

Herbicide safety relative to common targets in plants and mammals^{†‡}

Dale L Shaner*

USDA-ARS Water Management Unit, AERC Colorado State University, Fort Collins, CO 80523, USA

Abstract: Most modern herbicides have low mammalian toxicity. One of the reasons for this safety is that the target site for the herbicides is not often present in mammals. There are approximately 20 mechanisms of action that have been elucidated for herbicides. Of these, some do share common target sites with mammals. The mechanisms include formation of free radicals, protoporphyrinogen oxidase (PROTOX), glutamine synthetase (GS) and 4-hydroxyphenylpyruvate dioxygenase (HPPD). PROTOX, HPPD and GS inhibitors have been shown to inhibit these enzymes in both plants and mammals and there are measurable effects in mammalian systems. However, the consequences of inhibiting a common target site in plants can be quite different than in animals. What may be a lethal event in plants, eg inhibition of HPPD, can have a beneficial effect in mammals, eg treatment for tyrosinemia type I. These chemicals also have low mammalian toxicity due to rapid metabolism and/or excretion of the herbicide from mammalian systems.

Published in 2003 for SCI by John Wiley & Sons, Ltd.

Keywords: toxicology; mechanism of action; site of action; disease; tyrosinemia; porphyria

1 INTRODUCTION

Herbicides are the most widely used class of pesticides, accounting for more than 60% of all pesticides applied in agriculture.¹ One of the primary concerns about the use of pesticides is their effect on non-target organisms, with particular emphasis on mammalian toxicity. Most herbicides have no or limited toxicity on mammals (Table 1). In many cases this low toxicity is related to the herbicide target site, since these chemicals affect biochemical pathways that do not exist in mammals, such as photosynthesis, essential amino-acids biosynthesis or chlorophyll biosynthesis. In the search for new herbicides, researchers often reject chemicals that affect metabolic pathways that are shared by mammals and plants. However, this rejection may not be justified. There are very effective herbicides that do affect common target sites between plants and mammals and also have low mammalian toxicity. This review will examine those herbicides that affect common target sites between plants and mammals to discuss their relative activity in plants and mammals and how this determines their toxicity to both classes of organism. There are factors (eg absorption, distribution, metabolism, excretion) besides site of action that determine herbicide toxicity to an organism, and these factors can be manipulated to overcome potential toxicity.

2 RELATIVE TOXICITY OF HERBICIDES

There are approximately 20 different target sites for herbicides.² Table 1 compares 11 different sites of action of some of the most widely used herbicides. Approximately half of these herbicide classes act at sites of action that can be found in both plants and mammals. However, most of these herbicides have very low mammalian toxicity based on the acute toxicity, NOEL (no adverse effect level) and label statements.

3 LIPID BIOSYNTHESIS INHIBITORS

3.1 Acetyl-CoA carboxylase inhibitors

There are two major classes of herbicide that are widely used to control grasses, the cyclohexanediones and the aryloxyphenoxypropionates. These herbicides kill plants by inhibiting acetyl-CoA carboxylase (ACCase).³ ACCase catalyzes the first step in fatty acid biosynthesis and produces malonyl-CoA, a pathway which exists in both plants and mammals (Fig 1). Plants appear to have two forms of this enzyme.⁴ One is located in the plastids and is responsible for fatty acid biosynthesis, and the other is in the cytosol. The enzyme located in the plastid can occur in two forms. In grasses, such as wheat and maize, ACCase is a high molecular weight, multi-domain enzyme, whereas in broadleaf species (eg pea, soybean, and

* Correspondence to: Dale L Shaner, USDA-ARS Water Management Unit, AERC Colorado State University, Fort Collins, CO 80523, USA
E-mail: shaner@wmu.aerc.colostate.edu

[†]Based on a presentation at the 10th IUPAC International Congress on the Chemistry of Crop Protection: Innovative Solutions for Healthy Crops, held on 4–9 August, 2002, in Basel, Switzerland

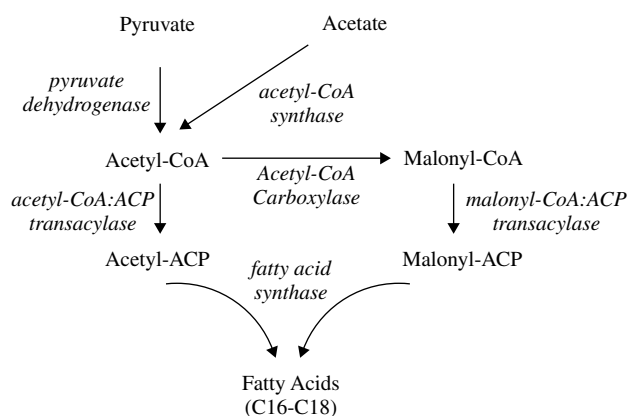
[‡]This article is a US government work and is in the public domain in the USA

(Received 1 May 2003; revised version received 29 May 2003; accepted 9 July 2003)

Published online 3 November 2003

Table 1. Herbicide target sites and mammalian toxicity (toxicity data compiled from WSSA Herbicide Handbook⁵⁹)

Mechanism of action	Herbicide family	Present in mammals	Toxicity category	Oral toxicity category	Acute LD ₅₀ (rat) (mg kg ⁻¹)	NOEL (rat) (mg kg ⁻¹ d ⁻¹)
EPSPS inhibition	Glyphosate	No	Caution	IV	5400	>1400
ALS inhibition	Sulfonylurea	No	Caution	IV	>5000	165
	Imidazolinone	No	Caution	IV	>5000	500
PSII inhibition	Triazine	No	Caution	IV	3090	0.7
PDS inhibition	Pyridazinone	No	Caution	IV	>5000	19
Auxenic mimics	Phenoxyacetic acid	No	Caution	III	764	15
	Picolinic acid		Caution	IV	>5000	300
ACCase inhibition	Cyclohexanedione	Yes	Caution	III	1630	19
	Aryloxyphenoxy propionate		Caution	III	4096	10
HPPD inhibition	Triketone	Yes	Caution	IV	>5000	2.1
	Isoxazole		Caution	IV	>5000	1000
Protox inhibition	Aryltriazolinone	Yes	Caution	IV	>5000	226
	<i>N</i> -Phenylphthalimide		Caution	IV	>5000	70
	Diphenyl ether		Caution	IV	5960	10
Lipid biosynthesis	Acetanilide	?	Caution	III	2780	90
	Oxyacetamides		Caution	III	1356	1.7
GS inhibition	Glufosinate	Yes	Warning	III	2170	0.4
Electron Transfer	Bipyridilium	Yes	Danger	I	115	1.25

**Figure 1.** General schematic of fatty acid biosynthesis.

tobacco) it exists as a multi-subunit enzyme. The cytosolic form of ACCase is a multi-subunit enzyme. The herbicidal ACCase inhibitors specifically inhibit the multi-domain enzyme that is found specifically in the Gramineae. These herbicides have little or no activity on the forms of ACCase found in other plant genera, so they can be used selectively to control grasses in broadleaf crops.

ACCase plays a vital role in mammalian systems for fatty acid biosynthesis, the enzyme existing in two isozymic forms.⁵ This enzyme is highly regulated in the brain and non-neural tissue. None of these forms are inhibited by cyclohexanediones or aryloxyphenoxypropionates. Although these herbicides affect common target sites in plants and mammals, they have no effect on ACCase in mammals.

It is interesting that certain parasites, such as *Toxoplasma* and *Plasmodium*, have non-photosynthetic plastids, known as apicomplexans, that contain the same multi-domain form of ACCase as found in grasses, and the herbicides that are effective in plants

are also active on these parasites.⁶ Such compounds, or ones with a similar mechanism of action, could potentially be used as control agents for these parasites.⁷

3.2 Very-long-chain fatty acid biosynthesis inhibitors

The acetanilides and oxyacetamides are two classes of herbicide that appear to act at the same target site. Early observations showed that these two herbicides classes interfered with lipid biosynthesis, particularly with the formation of leaf waxes.⁸ However, the actual site of action was not known, although it was suspected that the herbicides might be interfering with acetyl-CoA in some manner, since it had been shown that the acetanilides could alkylate CoA *in vitro*. Weisshaar *et al*⁹ reported that the first effect of chloroacetamide herbicides was a reduction in acetate incorporation into fatty acids and polar lipids.

In plants, fatty acid synthase (FAS), which is localized in the plastid, synthesizes fatty acids through a four-step process that condenses an acyl-ACP (acyl-carrier protein) with one molecule of malonyl-ACP, elongating the fatty acid chain stepwise by two methylene groups until the chain length reaches 16 or 18 carbon atoms. Some of this transesterified palmitic- or stearic-CoA is then used for lipid synthesis within the organelle, but the majority of these compounds are exported to the endoplasmic reticulum in the cytosol where they are further modified.^{10,11} One of the enzymes involved in these modifications is fatty acid elongase that produces very-long-chain fatty acids (C20-C36; VLCFA), which add additional carbons to the fatty acid chain via a process very similar to FAS but which utilizes malonyl-CoA instead of malonyl-ACP (Fig 2). These long-chain fatty acids are stabilizing constituents in the plasma membrane of

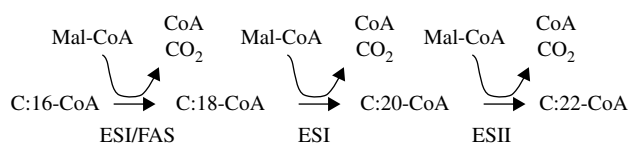


Figure 2. Very-long-chain fatty acid biosynthesis. FAS—fatty acid synthase; ES1—Elongase 1, ES2—Elongase 2. Modified from Schmalfluss *et al.*¹⁸

higher plants and are a major component of cuticular waxes.¹² In mammals, fatty acid elongation greater than C18 also occurs, primarily on the endoplasmic reticulum, and utilizes CoA derivatives, as is found in plants. In mammals, long-chain fatty acids are important for membrane phospholipids and for neural growth and myelination.¹³

Thiocarbamate herbicides appear to be pro-herbicides that are activated to sulfoxide metabolites in the plant. Kern *et al.*¹⁴ showed that the mechanism of triallate resistance in wild oats is due to differences in the rate of sulfoxidation. Wild oats produce much higher levels of triallate-sulfoxide than do resistant biotypes. Rashid *et al.*¹⁵ compared the effects of triallate on fatty acid composition in susceptible and resistant wild oat populations and found that the fatty acid levels of C18 and longer decreased with triallate treatment in susceptible plants, but there was no effect in resistant plants. Baldwin *et al.*¹⁶ found that pebulate decreased the incorporation of ¹⁴C-acetate into C22 and longer fatty acids in barley and wild oats and that this effect could be reversed by the safener, dichlormid.

Matthes *et al.*¹⁷ reported that ¹⁴C-malonyl incorporation in VLCFA was inhibited by *S*-metolachlor in cucumber and barley while fatty acid biosynthesis was not affected. Schmalfluss *et al.*¹⁸ examined the effect of metazochlor on ¹⁴C-malonyl incorporation into VLCFA in leeks and found a dose-dependent decrease in incorporation of label into C22–C24 fatty acids. They also found that *S*-metolachlor could inhibit elongation of 18:0-CoA *in vitro* ($I_{50} = 5$ mM), whereas *R*-metolachlor had no activity. Researchers in this laboratory have shown that other acetanilide herbicides also stereospecifically inhibit VLCFA elongation.^{19,20} The actual mechanism of inhibition of these fatty acid elongases has yet to be elucidated, but preliminary evidence suggests that the herbicides may bind irreversibly to the enzyme.¹⁸

The acetanilide and thiocarbamate herbicides are relatively non-toxic to mammals but some effects have been noted. Molinate, a thiocarbamate, has caused testicular lesions in rats with a single dose, after sulfoxidation within the organism.²¹ The lesion was characterized by failed spermiation and phagocytosis of spermatids. In a 2-year rat study, metolachlor, an acetanilide, caused the wasting of testicles at doses of 150 mg kg⁻¹ day⁻¹.²² Acetochlor has also been shown to cause testicular toxicity in male dogs given 10 and 50 mg kg⁻¹ day⁻¹ with a decrease in testes weight, atrophy and degeneration of seminiferous tubules and hypospermia.²³ There were also effects on the kidneys

and severe neurological effects at 50 mg kg⁻¹ day⁻¹ consisting of abnormal head movements, stiffness and rigidity of hindlimbs, ataxia tremor and other symptoms. These effects were accompanied by histopathological findings in the vermis cerebellum.

The toxic effect of the sulfoxide metabolite of molinate was attributed to inhibition of esterase activity, which decreased plasma and testicular testosterone concentrations.²⁴ However, this metabolite seems to be selectively produced in rodents and is not found in other mammals, including humans.²⁴

No connection has ever been made between the toxic effects of acetanilides and thiocarbamates on mammals and inhibition of VLCFAs. However, very-long-chain polyunsaturated fatty acids (>24) are normally found in excitatory tissues, and myelin-deficient mouse mutants have very low fatty acid elongation activity.²⁵ In addition, very-long-chain fatty acids are highly important in rat sperm maturation.²⁶ During their transit from the caput to the cauda segments of the epididymis, rat spermatozoa lipid content and composition change significantly. The proportions of oleate and linoleate fatty acids decrease and there is an increase in the longer-chain fatty acids (C20–C24) as well as the uncommon long-chain polyenoic fatty acids of the n-9 series. It might be highly informative to determine whether these two classes of herbicides inhibit very-long-chain fatty acid biosynthesis in mammals as well as in plants, and to see whether there is any connection between the mammalian toxicity of these chemicals and very-long-chain fatty acid synthesis.

4 GLUTAMINE SYNTHETASE

Glutamine plays a vital role in both plants and mammals, and glutamine synthesis depends on glutamine synthetase (GS). In plants GS is critical for the assimilation of ammonia and in recycling ammonia released during photorespiration (Fig 3). In mammals, GS is located throughout the organism and has two roles. It regulates ammonia levels in the animal, and it is critical for the recycling of glutamate, a neurotransmitter, in the brain (Fig 4).

Several compounds have been found which are potent inhibitors of GS in both plants and mammals. The first to be discovered was methionine sulfoxamine

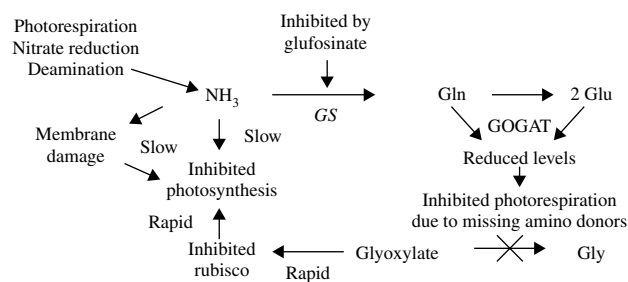


Figure 3. Schematic of mechanism of action of glufosinate in plants. Modified from Wild and Wendler.³¹

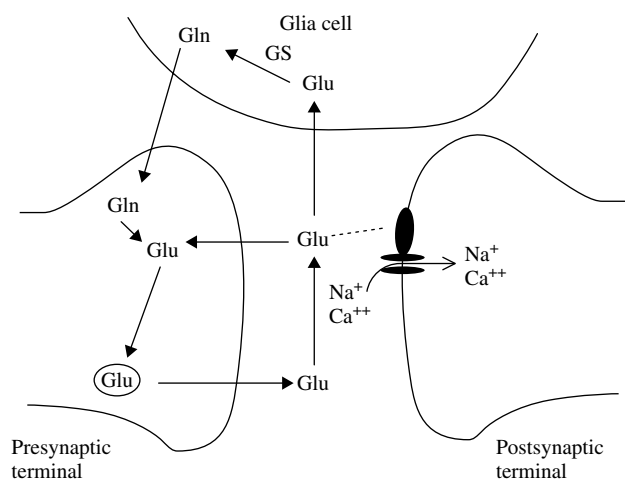


Figure 4. Role of glutamate and recycling by GS in brain. Modified from Palmada and Centelles.⁶⁰

(MSO) as a byproduct of the bleaching process of wheat in the late 1950s. Animals fed this wheat developed abnormal behavior, including seizures.²⁷ MSO was demonstrated to be responsible for these effects due to inhibition of GS in the brain.²⁸ MSO also has herbicidal activity via the inhibition of GS.²⁹

In the 1980s a natural product, bialophos, was isolated from *Streptomyces* spp.²⁹ Bialophos was found to be inactive at the molecular level, but in the plant it is metabolized to another product, phosphinothricin (PPT), which proved to be a more potent inhibitor of GS than MSO in both plants and animals. Bialophos was registered as a non-selective herbicide in Japan but was later replaced by a synthetic form of PPT, glufosinate, which is registered for use as a non-selective herbicide in several countries.²⁹ Animal studies with glufosinate showed that administration of the herbicide resulted in a measurable inhibition of GS in the brain, liver and kidney³⁰ but the effect was reversible. However, there was little increase in the levels of ammonia, unlike the effect seen in plants.

In plants the toxicity of these GS inhibitors appears to be due to multiple, indirect effects of the herbicide (Fig 3). Ammonia levels rise very rapidly in treated tissue and these high levels of ammonia may cause slow toxicity due to the uncoupling of photosynthesis. However, the primary effect of GS inhibitors appears to be due to the rapid increase in the levels of glyoxylate arising from the loss of glutamine, which acts as the amino donor to convert glyoxylate to serine in photorespiration. Glyoxylate is an inhibitor of ribulose-1,5-bisphosphate carboxylase, a key enzyme in carbon fixation in photosynthesis. Inhibition of carbon fixation reduces the pools of ADP and other compounds that are normally reduced by PSI, leading to the dissipation of light energy absorbed by photosynthetic pigments by destructive photo-oxidation of membranes in the light.³¹ In mammals the primary toxicological response to GS inhibitors is changes in neurological behavior, indicating that the CNS is the primary target site.³² GS is located

throughout the organism, but is at particularly high levels in the astrocytes in the brain. In the organs (eg muscles, liver) the primary role of GS appears to be regulation of ammonia levels.^{33,34} In liver, GS is confined to a small population of cells that form a continuous layer around the central vein.³³ In the brain, GS is critical for nerve function. Glutamate is the major fast excitatory neurotransmitter in the brain.³⁴ However, glutamate is also a neurotoxin and needs to be rapidly converted to glutamine via the glutamate/glutamine cycle.³⁵ In a normal cell, glutamate is rapidly absorbed by astrocytes, which surround the synapses, where it is converted to glutamine via GS. Then the glutamine moves back into the neuron (Fig 4). GS activity is also very high in the caput and cauda regions of rat epididymis, with the specific GS activity in the caput being 27-fold higher than in the cauda and similar to the levels found in the brain.³⁶

Methionine sulfoxamine is routinely used as a means to decrease GS activity in mammals to determine the role of glutamine and ammonia levels in various physiological functions. Patients with advanced liver disease lose the ability to process ammonia and this appears to lead to an accumulation of glutamine in the astrocytes in the brain that, in turn, leads to accumulation of water and swelling.³⁷ Studies^{38,39} have shown that MSO pretreatment could prevent this swelling in astrocytes in mice in which hyperammonemia was induced. MSO could have clinical utility in alleviating brain swelling that is associated with acute liver disease by preventing the accumulation of glutamine.

There have been cases where people have ingested large quantities of glufosinate in suicide attempts. Much of the toxicity of these ingestions has been related to the surfactant that is included in the formulation of the herbicide rather than to the active herbicide.⁴⁰ There are some neurological effects (eg short-term memory loss) noted in these suicide cases, suggesting that high concentrations of the herbicide may have toxic effects in the brain through inhibition of GS.⁴¹ However, work by Blin *et al*⁴² showed that chronic inhibition of GS in the brain by MSO was not associated with impairment of learning and memory in mice. In mammals, glufosinate is rapidly excreted from the system and does not appear to be able to cross the blood-brain barrier, so the effects of glufosinate on GS in the brain is probably minimal.³⁰

5 PORPHYRIN BIOSYNTHESIS

Porphyryn biosynthesis is critical in plants and animals for the production of chlorophyll and heme. Protoporphyrinogen IX oxidase (PROTOX) catalyzes the last common step in the biosynthesis of chlorophyll and heme and is important in both plants and animals (Fig 5).⁴³ A number of herbicides have been identified as acting through the inhibition of PROTOX in plants. These include the diphenyl

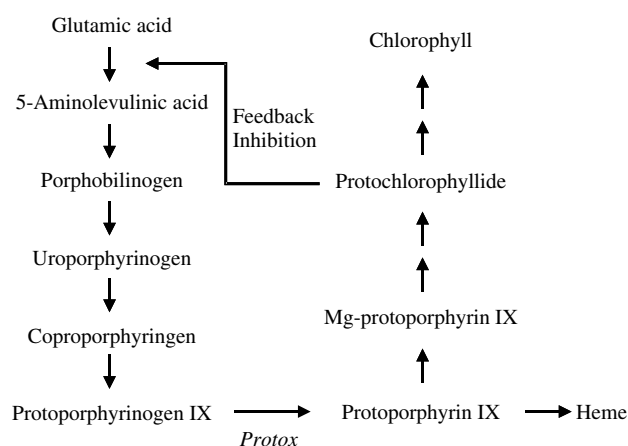


Figure 5. General biosynthetic pathway of porphyrin biosynthesis. Modified from Duke and Rebeiz.⁴³

ethers, *N*-aryltetrahydrophthalimides, oxadiazon and flumioxazin. These herbicides are potent inhibitors of PROTOX from both plants and animals.⁴⁴

In plants the toxicity of PROTOX inhibitors is light dependent, due to the accumulation of protoporphyrin IX in the cytoplasm.⁴⁵ PROTOX is located in the chloroplast and, when this enzyme is inhibited, protoporphyrinogen IX rapidly accumulates and then diffuses out of the chloroplast to the cytoplasm. In the cytoplasm, protoporphyrinogen IX is oxidized to protoporphyrin IX (Proto IX) via an unknown oxidase that is insensitive to these herbicides. Proto IX readily interacts with oxygen and light to form singlet oxygen, which causes lipid peroxidation and cell membrane disruption. The rate of protoporphyrin IX accumulation is enhanced by the deregulation of the porphyrin pathway due to the loss of protochlorophyllide that normally feeds back and regulates the conversion of glutamate to delta-aminolevulinic acid.

Protoporphyrinogen IX oxidase is also an important enzyme in mammals. A genetic disease in humans, variegate porphyria, is due a deficiency of PROTOX. Individuals with this genetic defect accumulate high levels of Proto IX and have heme deficiencies. However, many people carrying this defect can be asymptomatic. Feeding studies with various PROTOX inhibitors show that these herbicides cause an increase in the levels of porphyrins in the liver, bile and feces but the levels return to normal once the herbicides are removed from the diet.⁴⁶

Toxicology studies with PROTOX inhibitors have shown that certain chemicals cause embryo lethality, teratogenicity and growth retardation in rats but not in other mammals such as rabbits.⁴⁷ In these studies it was shown that the effect of 30 mg kg⁻¹ of S-52482, a phenylimide PROTOX inhibitor, on embryo development in rats was correlated with the accumulation of Proto IX in the embryo with a concomitant loss of heme. However, 3000 mg kg⁻¹ of S-52482 caused no accumulation of Proto IX in rabbit embryos and there was no adverse effect

on the embryos. The authors concluded that this difference was due to the relative sensitivity of PROTOX in rats versus rabbits. Thus, the effects of PROTOX-inhibiting herbicides on mammals is species-dependent. The mammalian toxicity of these herbicides appears to be minimal at the rates they are used.

There may be a potentially useful application of PROTOX inhibitors in human disease as a treatment for destroying cancer cells via photodynamic therapy (PDT). Currently PDT is done by administering photosensitizers to patients and attempting to establish high concentrations in the tumors. These tumors are then exposed to irradiation with light with the appropriate wavelength to activate the photosensitizers and destroy the cells.⁴⁸ Proto IX is an extremely effective photosensitizer, but it cannot be used since it does not accumulate within tumors after parenteral administration. Halling *et al*⁴⁸ showed that PROTOX inhibitors could cause the accumulation of Proto IX within tumor cells. The levels reached after treatment with certain PROTOX analogs was tenfold higher than the critical levels needed for effective PDT. The use of PROTOX inhibitors for PDT is being explored further.

6 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE (HPPD)

The triketones and isoxazoles are two relatively new classes of herbicides that inhibit carotenoid biosynthesis in plants via a unique mechanism. Plants treated with these herbicides show all the symptoms normally associated with inhibition of phytoene desaturase (PDS), the last enzyme in the carotenoid biosynthetic pathway that converts phytoene to carotenoids, with the loss of carotenoids and the accumulation of phytoene.⁴⁹ However, these herbicides also cause the loss of plastoquinones and alpha-tocopherol. Furthermore, supplying the plant with homogentisic acid can prevent the toxicity of these herbicides, indicating that the site of action of these herbicides is 4-hydroxyphenylpyruvate dioxygenase (HPPD), which converts 4-hydroxyphenylpyruvic acid to homogentisic acid. Homogentisic acid is the precursor that leads to the biosynthesis of the plastoquinones and alpha-tocopherol (Fig 6). Plastoquinones are vital cofactors for phytoene desaturase and their loss results in the inhibition of PDS and a decrease in carotenoid levels.

4-Hydroxyphenylpyruvate dioxygenase plays a vital role in mammalian systems. It is important for the catabolism of tyrosine (Fig 6). The herbicidal HPPD inhibitors have also been shown to inhibit mammalian HPPD. Rats treated with 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC), a triketone, accumulated tyrosine and excreted 4-hydroxyphenylpyruvate and 4-hydroxyphenyllactate due to the inhibition of HPPD.⁵⁰ One of the toxic effects of NTBC in rats is the development

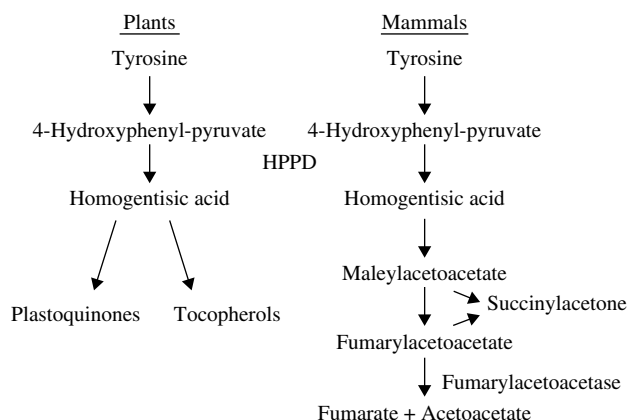


Figure 6. Role of HPPD in plants and mammals. Modified from Lock *et al.*⁵¹ and Pallett *et al.*⁴⁹

of corneal lesions. These lesions appear to be due to the accumulation of tyrosine in the aqueous humor of the eye. The effect is particularly acute if the animals were fed a low protein diet.⁵¹ However, this effect seems to be peculiar to rats. Similar lesions have not been observed in rabbits or humans although HPPD inhibitors will cause highly elevated levels of tyrosine in the blood.⁵² There is a genetic disease, tyrosinemia type I, which is caused by the loss of fumarylacetoacetase, an important enzyme in tyrosine catabolism (Fig 6). People suffering from this disease accumulate hepatotoxic and nephrotoxic compounds, such as succinylacetone, and the only effective treatment has been a liver transplant. However, it has been shown that these HPPD inhibitors can alleviate the symptoms of this disease by preventing the catabolism of tyrosine. The high levels of tyrosine are readily excreted and there do not appear to be any toxic side-effects. Treatment with NTBC has been used with great success to treat this disease and there have been no side-effects other than an increase in blood tyrosine levels.⁵³

7 REACTIVE OXYGEN GENERATORS

Bipyridilium herbicides, paraquat and diquat, kill plants by accepting electrons from Photosystem I and transferring those electrons to oxygen, producing reactive oxygen species that then peroxidize lipids in membranes (Fig 7). This peroxidation, in turn, disrupts the membranes and cause rapid plant death.⁵⁴

This acceptance of electrons by the bipyridiliums is not unique to plants (Fig 7). The bipyridilium herbicides can also accept electrons from the electron pathway in the mitochondria and then form reactive oxygen species that peroxidize membranes.⁵⁵ These herbicides are extremely toxic to mammals, including humans. The estimated lethal dose (via ingestion) in humans is 3–5 g.⁵⁶ In animals these herbicides appear to target the lungs where the chemical accumulates in the alveolar epithelium. Once in these tissues, these herbicides generate reactive oxygen species that appear to induce apoptosis in these cells.⁵⁷ There

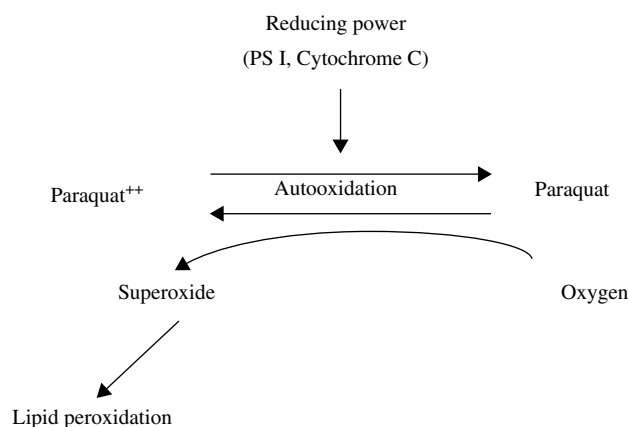


Figure 7. Mechanism of action of paraquat.

is no known antidote to paraquat ingestion, but the rapid administration of absorbents, such as clays or activated charcoal, can be an effective treatment since they can bind the herbicide and prevent absorption.⁵⁸ Most deaths related to paraquat are due to deliberate ingestion of the herbicide in suicide attempts or to accidental ingestion from unmarked containers.

8 OTHER FACTORS AFFECTING HERBICIDE TOXICITY

As discussed above, there are a number of herbicides that affect similar target sites in plants and mammals, and yet most of these herbicides, with the exception of the bipyridiliums, have minimal toxicity to mammals. There are many reasons for this difference between plants and mammals. First, most commercially available herbicides are rapidly metabolized and excreted by mammals. The residence time of many herbicides in mammals is less than 72 h (Table 2). Since the herbicides do not accumulate in mammalian tissue, they cannot affect the biosynthetic pathways.

Second, the effects of inhibiting a particular enzyme in plants can be quite different than in mammals. For example, PROTOX inhibitors cause the accumulation of Proto IX which then absorbs light energy and produces reactive oxygen species that disrupt membranes. In mammals, the accumulation of Proto IX also occurs in the liver and bile, but these organs are not normally exposed to high light intensities, unlike plant leaves, so there is little opportunity for the Proto IX to accept light energy and produce reactive oxygen. Similarly, inhibiting HPPD in plants disrupts carotenoid biosynthesis, which leads

Table 2. Excretion of selected herbicides from mammals

Herbicide	Excretion in 24–72 h (%)	Reference
Glufosinate	>90	32
Mesotrione	75	61
Oxyfluorfen	>90	62
Paraquat	>90	56

to the loss of chlorophyll and plant death. In mammals, inhibition of HPPD results in the accumulation of tyrosine, which in most species has little toxic effect.

REFERENCES

- Zimdahl RL, My view. *Weed Sci* 50:687 (2002).
- Heap I, The international survey of herbicide resistant weeds, Online, Internet, available at www.weedscience.com (2002).
- Burton JD, Acetyl-Coenzyme A carboxylase inhibitors, in *Herbicide activity: Toxicology, biochemistry and molecular biology*, ed by Roe RM, Burton JD and Kuhr RJ, IOS Press, Washington, DC, USA pp 187–205 (1997).
- Konishi T, Shinohara K, Yamada K and Sasaki Y, Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. *Plant Cell Physiol* 37:117–122 (1996).
- Spencer EB, Antonella B, Widmer J and Witters LA, Brain acetyl-CoA carboxylase: isozymic identification and studies of its regulation during development and altered nutrition. *Biochem Biophys Res Commun* 192:820–825 (1993).
- Wilson RJM, Progress with parasite plastids. *J Mol Biol* 319:257–274 (2002).
- Ralph SA, D'Ombrain MC and McFadden GI, The apicoplast as an antimalarial drug target. *Drug Res Updates* 4:145–151 (2001).
- Fuerst EP, Understanding the mode of action of chloroacetamide and thiocarbamate herbicides. *Weed Technol* 1:270–280 (1987).
- Weisshaar H, Retzlaff G and Böger P, Chloroacetamide inhibition of fatty acid synthesis. *Pestic Biochem Physiol* 32:212–216 (1988).
- Cassagne C, Lessire R, Bessoule JJ and Moreau P, Plant elongases, in *The metabolism, structure and function of plant lipids*, ed by Stumpf PK, Mudd JB and Nes WD, Plenum Press, New York, pp 481–488 (1987).
- Ohlrogge JB and Jaworski JG, Regulation of fatty acid synthesis. *Annu Rev Plant Physiol Plant Mol Biol* 48:109–136 (1997).
- Post-Beittenmiller D, Biochemistry and molecular biology of wax production in plants. *Annu Rev Plant Physiol Plant Mol Biol* 47:405–430 (1996).
- Cook HW and McMaster CR, Fatty acid desaturation and chain elongation in eukaryotes, in *Biochemistry of lipids lipoproteins and membranes*, ed by Vance DE and Vance JE, Elsevier Science, London, pp 181–204 (2002).
- Kern AJ, Peterson DW, Miller EK, Colliver CC and Dyer WE, Triallate resistance in *Avena fatua* L is due to reduced herbicide activation. *Pestic Biochem Physiol* 56:163–173 (1996).
- Rashid A, Johnson CI, Khan AA and O'Donovan JT, Effects of triallate and difenzoquat on fatty acid composition in young shoots of susceptible and resistant *Avena fatua* populations. *Pestic Biochem Physiol* 57:79–85 (1997).
- Baldwin A, Francis D, Rogers HJ and Harwood JL, The inhibition of fatty acid elongation by pebulate can be effectively counteracted by the safener dichlormid. *Biochem Soc Trans* 28:650–651 (2000).
- Matthes B, Schmalfluss J and Böger P, Chloroacetamide mode of action, II: Inhibition of very long chain fatty acid synthesis in higher plants. *Z Naturforsch* 53:1004–1011 (1998).
- Schmalfluss J, Matthes B, Knuth K and Böger P, Inhibition of acyl-CoA elongation by chloroacetamide herbicides in microsomes from leek seedlings. *Pestic Biochem Physiol* 66:161–169 (2000).
- Couderchet M, Bocion PF, Chollet R, Seckinger K and Böger P, Biological activity of two stereoisomers of the *N*-thienyl chloroacetamide herbicide dimethenamid. *Pestic Sci* 50:221–227 (1997).
- Takahashi H, Ohki A, Kato S, Tanaka A, Sato Y, Matthes B, Böger P and Wakabayashi K, Inhibition of very-long-chain fatty acid biosynthesis by 2-chloro-*N*-(3-methoxy-2-thienyl)-2',6'-dimethylacetanilide, thienylchlor and its analogs. *Pestic Biochem Physiol* 71:140–146 (2001).
- Jewell WT, Hess RA and Miller MG, Testicular toxicity of molinate in the rat: metabolic activation via sulfoxidation. *Toxicol Appl Pharmacol* 149:159–166 (1998).
- Anon, US Environmental Protection Agency, Health Advisory Draft Report: Metolachlor, Office of Drinking Water, Washington, DC, pp 10–102 (1987).
- Anon, Acetochlor. Integrated Risk Information System, US Environmental Protection Agency, pp 1–10 (1993).
- de S Wickramaratne GA, Foster JR, Ellis MK and Tomenson JA, Molinate: rodent reproductive toxicity and its relevance to humans—a review. *Regul Toxicol Pharm* 27:112–118 (1998).
- Cook HW and McMaster CR, Fatty acid desaturation and chain elongation in eukaryotes, in *Biochemistry of lipids, lipoproteins and membranes*, (4th edn) ed by Vance DE and Vance JE, Elsevier Science BV, London, UK, pp 181–204 (2002).
- Aveldano MI, Rotstein NP and Vermouth NT, Lipid remodeling during epididymal maturation of rat spermatozoa. Enrichment in plasmalogen lipids containing long-chain polyenoic fatty acids of the n-9 series. *Biochem J* 283:235–241 (1992).
- Silver ML, Johnson RE, Kark RM, Klein JR, Monahan EP and Zevin SS, White bread and epilepsy in animals. *J Amer Med Assoc* 135:757–760 (1947).
- Campbell PN, Work TN and Mellanby E, Isolation of crystalline toxic factor from agonized wheat flour. *Nature (London)* 165:345–346 (1950).
- Lydon J and Duke SO, Inhibitors of glutamine biosynthesis, in *Plant amino acids: biochemistry and biotechnology*, ed by Singh BK, Marcel Dekker, New York, USA, pp 445–464 (1999).
- Hack R, Ebert E, Ehling G and Leist KH, Glufosinate ammonium—some aspects of its mode of action in mammals. *Food Chem Toxicol* 32:461–470 (1994).
- Wild A and Wendler C, Inhibitory action of glufosinate on photosynthesis. *Z Naturforsch* 48:369–373 (1993).
- Ebert E, Leist KH and Mayer D, Summary of safety evaluation toxicity studies of glufosinate ammonium. *Food Chem Toxicol* 28:339–349 (1990).
- Gebhardt R and Mecke D, Cellular distribution and regulation of glutamine synthetase in liver, in *Glutamine metabolism in mammalian tissues*, ed by Haussinger D and Sies H. Springer-Verlag, Berlin, Germany, pp 98–121 (1984).
- Fonnum F, Glutamate: a neurotransmitter in mammalian brain. *J Neurochem* 42:1–11 (1984).
- Pow D and Robinson S, Glutamate in some retinal neurons is derived solely from glia. *Neuroscience* 60:355–366 (1994).
- Kvidera MD and Carey GB, Glutamine synthetase activity in rat epididymis. *Proc Soc Exp Biol Med* 206:360–364 (1994).
- Haussinger D, Kircheis G, Fischer R, Schliess F and vom Dahl S, Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocytes swelling and low-grade cerebral edema? *J Hepatol* 32:1035–1038 (2000).
- Hawkins RA, Jessy J, Mans AM and De Joseph MR, Effect of reducing brain glutamine synthetase on metabolic symptoms of hepatic encephalopathy. *J Neurochem* 60:1000–1006 (1993).
- Takahashi H, Koehler RC, Brusilow SW and Traystman RJ, Inhibition of glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am J Physiol* 26:825–829 (1991).
- Koyama K, Koyama K and Goto K, Cardiovascular effects of a herbicide containing glufosinate and a surfactant: *in vitro* and *in vivo* analyses in rats. *Toxicol Appl Pharmacol* 145:409–414 (1997).
- Watanabe T and Sano T, Neurological effects of glufosinate poisoning with a brief review. *Human Exptl Toxicol* 17:35–39 (1998).
- Blin M, Crusio WU, Hevor T and Cloix J-F, Chronic inhibition of glutamine synthetase is not associated with impairment of learning and memory in mice. *Brain Res Bull* 57:11–15 (2002).

- 43 Duke SO and Rebeiz CA, Porphyrin biosynthesis as a tool in pest management: an overview, in *Porphyric pesticides: Chemistry, toxicology, and pharmaceutical applications*, ed by Duke SO and Rebeiz CA, American Chemical Society, Washington, DC, USA, pp 1–17 (1994).
- 44 Birchfield NB and Casida JE, Protoporphyrinogen oxidase of mouse and maize: target site selectivity and thiol effects on peroxidizing herbicide action. *Pestic Biochem Physiol* **57**:36–43 (1997).
- 45 Duke SO, Handihalli UB, Lee HJ and Duke MV, Protoporphyrinogen oxidase as the optimal herbicide site in the porphyrin pathway, in *Porphyric pesticides: chemistry, toxicology and pharmaceutical applications*, ed by Duke SO and Rebeiz CA American Chemical Society, Washington, DC, USA, pp 191–205 (1994).
- 46 Kritz J, Pleskot R, Santittrak J and Janousek V, Experimental hepatic porphyria induced by oxadiazon in male mice and rats. *Pestic Biochem Physiol* **42**:180–187 (1992).
- 47 Kawamura S, Kato T, Matsuo M, Katsuda Y and Yasuda M, Species difference in protoporphyrin IX accumulation produced by an *N*-phenylimide herbicide in embryos between rats and rabbits. *Toxicol Appl Pharmacol* **141**:520–525 (1996).
- 48 Halling BP, Yuhua DA, Fingar VF and Winkelmann JW, Protoporphyrinogen oxidase inhibitors for tumor therapy, in *Porphyric pesticides: Chemistry, toxicology and pharmaceutical applications*, ed by Duke SO and Rebeiz CA, American Chemical Society, Washington, DC, USA, pp 280–290 (1994).
- 49 Pallett KE, Little JP, Sheekey M and Veerasekaran P, The mode of action of isoxaflutole I. Physiological effects, metabolism and selectivity. *Pestic Biochem Physiol* **62**:113–124 (1998).
- 50 Ellis MK, Whitfield AC, Gowan LA, Auton T, Provan WM, Lock EA and Smith LL, Inhibition of 4-hydroxyphenylpyruvate dioxygenase by 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione and 2-(2-chloro-4-methanesulphonylbenzoyl)cyclohexane-1,3-dione. *Toxicol Appl Pharmacol* **133**:12–19 (1995).
- 51 Lock EA, Gaskin P, Ellis MK, Robinson M, McLean Provan W and Smith LL, The effect of a low protein diet and dietary supplementation of threonine on tyrosine and 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione-induced corneal lesions, the extent of tyrosinemia and the activity of the enzymes involved in tyrosine metabolism in the rat. *Toxicol Appl Pharmacol* **150**:125–132 (1998).
- 52 Holme E, Lindstedt S and Lock EA, Treatment of tyrosinemia type I with an enzyme inhibitor (NTBC). *Int Pediatr* **10**:41–43 (1995).
- 53 Al-Dhalimy M, Overturf K, Finegold M and Grompe M, Long term therapy with NTBC and tyrosine-restricted diet in a murine model of hereditary tyrosinemia type I. *Mol Genet Metab* **75**:38–45 (2002).
- 54 Chase CA, Bewick TA and Shilling DG, Characterization of paraquat resistance in *Solanum americanum* Mill. II. Evidence for a chloroplast mechanism. *Pestic Biochem Physiol* **60**:23–30 (1998).
- 55 Bus JS, Cagen SZ, Olgaard M and Gibson JE, A mechanism of paraquat toxicity in mice and rats. *Toxicol Appl Pharmacol* **35**:501–513 (1976).
- 56 Smith LL, The toxicity of paraquat. *Adv Drug React Acut Poison Rev* **1**: 1–17 (1988).
- 57 Cappelletti G, Grazia Maggioni M and Maci R, Apoptosis in human lung epithelial cells: triggering by paraquat and modulation by antioxidants. *Cell Biol International* **22**:671–678 (1998).
- 58 Vanholder R, Colardyn F, DeReuck J, Praet M, Lameire N and Ringoir S, Diquat intoxication: report of two cases and review of the literature. *Am J Med* **70**:1267–1271 (1981).
- 59 Vencill WK, *Herbicide handbook*, 8th edn, Weed Science Society of America, Lawrence, KS, USA, 493 pp (2002).
- 60 Palmada M and Centelles JJ, Excitatory amino acid neurotransmission. Pathways for metabolism, storage, and reuptake of glutamate in brain. *Frontiers of biosciences*, July 20 1998, pp 701–718 (1998).
- 61 Anon, Mesotrione; Pesticide Tolerance. US Environmental Protection Agency, Federal Register Environmental Documents, 40 CFR Part 180 (OPP-301138; FRL-6787-71) RIN 2070-AB78 (2001).
- 62 Anon, Oxyfluorfen. Toxicology Chapter for RED, US Environmental Protection Agency, available at www.epa.gov/REDS/oxyfluorfen_red.pdf (2002).