 3).

The rate of glutathione conjugation with EPTC did not appear to be



EPTC EPTC sulfoxide

Figure 2. Cytochrome P450-mediated sulfoxidation of EPTC.

dependent on GST activity in crude enzyme extracts of maize leaves (Carringer et al., 1978). However, the *S*-alkyl and *S*-benzyl thiocarbamates butylate(S-ethyl bis (2-methylpropyl) carbamothioate), cycloate (S-ethyl cyclohexylethylcarbamothioate), EPTC, molinate (S-ethylhexahydro-1H-azepine-1-carbothioate), pebulate (S-propyl butylethylcarbamothioate), and vernolate (S-propyl dipropylcarbamothioate) were shown to undergo sulfoxidation in a rat microsome system, followed by conjugation with GSH in the liver glutathione transferase system (Casida et al., 1975). After GSH conjugation, subsequent metabolites were not identified. Similarly, Lay and Casida (1976) showed that GSH thiol conjugation with diallate sulfoxide was dependent on GST activity in maize roots. Other *in vivo* work showed that maize seedlings treated with dichloroacetamide herbicide antidotes (which elevate GST activity) rapidly metabolized EPTC sulfoxide in the presence of GSH, indicating that GST activity (dependent on the presence of GSH) increased the rate of sulfoxide cleavage

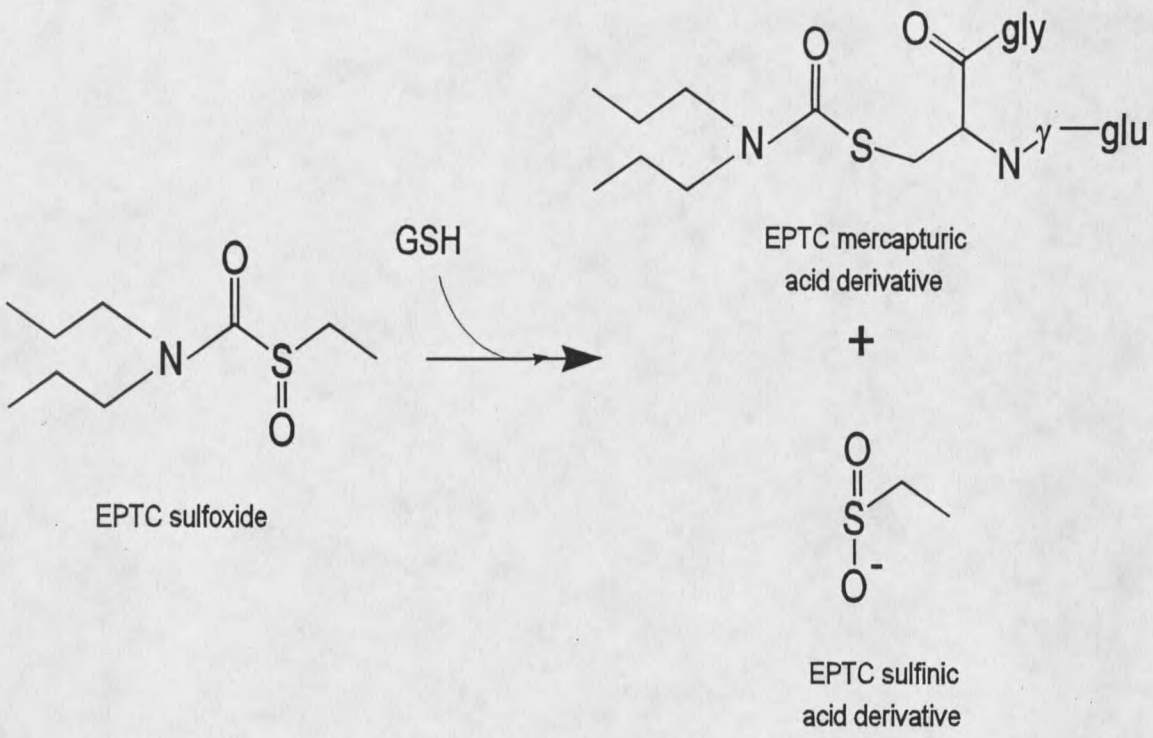


Figure 3. Glutathione conjugation and secondary metabolism of EPTC, as proposed by Leavitt and Penner (1979).

(Carringer et al., 1978).

Metabolism of *S*-chloroallyl thiocarbamates such as diallate and triallate is not clearly understood. While these compounds may undergo sulfoxidation *in vivo* like the *S*-alkyl and *S*-benzyl thiocarbamates, the sulfoxide derivatives have not been isolated from any plant or animal species. Work done by Casida and Lay (1978), Schuphan and Casida (1979a), and Schuphan et al. (1979) showed that the diallate and triallate sulfoxides have half-lives of less than three hours at room temperature. The authors suggested that these primary metabolites could not be isolated in living systems because of immediate *in vivo* conjugation with