

chapter three

Pesticides interfering with processes important to all organisms

Energy production in mitochondria and the mechanisms behind cell division are very similar in all eukaryotic organisms. Furthermore, some inhibitors of enzymes have so little specificity that many different enzymes in a great variety of organisms may be targets. Many of the pesticides with such general modes of action have considerable historic interest. Not all of them are simple in structure, and many are used for purposes other than combating pests.

3.1 Pesticides that disturb energy production

3.1.1 Anabolic and catabolic processes

Green plants are anabolic engines that produce organic materials from carbon dioxide, other inorganic substances, water, and light energy. New organic molecules are made by anabolic processes, whereas organic molecules are degraded by catabolic processes. Plants are also able to degrade complicated organic molecules, but the anabolic processes dominate. Animals, bacteria, and fungi may be called catabolic engines. Their task is to convert organic materials back to carbon dioxide and water. Most of the energy from the catabolism is released as heat, but much is used to build up new molecules for growth and reproduction. Almost all of the energy required for these many thousand chemical reactions is mediated through adenosine triphosphate (ATP), which is broken down to adenosine diphosphate (ADP) and inorganic phosphate in the energy-requiring biosynthesis. ADP is again rebuilt to ATP with energy from respiration and glycolysis. The basic catabolic processes that deliver ATP are very similar in all organisms and are carried out in small intracellular organelles, the mitochondria. We should suppose, therefore, that pesticides disturbing the processes are

not very selective, which is indeed the case. We find very toxic and nonselective substances such as arsenic, fluoroacetate, cyanide, phenols, and organic tin compounds, but also substances with some selectivity due to different uptake and metabolism in various organisms. Examples are rotenone, carboxin, diafenthiuron, and dinocap.

3.1.2 *Synthesis of acetyl coenzyme A and the toxic mechanism of arsenic*

Acetyl coenzyme A (Ac-CoA) plays a central role in the production of useful chemical energy, and about two thirds of all compounds in an organism are synthesized via Ac-CoA. Degradation of sugars leads to pyruvate, which reacts with thiamine pyrophosphate, and the product reacts further with lipoic acid. The acetyl-lipoic acid reacts with coenzyme A to give Ac-CoA and reduced lipoic acid. Lipoic acid, in its reduced form, has two closely arranged SH groups that easily react with arsenite to form a cyclic structure that is quite stable and leads to the removal of lipoic acid (Figure 3.1). Arsenic is toxic to most organisms because of this reaction. It is not used much as a pesticide anymore, but in earlier days, arsenicals, such as lead arsenate, were important insecticides. Natural arsenic sometimes contaminates groundwater, which led to a tragedy in Bangladesh. Wells were made with financial support from the World Health Organization (WHO), but their apparent pure and freshwater was strongly contaminated with the tasteless and invisible arsenic and many were poisoned. In Europe, arsenic is perhaps best known as the preferred poison of Agatha Christie's murderers, but it is also valuable for permanent wood preservation, together with copper and other salts. This use, however, also seems to have been terminated because of arsenic's bad reputation as a poison and carcinogen.

3.1.3 *The citric acid cycle and its inhibitors*

3.1.3.1 *Fluoroacetate*

Fluoroacetate is produced by many plants in Australia and South Africa and has an important function as a natural pesticide for the plants. It is highly toxic to rodents and other mammals. In certain parts of Australia, where such plants are abundant, opossums have become resistant to fluoroacetic acid. Good descriptions are presented by several authors in Seawright and Eason (1993).

The mode of action of fluoroacetate is well understood: it is converted to fluoroacetyl-CoA, which is thereafter converted to fluorocitric acid. This structure analogue to citric acid inhibits the enzyme that converts citric acid to cis-aconitic acid, and the energy production in the citric acid stops. Citric acid, which accumulates, sequesters calcium. α -Ketoglutaric acid and therefore glutamic acid are depleted. These changes are, of course, detrimental for the organism. The nervous system is sensitive to these changes because glutamic acid is an important transmitter substance in the so-called

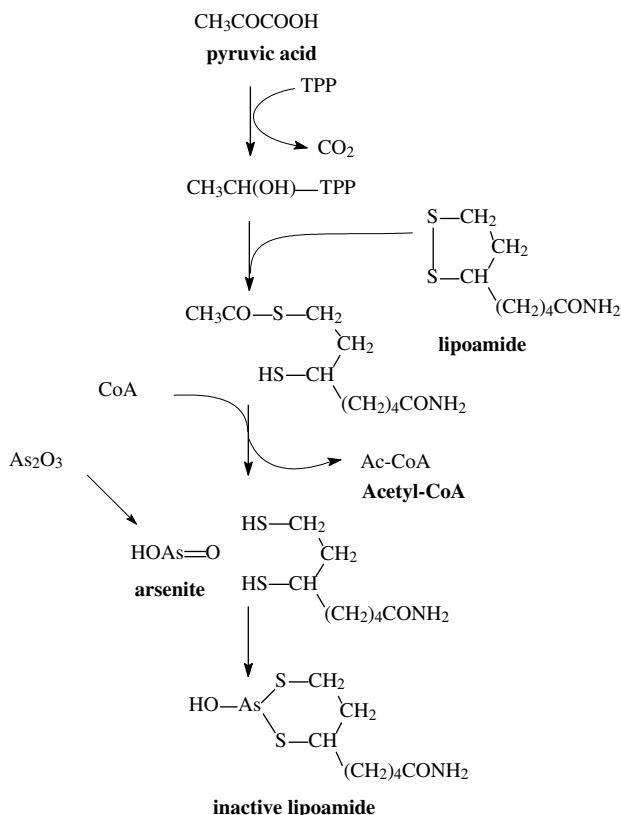
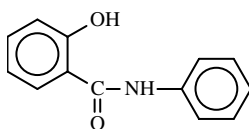


Figure 3.1 The mode of action of arsenic.

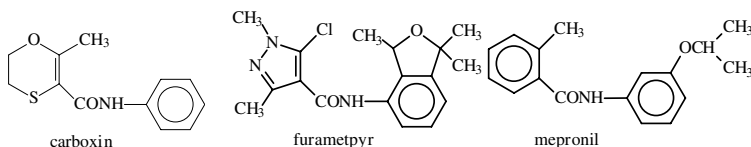
glutamnergic synapses, and calcium is a very important mediator of the impulses. Furthermore, the halt of aerobic energy production is very harmful.

3.1.3.2 Inhibitors of succinic dehydrogenase

Inhibitors of succinic dehydrogenase constitute an important group of fungicides. In 1966, carboxin was the first systemic fungicide to be marketed. A systemic pesticide is taken up by the organism it shall protect and may kill sucking aphids or the growing fungal hyphae. The older fungicides are active only as a coating on the surface of the plants and do not fight back growing mycelia inside the plant tissue. Carboxin and the other anilides, or oxathiin-fungicides, as they are often called, inhibit the dehydrogenation of succinic acid to fumaric acid — an important step in the tricarboxylic acid cycle. The toxicity to animals and plants is low in spite of this very fundamental mode of action. The fungicides in this group are anilides of unsaturated or aromatic carboxylic acids. The first compound in this group to be synthesized was salicylanilide, which since 1930 had a use as a textile protectant.



Other phenylamides with the same mode of action are fenfuram, flutalonil, furametpyr, mepronil, and oxycarboxin.

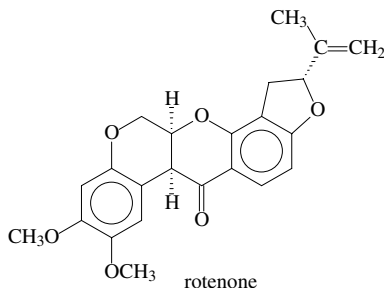


3.1.4 The electron transport chain and production of ATP

When compounds are oxidized through the tricarboxylic acid cycle (Figure 3.2) to carbon dioxide and water, electrons are transferred from the compounds to oxygen through a well-organized pathway, which ensures that the energy is not wasted and, more importantly, that electrons are not taken up by compounds that make them into reactive free radicals. The electrons are first transferred to nicotinamide-adenine dinucleotide (NAD⁺) and flavine adenine dinucleotide (FAD), and from these co-substrates the electrons are passed on to ubiquinone and further on to the cytochromes in the electron transport chain. Their ultimate goal is oxygen, which is reduced to water. The energy from this carefully regulated oxidation is used to build up a hydrogen ion gradient across the inner mitochondrial membrane, with the lower pH at the inside. This ion gradient drives an ATP factory.

3.1.4.1 Rotenone

Rotenone is an important insecticide extracted from various leguminous plants. It inhibits the transfer of electrons from nicotinamide-adenine (NADH) to ubiquinone.



It is also highly toxic to fish and is often used to eradicate unwanted fish populations, for instance, minnows in lakes before introducing trout, or

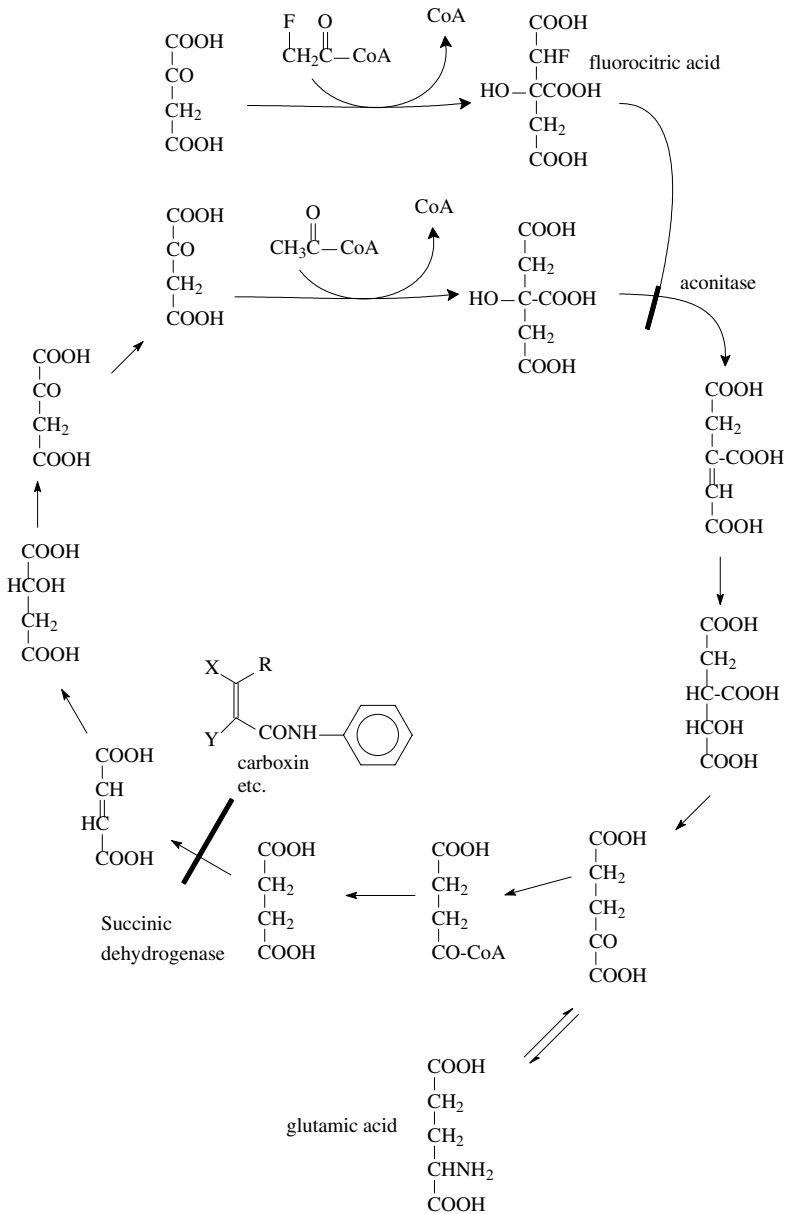


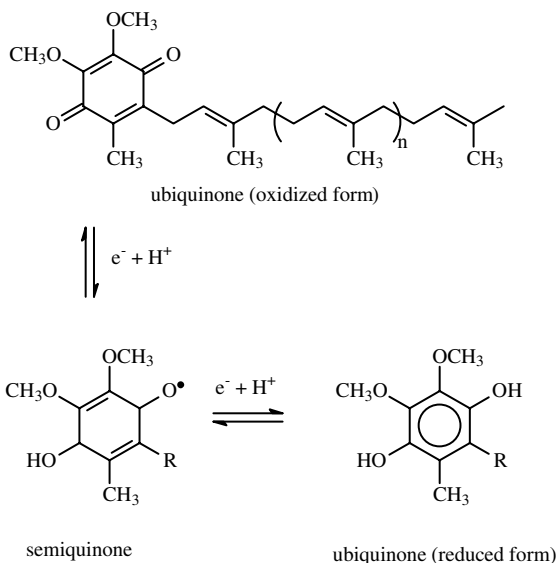
Figure 3.2 A simple outline of the citric acid cycle and the sites of inhibition by the insecticide/rodenticide fluoroacetic acid, and the fungicide carboxin.

to eradicate salmon in rivers in order to get rid of *Gyrodactylus salaricus*, an obligate fish parasite that is a big threat to the salmon population. The noninfected salmon coming up from the sea to spawn will not be infected if the infected fish present in the river have been killed before they arrive.

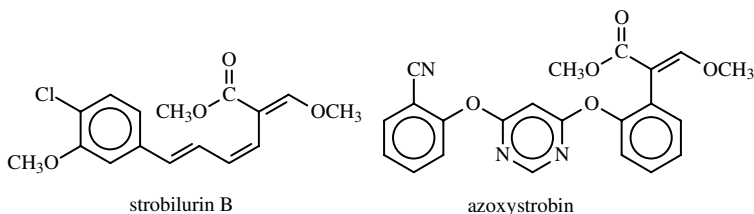
3.1.4.2 Inhibitors of electron transfer from cytochrome *b* to *c*₁

The strobilurins are a new class of fungicides based on active fungitoxic substances found in the mycelia of basidiomycete fungi. The natural products, such as strobilurin A and strobilurin B, are too volatile and sensitive to light to be useful in fields and glasshouses. However, by manipulating the molecule, notably changing the conjugated double bonds that make them light sensitive, with more stable aromatic ring systems, a new group of fungicides have been developed in the last decade. At least four are on the market (azoxystrobin, famoxadone, kresoxim-methyl, and trifloxystrobin). Their mode of action is the inhibition of electron transfer from cytochrome *b* to cytochrome *c*₁ in the mitochondrial membrane. They are supposed to bind to the ubiquinone site on cytochrome *b*.

The reactions inhibited by strobilurin fungicides:



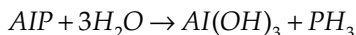
The fungicides are very versatile in the control of fungi that have become resistant to the demethylase inhibitor (DMI) fungicides described later. They have surprisingly low mammalian toxicity, but as with many other respiratory poisons, they show some toxicity to fish and other aquatic organisms. They may also be toxic to earthworms. In fungi they inhibit spore germination. The structures show the natural products strobilurin B and azoxystrobin, which has been marketed since 1996.



3.1.4.3 Inhibitors of cytochrome oxidase

Cyanide may still have some use against bedbugs and other indoor pests in spite of its high toxicity to man, but in the past it was used much more. In the 19th century, doctors prescribed it as a sedative and, of course, caused a lot of fatal poisoning (Otto, 1838). The recommended treatment was to let the patient breathe ammonia. Today we have very efficient antidotes, such as sodium nitrite and amyl nitrite. They cause some of the Fe^{2+} of hemoglobin to be oxidized to Fe^{3+} , which then binds the CN^- ion. Cyanide inhibits the last step in the electron transport chain catalyzed by cytochrome oxidase by binding to essential iron and copper atoms in the enzyme. Cyanide is very fast acting and blocks respiration almost totally.

Phosphine is used extensively as a fumigant and is very efficient in the control of insects and rodents in grain, flour, agricultural products, and animal foods. It is used to give continual protection during shipment of grain. The gas is flammable and very unstable and is changed into phosphoric acid by oxidation. By using pellets of aluminum phosphide at the top of the stored product, phosphine is slowly released by reacting with moisture. Other phosphine salts are also used. Phosphine is reactive and is probably involved in many reactions, but the inhibition of cytochrome oxidase is the most serious. The gas is very toxic to man, but residues in food cause no problems because it is oxidized rapidly.



3.1.4.4 Uncouplers

As discussed in Chapter 2, Section 1.4, uncoupling energy production and respiration is one of the fundamental toxic mechanisms. Weak organic acids or acid phenols can transport H^+ ions across the membrane so that energy is wasted as heat, and not used to produce ATP.

The name *uncouplers* arose from their ability to separate respiration from ATP production. Even when ATP production is inhibited, the oxidation of carbohydrates, etc., can continue if an uncoupler is present. Although the uncouplers are biocides, in principle toxic to all life-forms, many valuable pesticides belong to this group. However, few of them are selective, and they have many target organisms. The inner mitochondrial membranes are their most important sites of action, but chloroplasts and bacterial membranes will also be disturbed.

Figure 3.3 shows how weak acids can transport H^+ ions across the membrane.

Pesticides with this mode of action include such old products as the dinitrophenols (dinitroortho-cresol [DNOC], dinoterb, and dinoseb) and other phenols such as pentachlorophenol and ioxynil. DNOC is a biocide useful against mites, insects, weeds, and fungi. The mammalian toxicity is rather high, with a rat oral LD50 (lethal dose in 50% of the population) of 25 to 40 mg/kg of the sodium salt. The typical symptom is fever, which is

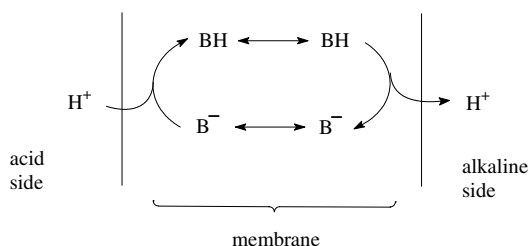
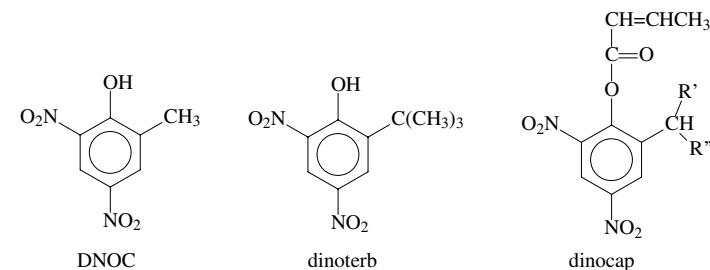


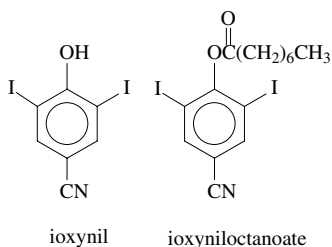
Figure 3.3 Transportation of H^+ ions across a biological membrane by a weak acid.

in accordance with its biochemical mode of action. The uncouplers have been tried in slimming treatments with fatal consequences.



Dinocap is an ester that is taken up by fungal spores or mites. It is hydrolyzed to the active phenol. It has low toxicity to plants and mammals. Dinocap is a mixture of several dinitrophenol esters, and the structure of one is shown.

Ioxynil is a more important uncoupler that is widely used as an herbicide. It acts in both mitochondria and chloroplasts. Bromoxynil is similar to the ioxynil, but has bromine instead of iodine substitutions.



3.1.5 Inhibition of ATP production

ATP is produced from ADP and phosphate by an enzyme, ATP synthase, located in the inner mitochondrial or chloroplast membrane. The energy is delivered from a current of H^+ ions into the mitochondrial matrix. Some important pesticides inhibit this enzyme, leading to a halt in ATP production.

Table 3.1 Diafenthiuron and Organotin Compounds Used as Pesticides

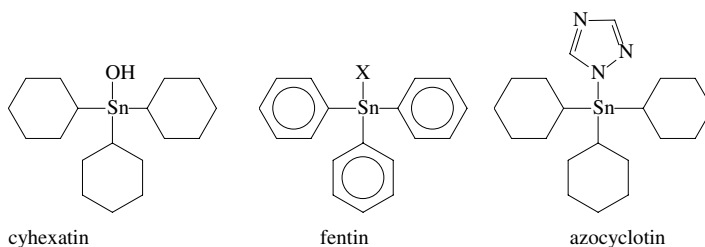
Pesticide	Fish (Various Species)	Daphnia	Rodents
	LC50 (24–96 h) (mg/l)	EC50 (48 h) (mg/l)	(Various Species or Sex) Oral LD50 (mg/kg)
Cyhexatin	0.06–0.55 (24 h)	—	540–1000
Azocyclotin	0.004 (96 h)	0.04	209–980
Fentin (acetate)	0.32 (48 h)	0.0003–0.03	20–298
Tributyltin	0.0021 (96 h)	0.002	—
Diafenthiuron	0.0013–0.004 (96 h)	<0.5	>2000

Note: LC50 = lethal concentration in 50% of the population; EC50 = effective concentration in 50% of the population.

Source: Data from Tomlin, C., Ed. 2000. *The Pesticide Manual: A World Compendium*. British Crop Protection Council, Farnham, Surrey. 1250 pp.

3.1.5.1 Organotin compounds

Organotin compounds have been used extensively as pesticides for special purposes. At least some of them owe their mode of action to the inhibition of ATP synthase in the target organism. Tricyclohexyltin (cyhexatin) and azocyclotin are used as selective acaricides. Cyhexatin is toxic to a wide range of phytophageous mites, but at recommended rates it is nontoxic to predacious mites and insects. Triphenyltin acetate or hydroxide may be used as fungicide, algicide, or molluscicide. The toxicity of these compounds to fish is very high, but they have moderate toxicity to rodents. The data in Table 3.1 are taken from *The Pesticide Manual* (Tomlin, 2000).



Tributyltin and tributyltin oxide are still used on boats and ships to prevent growth of barnacles. They are extremely toxic for many invertebrates in the sea, notably some snails whose sexual organs develop abnormally. In these snails the female develops a penis. In oysters and other bivalves, their shells become too thick. Tributyltin must be regarded as one of the most serious environmental pollutants, but contrary to the lower analogues, trimethyltin and triethyltin, they are not very toxic to man and other mammals. Trimethyltin is of considerable interest for neurotoxicologists because it leads specifically to atrophy of the center for short-term memory, the hippocampus. The ethyl analogue has other serious detrimental effects on the brain.

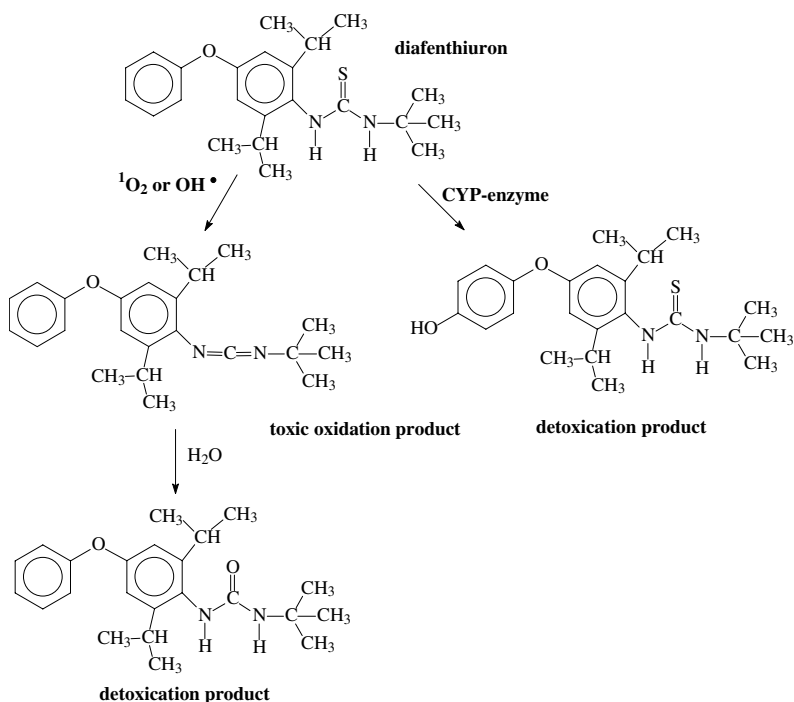
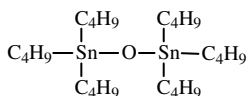
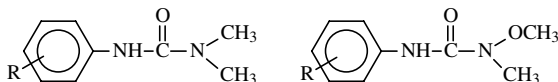


Figure 3.4 Activation and detoxication of diafenthiuron.



3.1.5.2 *Diafenthiuron*

Diafenthiuron inhibits ATP synthesis in the mitochondria (Ruder et al., 1991). This pesticide is interesting because, as is the case for the phosphorothioates, it needs to be activated by oxidation, which can occur abiotically by, for instance, singlet oxygen generated by sunlight or inside the organism by hydroxyl radicals generated by the Fenton reaction:



H_2O_2 may be produced as a by-product in the catalytic cycle of the CYP enzymes described later. Diafenthiuron therefore becomes more active in sunshine, and piperonyl butoxide that inhibits CYP enzymes makes diafenthiuron less toxic. However, some CYP enzymes are also important in the detoxication of diafenthiuron, as shown in Figure 3.4. Diafenthiuron may

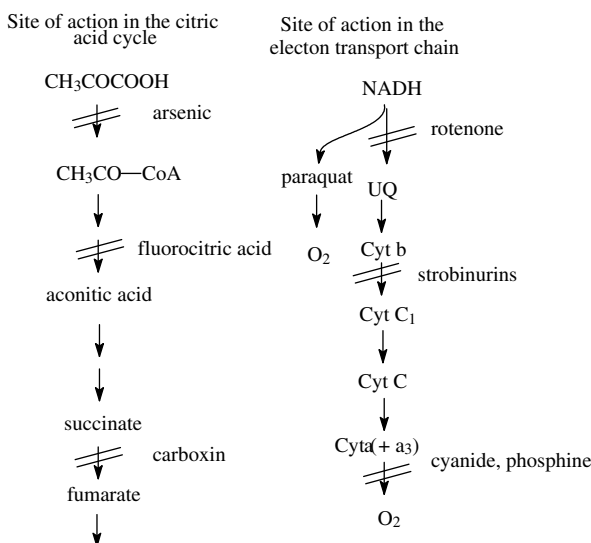


Figure 3.5 The site of inhibition of various pesticides in the citric acid cycle and the electron transport chain.

be used against mites, aphids, and other insects on several crops such as cotton, vegetables, and fruit. Table 3.1 shows that it has very high fish toxicity.

3.1.5.3 Summary

The mitochondrial poisons include such a variety of compounds with so many different activities on the organismic level that a summary may help. Figure 3.5 and Table 3.2 may help to identify the site of reaction.

In Figure 3.5, the arrows show the electron flow. When reaching oxygen the normal way, water is formed, while in the sideline via paraquat, superoxide radicals are formed.

3.2 Herbicides that inhibit photosynthesis

About half of all herbicides inhibit photosynthesis. Most of them disturb one particular process, i.e., the transfer of electrons to a low molecular quinone called plastoquinone. The inhibition occurs by the binding of the inhibitor to a specific protein called D_1 that regulates electron transfer. This protein has 353 amino acid residues and spans the thylakoid membrane in the chloroplasts. In atrazine-resistant mutants of certain plants, a serine residue at position 264 in the D_1 protein of the wild type has been found to be substituted by glycine. It is now possible to replace the serine 264 with glycine by site-directed mutagenesis in its gene and to reintroduce the altered gene to engineer atrazine resistance in plants.

The inhibitors of photosynthesis are all nitrogen-containing substances with various structures. They may be derivatives of urea, s-triazines, anilides,

Table 3.2 Site of Action of Some Mitochondrial Poisons

Site of Action	Compounds	Toxic for
Inhibition of acetyl-CoA synthesis	Arsenic	Most animals
Inhibition of akonitase	Fluoroacetic acid (fluorocitrate)	Most animals
Inhibition of succinic dehydrogenase	Salicylanilide and oxathiin fungicides	Fungi
Inhibition of NADH dehydrogenase	Rotenon	Insects, fish
Inhibiting cytochrome b	Strobinurins	Fungi
Inhibiting cytochrome oxidase	Cyanide Phosphine	All aerobic organisms
pH gradient in mitochondrial membranes (uncouplers)	Phenols	Most organisms
Inhibitors of ATP synthase in the mitochondrial membrane	Organonotin compounds Diafenthiuron metabolite	Fungi, mites, aquatic organisms; some have high mammalian neurotoxicity Insects, fish
Superoxide generators	Copper ions	Most organisms
Takes electrons from the transport chain and delivers them to O ₂	Paraquat	Most aerobic organisms

as-triazinones, uraciles, biscarbamates, pyridazinones, hydroxybenzoenitriles, nitrophenols, or benzimidazols. We shall describe just a few of them and give a very brief outline of the photosynthetic process. Textbooks of cell biology, biochemistry, and plant physiology (e.g., Alberts et al., 2002; Nelson and Cox, 2000; Taitz and Zaiger, 1998) describe the process in detail. The action of the herbicides may be read in more detail in Fedke (1982) or Devine et al. (1993).

In photosynthesis, light energy is trapped and converted to chemical energy as reduced coenzymes (e.g., nicotineamide-adenine dinucleotide phosphate [NADPH]), triphosphates (e.g., ATP), and O₂. Oxygen is a poisonous waste product in plants, although they also need some oxygen in mitochondrial respiration.

The chlorophyll takes up light energy (photons) directly or through so-called antennae molecules. (All colored substances take up light energy, but convert it to heat and not to chemical energy.) Electrons that jump to another orbit requiring more energy take up the energy and are said to have become excited. Such excited electrons may be lost by being taken up by an acceptor molecule, leaving chlorophyll as a positively charged ion. According to this scheme, chlorophyll will have three different states: the normal form that can pick up light energy, the excited molecule that is a very strong reducing substance, and the positively charged ion that is a very strong oxidant. The reducing power in the excited chlorophyll molecule is used to produce ATP and NADPH, while the oxidation power of the chlorophyll ion is used to

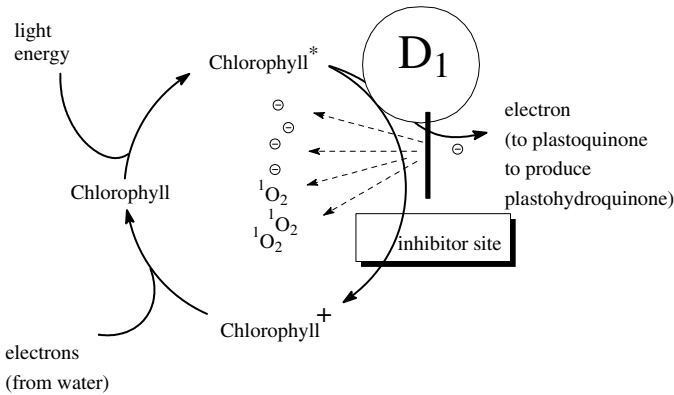


Figure 3.6 Blocking the passage of electrons from light-excited chlorophyll to plastoquinone by herbicides leads to production of singlet oxygen and electrons that may produce free radicals.

produce ATP and oxygen. ATP production is an indirect process coupled to the pH gradient between the inside and outside of the thylakoid membrane.

The photosynthetic apparatus is situated on and in the thylakoid membrane.

Four different and complicated protein complexes carry out the necessary chemical reactions: photosystem II, the cytochrome b_6f complex, photosystem I, and ATP synthase. These complexes are precisely oriented and fixed in the membrane. In addition, there are the plastoquinones, which easily undergo a redox cycle and can swim in the membrane's lipid phase. A manganese-containing complex in photosystem II is involved in the splitting of water and the generation of electrons and molecular oxygen. A small, copper-containing protein, plastocyanine (PC), is involved in transfer of electrons from $cytb_6f$ to photosystem I.

The thylakoid matrix is an extensive internal membrane system inside the chloroplasts, which are small organelles in the plant cells. The inner lumen of the membrane system maintains a pH of 5, while the outer compartment, called stroma, has a pH of 8. The energy picked up from the photons is used to establish and maintain this difference.

The chlorophyll pigments in photosystem II, organized in a structure called P680, catch energy from a photon and become excited. The electron is then transferred to a molecule called pheophytin and then to a tyrosine residue in protein D_1 called the reaction center. The oxidized form of plastoquinone (PQ) has a specific binding site on protein D_1 , where it is reduced and then diffuses to the lumen side of the membrane (now as plastoquinone (PQH_2)). Here it binds to an iron-sulfur protein in the cytochrome b_6f complex and reduces it. The hydrogen ions released in this process are delivered to the inside of the membrane. The plastoquinone/plastoquinone is thus functioning as a proton pump driven by light-excited electrons. A summary of the process is shown in Figure 3.7.

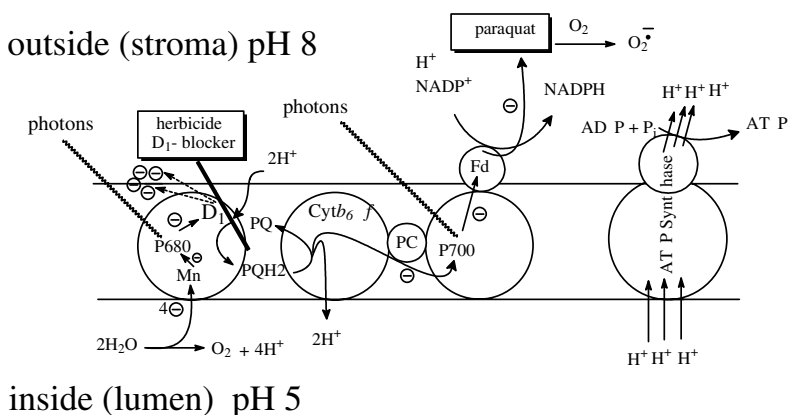


Figure 3.7 Schematic representation of photosynthesis and the site of action of D₁ blockers and paraquat.

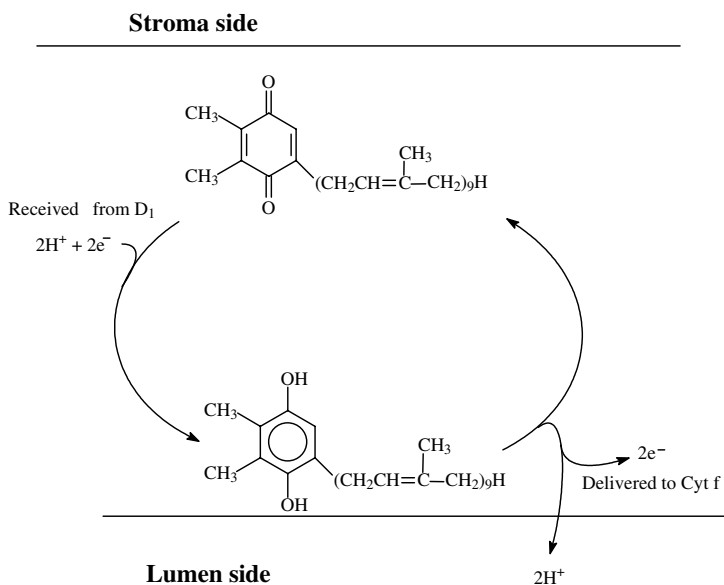


Figure 3.8 Schematic representation of the redox cycle of plastoquinone.

A simplified scheme of the redox cycle of plastoquinone is shown in Figure 3.8. The quinoid structure and the isoprene side chain make it possible for plastoquinone to take up one electron at a time, producing rather stable semiquinone radicals (which is not shown in the figure), and there are probably at least two plastoquinones involved.

The reduced cytochrome f delivers the electron to plastocyanine, a copper-containing, low-molecular-weight soluble protein, and then to special

chlorophyll pigments in photosystem I (P700). The P700 can be excited by a new photon and deliver the electron to an iron-containing protein called ferredoxin. The reduced ferredoxin delivers the electrons to NADP^+ to produce NADPH, or to a minor pathway that reduces nitrate to ammonia at the outer membrane surface. Some important herbicides (paraquat and diquat) can snatch the electrons before the delivery to ferredoxin and generate free radicals.

The chlorophyll ion in P680^+ takes electrons from water, via a manganese-containing enzyme complex, and is reduced to the neutral unexcited state, ready to pick up new photons. Water is then split to oxygen and hydrogen ions. Oxygen is a toxic waste product, while the hydrogen ions contribute to the buildup of the pH difference across the membrane.

ATP is produced from ADP and phosphate by ATP synthase, an enzyme located in the membrane. The difference in hydrogen ion concentration between the inside and outside is used as the energy source. Because there are approximately 1000 times more hydrogen ions on the inside than on the outside of the membrane, the hydrogen ions will tend to diffuse out. This would waste energy, so instead, hydrogen ions are forced to flow through a special proton channel in the ATP synthase that uses the energy from the hydrogen ion flow to produce ATP. ATP synthase is very similar in chloroplasts and mitochondria.

In summary, there are four main types of herbicides that disturb the photosynthetic apparatus:

1. Weak organic acids that destroy the hydrogen ion concentration gradient between the two sides of the membrane
2. Free radical generators
3. Compounds that bind to the D_1 protein at (or near) the plastoquinone-binding site
4. Substances that destroy or inhibit synthesis of protecting pigments such as carotenoids

3.2.1 Weak organic acids

Weak organic acids with a pK value between pH 5 and 8, or close to these values, will cause leakage of hydrogen ions if the acid dissolves in the thylakoid membrane. Ammonia also has this effect as a result of the reaction $\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+$. Instead of producing ATP, heat will be generated. These so-called uncouplers will act similarly in mitochondria, chloroplasts, and bacterial cell membrane. They may therefore also be toxic for animals and microorganisms, and some of them are described under mitochondrial poisons.

3.2.2 Free radical generators

These are a type of herbicide that is able to steal the electron on its long route from water to NADP^+ . The most important herbicides in this category

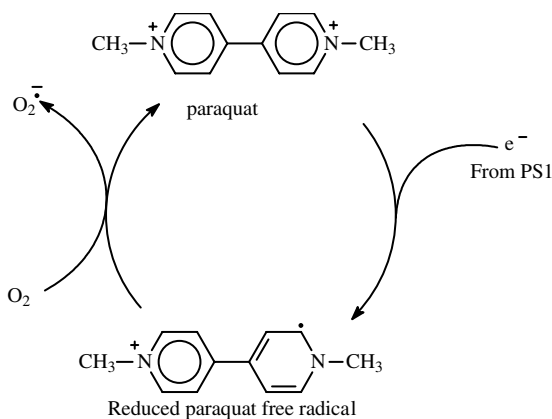
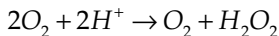


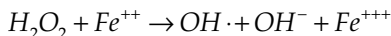
Figure 3.9 The toxic cycle of paraquat.

are paraquat (Figure 3.9) and diquat. They take up the electron at some stage before ferredoxin and deliver it to oxygen to produce the superoxide radical. Many other natural processes form superoxide radicals, and the cells have very efficient enzymes called superoxide dismutases that detoxify the superoxide radicals. However, the detoxication is not complete because another very reactive substance, namely, hydrogen peroxide (H_2O_2), is produced. Hydrogen peroxide must be detoxicated by catalases or glutathione peroxidases. If this does not happen fast enough, H_2O_2 may react through the Fenton reaction, producing the extremely reactive hydroxyl radical. Interestingly, paraquat is more toxic for plants when they are placed in light and is less toxic for bacteria when they are grown anaerobically, as shown by Fisher and Williams (1976). The superoxide generators are toxic to animals as well as to plants. Characteristically, the lung is the critical organ for paraquat in mammals, even when administered by mouth.

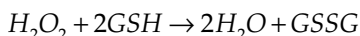
The partial detoxication of superoxide anions by superoxide dismutase is as follows:



The Fenton reaction produces the extremely reactive hydroxyl radical:



The detoxication of hydrogen peroxide with glutathione peroxidase is as follows:



Paraquat chloride has been marketed since 1962. It is nonselective as an herbicide and rather toxic to animals (rat oral LD50 is between 129 and 157

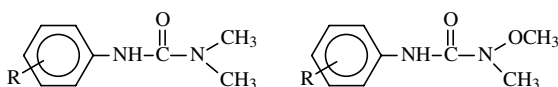
mg/kg). Its valuable properties are that it is fast acting, is quickly inactivated through binding to soil and sediment (in spite of its high water solubility), and can be used for destruction of potato haulm before harvest. Its high human toxicity, with the lungs being the most severely affected, has led to many fatalities. Diquat dibromide started to be marketed at approximately the same time as paraquat and has approximately the same uses. It has a slightly lower acute toxicity to rats ($LD_{50} \approx 234$ mg/kg). None of the bipyridylum herbicides have high dermal toxicity. They are placed in WHO toxicity class II.

3.2.3 D_1 blockers

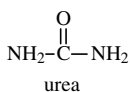
The herbicides that act at D_1 have a low toxicity to animals. As explained, they block a specific site only present in photosynthesizing plants. The binding site is, however, the same or nearly the same for all plants, and little degree of selectivity is expected inside the plant kingdom. The inhibitors are more active in strong sunshine and in warm and dry weather with good moisture in the soil — the reason being that the strong transpiration stream in the plant takes up the herbicides, and the conditions are good for active photosynthesis. Plants adapted to low illumination are very sensitive to a combination of herbicide and strong light. The cause of death is definitely not lack of energy due to inhibition of photosynthesis, but is rather due to production of reactive oxygen species. When excited chlorophyll cannot transfer the energy to plastoquinone, it is forced to react with oxygen. Singlet oxygen (1O_2) is formed. This can destroy beta-carotene and lipids in the thylakoid membrane. Excited chlorophyll can also react directly with unsaturated lipids.

3.2.3.1 Urea derivatives

This group is easy to recognize by their formulae:



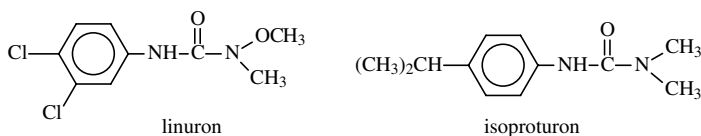
Urea is substituted with one aryl group in one nitrogen and two methyl groups or a methoxy and a methyl group on the other nitrogen.



The aryl group may be an unsubstituted simple phenyl ring, as in fenuron, or may be substituted with halogens, alkyl groups, and ring structures. The possibility of varying the aryl group and still retaining its activity makes

it possible to modulate the properties, such as water solubility, stability, and uptake in plants. Most ureas have very low toxicity to birds and mammals, but fish and crustaceans may be sensitive (linuron has an LC50 for the fathead minnow of ≈ 1 to 3 mg/l, and for *Daphnia*, ≈ 0.1 to 0.75 mg/l). The herbicides are, of course, very toxic to photosynthesizing algae, and leakage into lakes and rivers and contamination of groundwater must therefore be avoided.

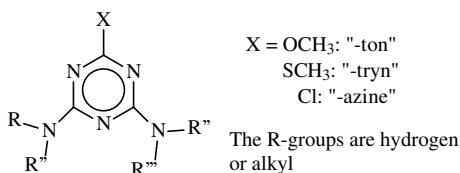
Linuron (left structure) was first marketed in the 1960s and has been one of the more popular herbicides in the culture of potatoes and vegetables:



The plants take it up by roots and leaves, and it has a high persistence in humus-rich soil in cool climates, with a half-life of 2 to 5 months. Microorganisms in soil degrade isoproturon, which can be used selectively in various cereal crops, and half-lives of 6 to 28 days have been reported under field conditions, depending on the microbial activity. The aliphatic substitution in the aryl ring is sensitive to microbial oxidative attack.

3.2.3.2 Triazines

Most of the triazines are derivatives of the symmetrical 1,3,5-triazine-2,4-diamine, but other possibilities also exist. In position 6 there is a methylthio (the -tryns), a methoxy (the -tons), or a chloro group (the -azines).



The triazines are also interesting because there is no negative correlation between water solubility and soil adsorbance, because of their cationic character. Atrazine has been used a lot in maize because maize is less sensitive due to a glutathione transferase that inactivates atrazine. The same mechanism, as well as the mutant variety of the D_1 described in the beginning of this chapter, that reduces the binding may also be the cause of resistance in weeds. The substituents in the 6 position and the 2- and 4-amino groups greatly influence important properties such as soil-binding capacity, water solubility, microbial degradation, and other factors of importance. Besides

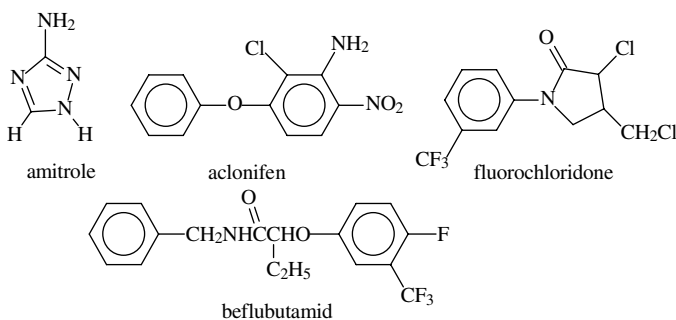
atrazine, simazine, simetryn, dimethametryn, terbumeton, terbuthylazin, terbutryn, and trietazin are all available on the market.

3.2.4 Inhibitors of carotene synthesis

Some herbicides act by inhibiting the synthesis of carotenoids that protect chlorophyll from being destroyed by photooxidation. Amitrole is not selective, whereas aclonifen has valuable selective properties.

3.2.4.1 Amitrole

This once very promising herbicide with very low acute toxicity is carcinogenic and has been reported to increase the incidence of soft-tissue cancers in people engaged in thicket clearing along railways tracks in Sweden. Amitrole and aclonifen may cause enlarged thyroid in high doses. In plants, amitrole inhibits lycopene cyclase, an enzyme necessary for the synthesis of carotenoids. It is not selective, as opposed to aclonifen.



3.2.4.2 Aclonifen

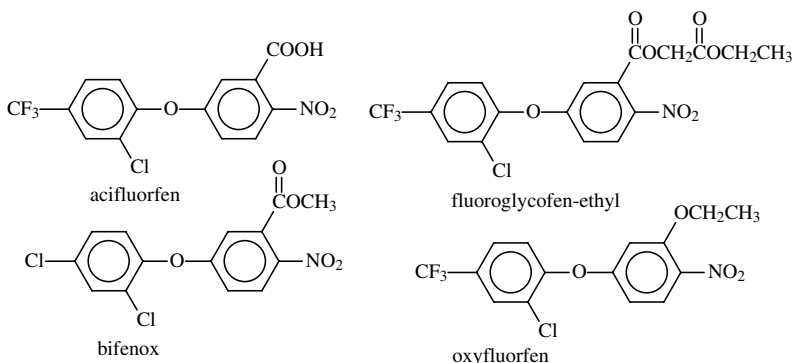
This herbicide is not toxic for potatoes, sunflowers, or peas. It may also be used selectively in other crops. It inhibits biosynthesis of carotene, but the exact mode of action is not known. In mammals, it is biotransformed to many different compounds; the nitro group is reduced, the rings can be hydroxylated, the amino group can be acetylated, and the hydroxy groups formed by ring hydroxylation can be conjugated to sulfate or glucuronic acid. Its acute toxicity is very low. In mice and rats it may produce some kidney injury at high doses (25 mg/kg), but the no-observed-effect level (NOEL) for 90 days in rat is 28 mg/kg of body weight, and France has determined an acceptable daily intake (ADI) of 0.02 mg/kg.

3.2.4.3 Beflubutamid

Beflubutamid is a newly described carotenoid synthesis inhibitor, inhibiting phytoene desaturase. It has a very low toxicity to animals but is very toxic to algae and plants. It is not mutagenic or teratogenic in standard tests.

3.2.5 Protoporphyrinogen oxidase inhibitors

Acifluorfen is used in soybeans, peanuts, and rice, which are more or less tolerant to this herbicide.



Bifenox, fluoroglycofen-ethyl, HC-252, lactofen, and oxyfluorfen have analogous structures and modes of action. The carboxyl group may be replaced by an ether group or an ester, and many other herbicides with related structures have been developed. Tetrapyrrol and protoporphyrin accumulate, act as photosensitizers, and cause photooxidation and necrosis. They are contact herbicides and are more active in strong sunlight.

3.3 General SH reagents and free radical generators

Sulfhydryl groups are reactive and very often important in the active sites of many enzymes. Some pesticides with rather unspecific action are often SH reagents.

3.3.1 Mercury

We remember from our lessons in inorganic chemistry that HgS is insoluble (the solubility product for the reaction $\text{Hg}^{++} + \text{S}^{2-} \rightleftharpoons \text{HgS}$ is 1.6×10^{-52} at 25°C). The very high affinity of Hg⁺⁺ to SH groups is also the reason for the high toxicity of mercury compounds. Almost all organisms may be killed by mercurials. Resistance in fungi is therefore very rare, but may occur and result from an increased level of glutathione in the fungal cells that trap the Hg compounds.

The Pesticide Manual still has a few entries with mercurials, although the significance of mercury as a poison and as a general environmental pollutant is widely recognized. Today crematoriums have to install air-cleaning devices, dentists cannot use amalgam anymore, mercury-free thermometers are to be used, etc. The public concern about chronic mercury poisoning is very high. Organized groups of patients are convinced that their pains and

problems are due to mercury released from their teeth even though most metal toxicologists believe that teeth amalgam gives too low a level of exposure to mercury to cause the claimed problems and think that patients who suffer from “amalgamism” should seek other reasons for their sufferings.

Pesticides with mercury also have a mixed reputation. Organic mercury compounds were used quite extensively as seed dressings for various cereals and other seeds to protect against fungal diseases. Very small amounts were effective in controlling fungus diseases. According to Mellanby (1970), as little as 0.5 kg of an organomercury preparation, containing 1% of mercury (5 g), was sufficient for 1 bushel of wheat grain. Only about 1 mg of mercury will be added to each square meter by this treatment — far below the natural level. Poultry or cattle fed on a moderate amount of dressed grain did not seem to suffer. No reason to wonder that organomercurials were popular. However, several epidemics of methylmercury poisoning have been reported, the most notable in Japan (1950s) and in Iraq. The Japanese case was due to mercury effluents from a factory. Microorganisms converted inorganic mercury to methylmercury, which poisoned fish, cats, and humans. The largest recorded epidemic of methylmercury poisoning took place in the winter of 1971–72 in Iraq, resulting in over 6000 patients and over 500 deaths (Goyer and Clarkson, 2001; WHO, 1974). The exposure was from bread containing wheat imported as seed grain and dressed with methylmercury fungicide. Several other serious accidents have been recorded. In Sweden, pheasant ate dressed seed and pike were poisoned or strongly contaminated by mercury from the chlorine–alkali process and by biocides used in the pulp industry. Mercury contamination was the big issue and changed public opinion and policy on effluents and agrochemicals in the late 1960s (Berlin, 1986; Borg et al., 1969; Fimreite, 1970, 1974; Mellanby, 1970).

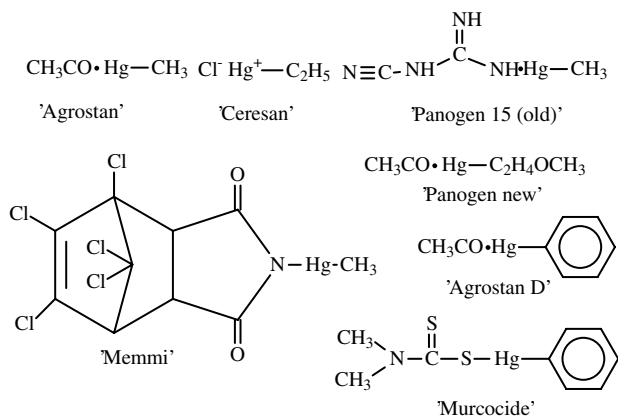
Mercuric chloride (HgCl_2), mercuric oxide (HgO), and mercurous chloride (Hg_2Cl_2) still have some limited use as fungicides. They are very toxic (WHO toxicity classes Ia, Ib, and II). Mercurous chloride has a lower toxicity (rat oral LD_{50} = 210 mg/kg) than HgCl_2 (LD_{50} = 1 to 5 mg/kg) because it has a very low solubility.

Salts of methylmercury were used as a fungicide but may also be formed by biomethylation.

Hg^{++} is methylated by several sulfate-reducing bacteria (*Desulfobacter*) by a reaction with the methyl–vitamin B_{12} complex, which the bacteria normally use for producing some special fatty acids. The detection of methylmercury in the environment led to a shift from methylmercury fungicides such as Agrostan, Memmi, and Panogen 15 to ethoxyethylmercury as Panogen new, the methoxyethylmercury silicate Ceresan, and phenylmercurials such as Ceresol, Phelam, and Murcocide. Because mercury compounds are all very toxic to fungi, it was easy to make new alternatives, and many compounds and trade names were on the market.

The target organ in mammals and birds is the central nervous system. Symptoms include tunnel vision, paresthesias, ataxia, dysarthria, and deafness. Phenyl- and alkoxyalkylmercurials are absorbed through the skin and

are almost as dangerous as the methyl analogue. Neurons are lost in the cerebral and cerebellar cortices. In the fungi, phenyl and alkoxyalkyl mercurials react with essential SH groups important in cellular division. Methylmercury interacts with DNA and RNA and binds with SH groups, resulting in changes of secondary structure of DNA and RNA.



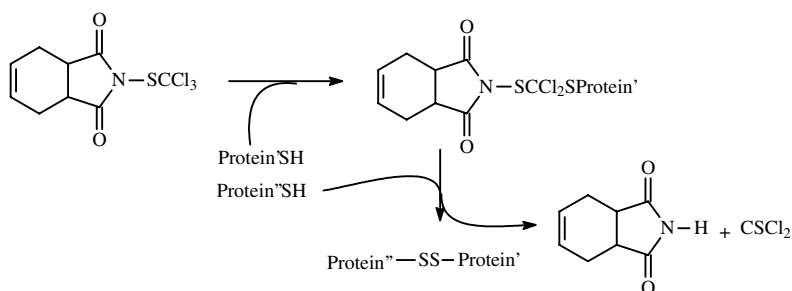
A selection of different organomercurials is shown above. They are taken from older issues of *The Pesticide Manual* (Worthing, 1979).

3.3.2 Other multisite fungicides

Dithiocarbamates, perhalogenmercaptans, sulfamides, copper salts, and ferric sulfate may be classified in this group. They all seem to be quite reactive against SH groups or are free radical generators. Detergents such as dodine and toxic salts such as sodium fluoride also have a multisite mode of action.

3.3.2.1 Perhalogenmercaptans

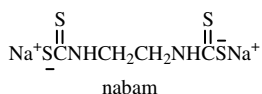
The fungicides in this group are good examples of pesticides that react with sulfhydryl groups in many enzymes:



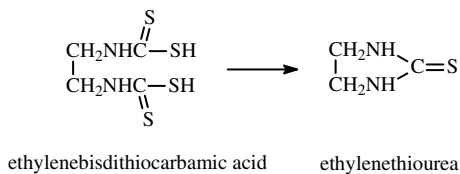
Other fungicides in this group are captafol, folpet dichlofluanid, and tolylfluanide. Captan has been widely used as a fungicide, but was blamed for being carcinogenic by the Environmental Protection Agency (EPA) in the U.S. in 1985. The perhalogenmercaptans' general toxicity toward animals is very low.

3.3.2.2 Alkylenebis(dithiocarbamate)s and dimethyldithiocarbamates

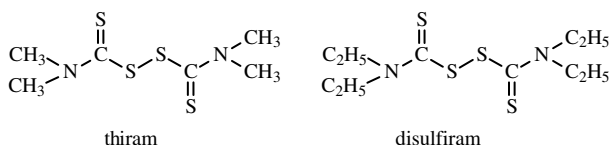
The pesticides in these groups are also regarded as unspecific SH reagents. Nabam is the disodium salt of alkylenebis(dithiocarbamate):



Nabam was originally described in 1943. When mixed with zinc or manganese sulfate, zineb or maneb is formed, respectively. The mixed salt of zinc and manganese, mancozeb is used quite extensively. The alkylenebis(dithiocarbamate)s have a low mammalian toxicity (e.g., LD50 = 8000 mg/kg for rats) but are considered to be carcinogenic, notably through the metabolite ethylenethiourea, which is formed by cooking.



The dimethyldithiocarbamates, thiram ferbam and ziram, are disulfides, which were also described and made commercially available during the Second World War. It may be of interest to know that a structural analogue to thiram, disulfiram, is used as an alcohol deterrent because it inhibits aldehyde dehydrogenase, and when taken, ethanol is converted to acetaldehyde, which is then only slowly further metabolized. The high level of acetaldehyde in the body gives an extremely unpleasant feeling. A curious use of thiram is as a protectant against deer and stags in orchards. They dislike its scent and keep away, probably because the smell reminds them of some dangerous carnivores.



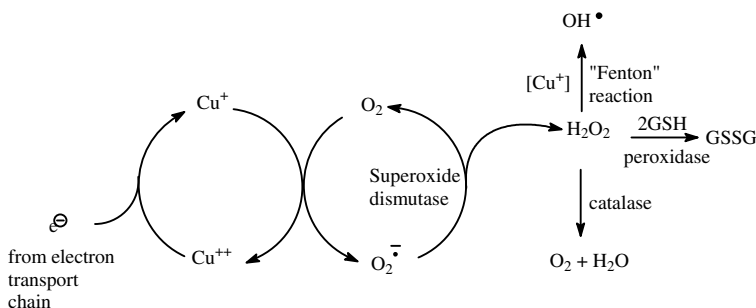


Figure 3.10 The toxic cycle of copper and the partial detoxification of superoxide by superoxide dismutase.

3.3.2.3 Fungicides with copper

Copper is an essential metal and all life-forms need it. It is a vital part of many enzymes such as cytochrome oxidase and the cytosolic form of superoxide dismutase. Some organisms are very sensitive to it. Among them, we find such different organisms as sheep and goats, marine invertebrates, many algae, and fungal spores. Humans and pigs belong to the less sensitive species. The mechanism for this tolerance is at least partially due to a strict regulation mechanism of active copper concentration. A small cystein-rich protein called metallothionein can sequester metals like zinc, copper, cadmium, and mercury and plays a very important role in reducing the toxicity of these metals. The amount of metallothionein increases by exposure to the metals, and the difference in this ability is one of the mechanisms behind the great variation in copper sensitivity. Bordeaux mixture is a slurry of calcium hydroxide and copper(II) sulfate and has been used as an efficient spray to control *Phytophthora infestans* on potatoes, *Venturia inaequalis* on apples, *Plasmopara viticola* on vine, and *Pseudoperonospora humuli* on hops. It has a strong blue color and you cannot fail to recognize the blue-colored leaves on the grape vines during hikes through the vineyards on the Greek islands. It was introduced in France already in 1885 and soon played an important role in vine growing. It has surprisingly low toxicity to mammals, but when used for many decades in vineyards, it may give rise to an unwanted buildup of copper in the soil.

Bordeaux mixture and other copper salts owe their toxicity to the ions' ability to make one-electron exchanges ($\text{Cu}^+ \rightleftharpoons \text{Cu}^{++} + e^-$) (Figure 3.10). An electron is taken up from the electron transport chain and delivered to O_2 to form the superoxide anion. The anion radical is further transformed to H_2O_2 by superoxide dismutase catalysing.

The hydrogen peroxide is normally destroyed by catalase and peroxidases, but some may be transformed to OH^\bullet , the hydroxyl radical, which is extremely reactive and modifies all biomolecules in its vicinity. Membrane lipids are destroyed. The mode of action of copper salts and paraquat is in many ways the same, although their target organisms are quite different.

It may be of interest to know that the most efficient peroxidase contains selenium and is the reason why selenium is a necessary trace element. The most efficient superoxide dismutase in eukaryotic cells contains copper. Copper is thus an important element for protection against free radicals but is also responsible for their formation. Copper ions form very stable SH compounds and would have been toxic without being superoxide generators.

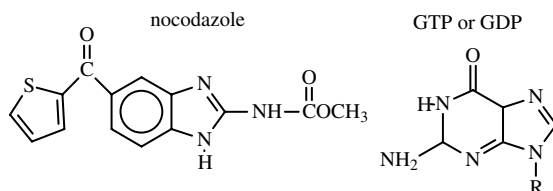
3.4 Pesticides interfering with cell division

Bhupinder, P.S. et al. (2000) give a short and inspiring account of these pesticides, whereas a textbook in cell biology (e.g., Alberts et al., 2002) describes the function of tubulin in greater detail. Paclitaxel (Taxol®) is a very strong poison extracted from pacific yew (*Taxus brevifolia*), where it is present together with many other compounds with similar structure. The substance is also present in fungi (*Taxomyces adreanea* and *Pestalotiopsis microsporia*) associated with the Pacific and Himalayan yews, respectively. It has recently been detected in other species as well. Despite its toxicity, paclitaxel has become a very promising anticancer drug. The compound has a very complicated structure and is difficult, but not impossible, to synthesize. It has a strong fungicidal activity against oomycetes, i.e., fungi causing blights.

All garden enthusiasts know about the nice autumn crocus (*Colchicum autumnale*), which flowers in late autumn. It is not difficult to understand that this very conspicuous plant profits by containing a strong poison that protects it from pathogens and herbivores. It contains colchicine, which is very toxic and has a complicated structure. The substance is well known to plant breeders because it is used to double the number of chromosomes artificially in plants. A synthetic benzimidazole derivative, 1-methyl-3-dodecylbenzimidazolium chloride, was developed in 1960 as a curative fungicide against apple scab. Thiabendazole, another synthetic benzimidazole derivative, has been used as an anthelmintic since 1962.

These synthetic and natural compounds are mentioned because they have a related mode of action. They react with *tubulin*, a protein that is the building block of the intracellular skeleton in eukaryotic cells. The shape and structure of a cell are dependent on microfilaments that keep the cell's constituents in the right place. It is different from a real skeleton because it is dynamic and changes structure. One important function of tubulin or, more precisely, the polymer of tubulin, called microtubules, is to make the spindle, a structure that pulls the chromosomes apart during mitosis. Two different tubulin subunits (α -tubulin and β -tubulin) make dimers that are stacked together and form the wall of hollow cylindrical microtubules. The α -/ β -tubulin dimers are present in unpolymerized form in the cell, together with the polymeric microtubules, and a balance of assembly and disassembly maintains the microtubules, but the poisons mentioned above disturb this balance by binding to various sites of the β -tubulin, impairing normal cell division. Maintenance of cell shape, cell movement, intracellular transport, and secretion is dependent of the microtubuli. Therefore, it is not difficult

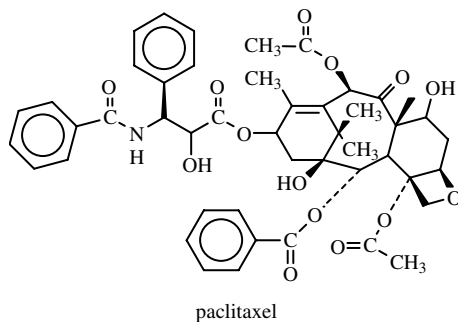
to understand that strong poisons and anticarcinogenic compounds can be found among the substances interfering with the function of tubulin. Taxol acts by stabilizing the microtubule too much, whereas colchicin and most of the benzimidazoles act by inhibiting the formation of the microtubules. Although the mode of action of the two types of compounds is different, the net result will be the same: impairment of cell division. Binding of guanosine triphosphate (GTP) to β -tubulin stabilizes the polymeric form, whereas hydrolysis of GTP to guanosine diphosphate (GDP) destabilizes the microtubules. Benzimidazoles have a structural resemblance to the guanosine phosphates and may compete for the same binding site. This has been shown experimentally in preparations of rat testicles (Winder et al., 2001). Cell biologists studying tubulin and its function often use nocodazole.



The biological activity of tubulin-interfering substances can be very high. The LC₅₀ of taxol for cultured liver cells (HL-60 cells) is <0.001 μM , whereas thiabendazole at 80 μM completely inhibits mitosis in hyphae of *Aspergillus nidulans*, when growing in liquid culture.

Benzimidazole fungicides with this mode of action include benomyl, carbendazim, dedacarb, fuberidazole, thiabendazole, and thiophanate-methyl. Other groups of fungicides that bind to tubulin include the fungicidal phenylcarbamate diethofencarb, swep and methyl 3,5-dichlorophenyl-carbamate (MDPC), and the herbicidal carbamate carbetamide. A group of preemergence herbicides, the dinitroanilides, also bind to tubulin: this group includes benzfluralin, butralin, dinitramine, ethalfluralin, fluazinam, fluchloralin, flumetralin, oryzalin, pendimethaline, prodiamine, and trifluralin.

Taxol and the other extremely biologically active derivatives of yews and fungi associated with yews have no value as fungicides, but may have evolved as natural pesticides:



3.4.1 Fungicides

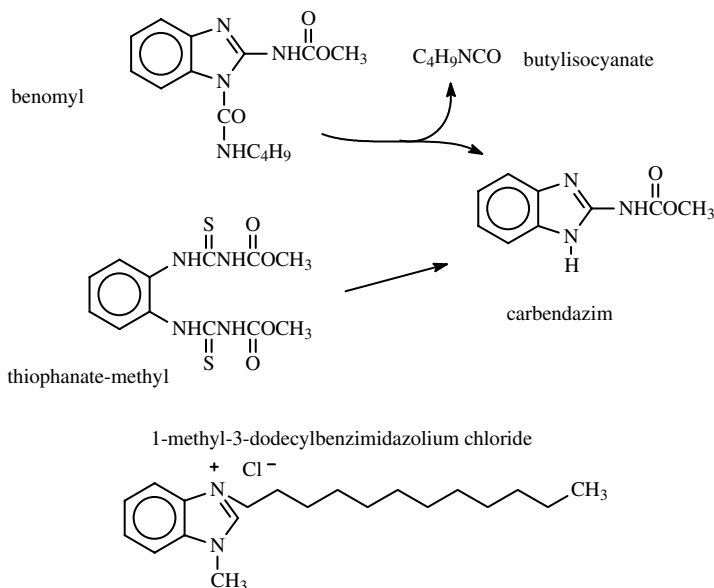
3.4.1.1 Benomyl

As opposed to the older protective fungicides, the benzimidazole fungicides will kill growing mycelia and can therefore stop an infection already in progress. Benomyl has low water solubility (4 mg/l) but is degraded in soil and water to carbendazim and butylisocyanate with a half-life of less than 1 day. Carbendazim works, as described, as a tubulin poison, whereas butylisocyanate is very toxic and reacts with many cell constituents. Benomyl, or its degradation product butylisothiocyanate, may cause the plants to produce so-called phytoalexins. Attacked by pathogens, plants have an inducible defense system in producing so-called phytoalexins, which are substances toxic for the fungi and protecting the plants against further attack. It has been shown that butylisocyanates induce production of such chemicals in plants and may be one of the mechanisms of the antifungal activity of benomyl.

Benomyl and the other benzimidazole fungicides are toxic to earthworms (Stringer and Wright, 1973) and may seriously disturb the earthworm population, for instance, in orchards, so that the leaf litter is not removed. Some benzimidazole fungicides (thiabendazole and mebendazole) may be used as anthelmintica.

3.4.1.2 Thiophanate-methyl

Thiophanate must also be transformed to carbendazim in order to be fungicidal. It is active against a wide variety of fungal pathogens and has low toxicity. In soil and plants it is slowly transformed into carbendazim.

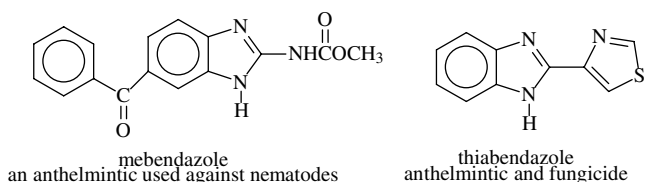


3.4.1.3 Carbendazim

Carbendazim was first described as a fungicide in 1973 and is active against a wide variety of fungi.

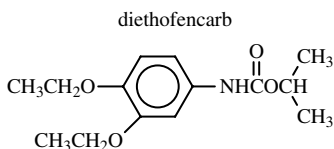
3.4.1.4 Thiabendazole

Thiabendazole's fungicidal properties had already been reported in 1964, but prior to that it had been used as an anthelmintic in human and veterinary medicine. In aqueous solution it is stable, but in mammals it is hydroxylated in the benzene ring by CYP enzymes.



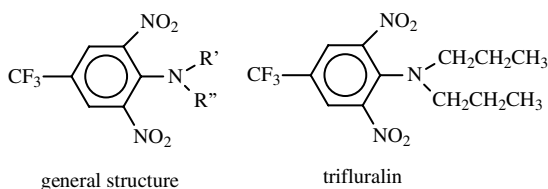
3.4.1.5 Diethofencarb

Diethofencarb can be used against benzimidazole-resistant strains of *Botrytis* spp. It also inhibits mitosis. It is quickly degraded in soil and in animals through oxidation of the 4-ethoxy group.



3.4.2 Herbicides

Many herbicides with a common structure inhibit cell division:



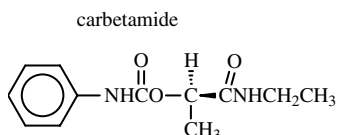
3.4.2.1 Trifluralin

Trifluralin has been marketed since 1961. It is a selective soil herbicide that acts by entering the seedlings in the hypocotyl region and disrupting cell division. It adsorbs in the soil and so is resistant to leaching, having a long residual activity. Trifluralin is much more stable under aerobic than anaerobic

conditions because microorganisms can reduce its nitro groups to amino groups anaerobically.

3.4.2.2 Carbetamide

This herbicide was first reported in 1963. It is highly selective against grasses and some broad-leaved weeds. Note that one enantiomer is regarded as the active ingredient:

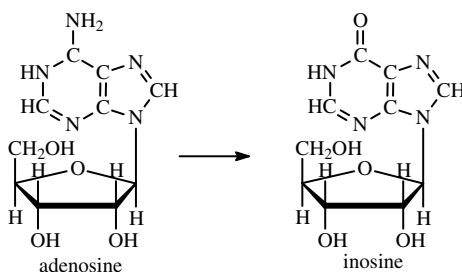


3.5 Pesticides inhibiting enzymes in nucleic acid synthesis

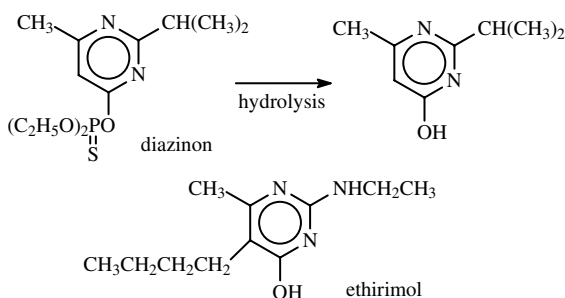
Important herbicides and fungicides may inhibit various enzymes in the synthesis of nucleic acids. One group of fungicides, the pyrimidinols, inhibits the synthesis of substances important for sporulation, whereas the acetanilides inhibit incorporation of nucleic acids in RNA.

3.5.1 Sporulation-inhibiting fungicides

Analogues of the insecticide diazinon were found to be active against powdery mildews, and studies of correlations between structure and activity led to the development of the pyrimidinol fungicides (ethirimol, bupirimate, and dimethirimol). The pyrimidinols appear to act by interfering with the metabolism of purines by inhibiting adenosine deaminase. The enzyme, which catalyzes the hydrolytic deamination of adenosine to inosine, is important in fungi but not in plants. Inhibition leads to a stop in the sporulation process. The fungicides are highly soluble in water and act systemically by being absorbed through the leaves and translocated. They are stable in soil, but their toxicity to animals is very low.

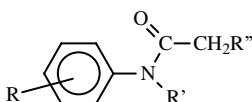


The structures below show the insecticide diazinon, its hydrolytic product, and ethirimol. Bupirimate and dimethirimol have very similar structures.



3.5.2 Inhibition of incorporation of uridine into RNA

Inhibition of the incorporation of uridine into RNA is caused by the herbicides referred to as the chloroacetanilides (e.g., acetochlor, alachlor, butachlor, and several others) and a group of fungicides referred to as phenylamides (metalaxyl, ofurace, and oxadixyl). They have similar structure and mode of action:



R is often one or two alkyl groups, R' may have various structures, and in the herbicides, R'' is chlorine in accordance with the name chloroacetanilides.

The discovery of the fungicides arose from the observation of the anti-fungal activity of chloroacetanilide herbicides, and it proved possible to retain and improve this activity while minimizing the herbicidal effects. These compounds were commercialized during the 1970s. The herbicides are valuable because of their selectivity, and the fungicides are particularly useful against powdery mildew on grapes. Acetochlor is selective because it is detoxified by being conjugated to the SH-group of the tripeptide, glutathione (GSH) in some plants, such as maize, or to homoglutathione in soya. In sensitive plants the protein synthesis stops. Glutathione and the enzymes responsible for conjugations with this tripeptide will be described later. Many plants, notably maize, owe their herbicide tolerance to a high level of glutathione transferase. It is even possible to treat the seeds with so-called safeners, which induce the plants to produce even more of this enzyme and thus make it safe to use herbicides.

