

## Biochemistry of herbicides and their relationships with animal toxicity

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### Summary

Pesticides, in general, perform their activity by acute toxicity to the target organisms. The toxicity is called the mode of action, which may be clarified from biochemical activity, these have a close relationship with acute toxicity to animals. On the other hand, the selectivity between target and non-target organisms may also be explained by biochemical activity.

In this review, the action of the main herbicides will be biochemically explained, including from the viewpoint of acute toxicities to animals.

### Introduction

The information obtained from researching mode of action of herbicides can be utilized for two purposes. In the first case, screening new herbicides the target enzyme or receptor may become the target for candidate compounds. On the other hand, the information can be used to estimate animal toxicity and environmental effects of the herbicide. Together with the biochemical and physiological mechanisms of herbicide selectivity, the mode of action is important information in understanding and utilizing herbicides. Modern pesticides are in general less toxic, used at lower dosages, highly selective and lower in cost and persistence. Pesticides perform their activity by acute toxicity to target organisms, then their mode of action has a direct relationship with acute toxicity to the animals. In this review, the mode of action of the main herbicides will be explained and related to animal toxicity.

**Disturbance of phyto-hormonal actions**  
During World War II, 2,4-D was developed

and its efficacy, herbicidal activity and especially its selectivity between graminaceous crops and broad-leaved weeds was found to be very useful. Thus began the modern age of herbicides.

Research on the mode of action of 2,4-D and indole acetic acid (IAA) has a long history. Although some molecular biochemical approaches can be found in the literature (10), we still have no conclusive evidence of its biochemical activity. Man-made, not natural, properties of such herbicides may increase resistance against metabolic degradation in plants. Also, the introduction of larger amounts of auxin type herbicides compared to the natural auxin compounds, make them effective as practical herbicides. The mode of action may be explained simply as the disturbance of phyto-hormonal activity in weeds.

The selectivity between crops and broad-leaved weeds may partially reflect the difference in mobility between the plants. When the mode of action is completely understood at the molecular level, a new selectivity mechanism between the two groups will be proposed.

Although auxin type herbicides show specific plant hormone action, and are assumed to have a low animal toxicity, the LD<sub>50</sub> (oral acute, rat) of 2,4-D and MCPA is 375 and 700 mg kg<sup>-1</sup>, respectively and are therefore relatively toxic.

In Japan, inclined barn-yards, such as mandarin orange farms, need ground cover plants to protect the soil from erosion. These should be cut several times to reduce the competition for mineral nutrients and water. To achieve this, non-selective foliar applied herbicides are used at present. However, the inhibitors of gibberellin biosynthesis for all weeds could be utilized.

### Inhibition of respiratory energy formation

Pentachlorophenol (PCP) was found to be effective on barnyard grass (*Echinochloa*

*oryzoides* (ARD.) Fritsh) in transplanted rice fields in Japan. Following the development of a granular formulation, PCP use expanded to over one million ha in 1964. However, after heavy rain, very severe fish toxicity was noted, and by the early 1970s its use suddenly decreased.

PCP severely inhibits oxidative phosphorylation in respiration as does 2,4-dinitrophenol (DNP). PCP stimulated the respiration of rice plant roots by 70 % at 20  $\mu\text{M}$ , which means ten times more activity than DNP (6). Inhibition of ATP formation causes an increase of ADP which stimulates the  $\text{O}_2$  uptake. The selectivity between rice and weeds was due to both the difference in growth stage and the granular formulation.

Because of their mode of action, the inhibitors of oxidative phosphorylation, PCP, DNOC, dinoseb, ioxynil, bromoxynil and organic tin herbicides have a high acute toxicity to mammals and fish. They should, therefore, not be used on sites connected with aquatic zones.

#### Formation of active oxygen by light (8)

Higher plants, both crops and weeds, are always exposed to a higher concentration of oxygen and to solar radiation in the day-time. These conditions are assessed as good and healthy by environmentalists, however, they are dangerous in plants because of the generation of active oxygen. Higher plants usually scavenge the active oxygen produced under such conditions through their own detoxifying systems. However if a chemical is used to accelerate the production of active oxygen in weeds, the weeds cannot detoxify it and thus, the chemical may be used as a herbicide.

Two examples of such herbicides are the bipyridiliums such as paraquat and diquat, and the diphenyl ethers such as chlornitrofen and acifluorfen-methyl. The former group produces a superoxide radical and the latter singlet oxygen.

Paraquat receives one electron from the photosynthetic electron transport system under conditions of light, producing a paraquat free radical, which is oxidized by triplet oxygen into original paraquat. Simultaneously the triplet oxygen is changed to a superoxide radical by receiving one electron. The active oxygen shows herbicidal activity. This herbicidal action can be inhibited by the presence of

Hill reaction inhibitors, darkness or absence of oxygen. Also, paraquat reduction into its free radical can be observed in animal tissues under appropriate conditions. It then shows very slow but strong acute toxicity also to animals, especially in the lung.

Diphenyl ether herbicides such as chlornitrofen and other compounds such as oxadiazon or chlorphthalim are active only under conditions of light (7). Both groups have quite different chemical structures, but show very similar modes of action. They can inhibit protoporphyrinogen IX oxidase by way of tetrapyrrole biosynthesis; this substrate being accumulated (5). The accumulated materials are auto-oxidized into protoporphyrin IX, which apparently accumulates in cells of weeds. These tetrapyrroles will photodynamically produce singlet oxygen which induces herbicidal activity in the weeds sprayed with such a herbicide.

The latter herbicides were developed to lower fish toxicity after the problems from PCP use in paddy fields in Japan. In practice, these herbicides showed lower animal toxicity, as shown in Table 1. One reason for such lower toxicity seems to be the requirement for light to produce singlet oxygen. Recently protoporphyrinogen oxidase, isolated from mouse mitochondria, was reported to be inhibited by these compounds. Our experiments show that in animals injected with these compounds, a small amount of tetrapyrroles is accumulated but that which is accumulated does not show any toxicity without light.

#### Inhibition of photosynthesis

There are two targets for photosynthetic inhibiting herbicides, one is the photosynthetic electron transport system and the other is the biosynthetic production of pigments. The electron transport system uses solar energy to produce ATP and NADPH to enable  $\text{CO}_2$  fixation. Biochemical research shows many chemical inhibitory possibilities in the transport system, however, practically only one is useful, point the herbicide binds with so-called 32-kDa D1 protein (1). This special protein attaches at a specific position in photosystem II and comprises 353 amino acids. Researchers using herbicide resistant mutants, have found that one of the binding sites for herbicides such as atrazine is serine at the 264th. Typical photosynthetic-inhibiting herbicides, such as

diuron (DCMU), simazine and others, have such modes of action.

On the other hand, many herbicides affect leaf pigment formation. Some directly inhibit of chlorophyll biosynthesis, such as pyrazolate; others inhibit the carotenoid

biosynthesis by causing loss of chlorophyll protection. Amitrole and methoxyphenone have such a mode of action.

In general, photosynthesis-inhibiting herbicides show very low animal toxicity, because animals do not photosynthesise, as shown by the LD<sub>50</sub>'s in Table 1.

*Table 1 The mode of action and acute toxicity of the main herbicides*

Mode of action (Action site)	Herbicides	Acute toxicity to rat (mg kg <sup>-1</sup> )
Inhibition of oxidative phosphorylation	PCP	210
	DNOC	40
	ioxynil	110
Hormonal disturbance	2,4-D	375
	MCPA	700
Formation of active oxygen		
	Superoxide formation	paraquat
Singlet oxygen formation	diquat	231
	chlornitrofen	10,800*
	chlomethoxynil	33,000
	bifenox	6,400
	oxadiazon	1,800
Photosynthesis		
	Electron transport system	simazine
Chlorosis	atrazine	3,080
	diuron(DCMU)	3,400*
	bromacil	5,200
	pyrazolate	9,550
	methoxyphenone	4,000
Amino acid biosynthesis		
	EPSP synthase	glyphosate
Glutamine synthase	bialaphos	7,300
	glufosinate	1,600
Acetolactate synthase	chlorosulfuron	5,900
	bensulfuron-methyl	11,000
	pyrazosulfuron-ethyl	5,000
	imazapyr	≥5,000
Dwarfing		
	Thiocarbamates	thiobencarb
Chloroacetamides	molinate	700
	butachlor	3,300
	pretilachlor	6,000
Acetyl coenzyme A carboxylase		
	Cyclohexanediones	alloxydim
Aryloxyphenoxypropionates	sethoxydim	3,200
	diclofop-methyl	557
	fluazifop-butyl	3,000

\* to mouse

### Inhibitors of biosynthesis of amino acids

Biosynthesis of essential amino acids are an increasingly important site of action for herbicides. Essential amino acids cannot be synthesized by humans. We can expect such herbicides to have lower toxicity to animals, as their sites of action inhibit such amino acid biosynthesis.

Glyphosate inhibits enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) which is essential for the biosynthesis of aromatic amino acids such as tryptophan, phenylalanine and tyrosine. On the other hand, bialaphos and glufosinate are glutamine synthase inhibitors, causing ammonia accumulation which is phytotoxic to weed cells.

Sulfonylurea and imidazolinone herbicides inhibit biosynthesis of branched chain amino acids, such as valine, isoleucine and leucine. Production of such amino acids is dependent on acetolactate synthase (ALS), the target of these herbicides. In our experiments (9), the sulfonylurea chlorsulfuron, completely inhibited the growth of tobacco cell culture at a concentration of 0.001 ppm while no adverse effect was observed in hamster tissue culture even at 100 ppm. This herbicide group shows very low animal toxicity (Table 1). Also, the sulfonylureas have extremely high biochemical activity. This enables extremely small dosages on farms or input into the environment. Bensulfuron-methyl and pyrazosulfuron-ethyl are sulfonylureas, and imazapyr an imidazolinone. Environmentalists should seriously consider these facts.

### Other modes of action

Dwarf malformed weeds are a typical symptom of the thiocarbamate and chloroacetamide herbicides. This mode of action is very important for the rice herbicides thiobencarb, molinate, esprocarb (thiocarbamates), and butachlor, pretilachlor (chloroacetamides). They inhibit the synthesis of lipids, isoprenoids, and other metabolic processes requiring coenzyme A (3), but the details are not clear. Animal toxicities of these compounds are relatively low ( $LD_{50}$ : thiobencarb: 1,900 and butachlor: 3,300 mg  $kg^{-1}$ ), however the reason is not clear either.

The grass-killers, cyclohexanediones and aryloxyphenoxypropionic acids, inhibit acetyl coenzyme A carboxylase, the second enzyme

common to both fatty acid and flavonoid biosynthesis pathways (2). The former herbicide group contains alloxydim, sethoxydim and clethodim, and the latter diclofop-methyl, fluazifop-butyl and quizalofop-ethyl. Monocotyledonous plants are very susceptible to these grass-killers, while broad-leaved crop plants are very tolerant. In general, animal toxicity of this group is also low, but the reason is not clear. Even higher plants demonstrate the above-described selectivity. Then, more differences in toxicity between plants and animals may be expected.

### Selectivity mechanisms of herbicides

The mode of action of herbicides and their selectivity between crop plants and weeds are mainly explained by biochemical reactions. Some of the selectivity can be explained by location on the target, or time when applied, or physiological differences in penetration or translocation. Biochemical selectivity may be classified in three ways; tolerance at the site of action, activation mechanism or inactivation. The last one is the most important, that is, when tolerant crop plants can inactivate or detoxify the herbicides, while weeds cannot.

Biotechnology is now producing some examples of herbicide resistant crops. Also, herbicide-safeners are being utilized to protect crop plants (4). In both areas biochemistry plays a very important role, and these processes will also contribute to the normal biochemistry of higher plants.

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