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Herbicides: The Face and the Reverse of the Coin. An *in vitro* Approach to the Toxicity of Herbicides in Non-Target Organisms

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1. Introduction

I. Historical aspects, benefits and disadvantages of herbicide use

During thousands of years up to about a hundred years ago, man expended most of required energy in arable farming with mechanical operations aiming to remove weeds and providing suitable conditions for the efficient growth of crop plants, considering that weeds compete with beneficial and desired vegetation, which means that weeds are plants growing where man does not wish them to grow. With the dawn of industrialization, labor to the factories decreased manpower on the farms, which forced to think of more efficient mechanical means of weed control.

The need of weed management is as old as agriculture itself. Six stages in the evolution of weed control practices can be considered: 1) 10,000 B.C. – removing weeds by hand; 2) 6,000 B.C. – the use of primitive hand tools to till the land and destroy weeds; 3) 1,000 B.C. – animal-powered implements like harrows; 4) 1920 A.D. – mechanically-powered implements like cultivators, blades, harrows, finger-weeders, rotary-hoes, rod-weeders, etc.; 5) 1930 A.D. – biological control and; 6) 1947 A.D. – chemical control, with the commercial development of organic herbicides such as 2,4-D and MCPA (Hay, 1974).

Especially in the last century, for various reasons among which the population explosion, in his effort to produce adequate supplies of food, man needed to combat efficiently the attacks of various pests on agricultural and horticultural crops. Pesticides, falling into three major classes: insecticides, fungicides, and herbicides (or weed killers), are required. Herbicides, specifically, are used for control of weeds.

At the end of the twentieth century, with an estimated world population of 6 billion people, some 700 million were undernourished and 1.3 billion exist on an inadequate diet. In 2009 FAO says that 1.02 billion people are undernourished, corresponding to 15 percent of the estimated world population of 6.8 billion. Undoubtedly, the first problem of Humankind is the lack of food, which affects especially underdeveloped countries. So, the urgent need for much greater application of herbicides and other agrochemicals is essential to increase food supply. Crops can duplicate or increase even more at the expenses of the agrochemicals use.

Another advantage of the greater efficiency of modern agricultural practice is the liberation of land. This can be of major importance in countries where the space dedicated to agriculture is limited, and where there is a very high population density or soil is inadequate for crop yields.

For more than a century chemicals have been employed for total weed control. For the removal of all plants from railway tracks, timber yards and car roads, crude chemicals such as rock salts, crushed arsenical ores, creosote, oil wastes, sulfuric acid, and copper salts were used (Whitten, 1966; Green et al., 1987). Under these conditions, all plants were killed and these compounds all function as total herbicides and treated areas remain toxic to plants for months or even years. However, for agricultural purposes, it was preferred that chemicals would selectively kill the weeds, but not harm the crop plants.

Selectivity can be based, for instance, in the fact that the larger rougher surfaces of weed leaves are more effectively wetted by the spray than the narrow, smooth cereal leaves in which there was much greater run-off of the toxicant. In a few cases selectivity is based on biochemical differences between weeds and crop plants, such that the latter have a unique defense mechanism. The absence of β -oxidases from leguminous plants enables 2,4-DB to be applied safely for selective weed control in these crops. Maize detoxifies the triazine herbicides (e.g. atrazine and simazine) by enzymic hydrolysis, but weeds do not possess this enzyme. Physicochemical properties may be important for selectivity. The soil-applied herbicide, triazine, have low aqueous solubility meaning that the chemical only penetrates some 5 cm downwards in the soil. Hence, germinating weed seeds are killed but more deep-rooted crops are unaffected. The phenoxyacetic acids were the first really effective selective herbicides, being the product of war-direct research. They came into use at a time when the maximum food production with the minimum labor force was a vital factor in the war effort (Cremlyn, 1991).

Herbicides and research related to their biochemical mechanisms of action have helped to unravel details of several of the biochemical pathways in plants. Historically, effects of various chemicals on photosynthesis and respiration have been studied since around 1878. Then, chloroform was described as reversibly inhibiting photosynthesis at concentrations that did not affect respiration (Moreland, 1993).

In France, in 1933, the first important discovery in the field of selective weed control was the use of DNOC, a contact herbicide that killed the majority of annual weeds infesting the crop without causing appreciable damage to the cereals. Its selectivity is explained by the fact that DNOC is not translocated in plants and perennial weeds were not killed because, although their top growth was desiccated, their extensive root systems survived and in due course sent up further shoots (Cremlyn, 1991). DNOC was developed for use in Europe and dinoseb (DNBP) for use in USA (Moreland, 1993). DNOC and other dinitro compounds played a big part in increasing food production during World War II as herbicides. They can also be used as insecticides, especially as winter wash for fruit trees (Cremlyn, 1991).

On field observations conducted in 1940 and 1941 by Templeman and Sexton, phenoxyacetic acids were toxic to dicotyledonous, but not monocotyledonous plants. Eventually, 2,4-D was commercially developed for use in U.S.A. and MCPA for use in Europe from 1947 (Rao, 2000). The discovery of phenoxyalcanoates revolutionized practices and the success achieved in their use probably stimulated industry to invest in research that led to the discovery of the large variety of herbicides that has been used until now (Moreland, 1993).

Templeman and Sexton also reported, on field observations made in 1940 with esters of arylcarbamates and thiocarbamates, that they were toxic to monocotyledonous but not to dicotyledonous species (Moreland, 1993).

In 1949, Macdowall reported inhibition of the Hill reaction, in illuminated chloroplasts, by phenylurethane ($I_{50}=2$ mM) and dinitrophenol (DNP) ($I_{50}=0.63$ mM). However, based on I_{50} , arylcarbamates and phenylureas were up to 2500 times more active than phenylurethane, as was detected by Wessels and van der Veen in 1956 (Moreland, 1993).

Herbicides are the most widely used class of pesticides accounting for more than 60% of all pesticides applied in agriculture (Zimdahl, 2002). One of the main concerns about the use of herbicides is their effects on non-target organisms, especially mammalian toxicity.

Most modern herbicides have low mammalian toxicity, because research for new herbicides often rejects chemicals affecting metabolic pathways that are shared by mammals. In many cases, the low mammalian toxicity has to do with the fact that these chemicals interfere with biochemical pathways that do not exist in mammals, such as photosynthesis, essential amino acids biosynthesis or chlorophyll biosynthesis (Shaner, 2003).

There are about 20 mechanisms of action that have been elucidated for herbicides. Of these, some do share common target sites with mammals. However, the consequence of inhibiting a common target site in plants can be quite different than in animals. What may be a lethal event in plants, e.g. inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), can even have a beneficial effect in mammals, e.g. treatment for tyrosinemia type I (Shaner, 2003). Also a common enzyme existing in plants and mammals, e. g. acetyl-CoA carboxylase (ACCase), can be inhibited by the herbicides cyclohexanediones and the aryloxyphenoxypropionates, killing plants, and not affecting ACCase in mammals. ACCase plays the same role in the fatty acid biosynthesis of the different organisms, despite vast differences in its regulation and protein structure (Inclendon & Hall, 1997; Shaner, 2003). These herbicides suppress *de novo* synthesis of fatty acids in sensitive species by inhibiting the activity of plastid-associated ACCase. Aryloxyphenoxypropionates were reported by Hoechst AG in 1971 (Nester, 1982), and cyclohexanediones (alloxydim and sethoxydim) were introduced by Nippon Soda in 1977 and 1978 (Iwataki & Hirono, 1979; Ishikawa et al., 1985), being used as postmergence herbicides for the control of annual and perennial grasses in broadleaf crops.

Other commercially available herbicides that affect similar target sites in plants and mammals, with the exception of the bipyridiliums, have minimal toxicity to mammals because they are rapidly metabolized and excreted by mammals. As the herbicides do not accumulate in mammalian tissue, they cannot affect the biosynthetic pathways (Shaner, 2003).

On the other hand, the effects of inhibiting a particular enzyme in plants can be quite different from those observed in mammals. For example, considering that protoporphyrin biosynthesis is critical in plants and animals for the production of chlorophyll and heme, PROTOX (protoporphyrinogen IX oxidase) inhibitors cause the accumulation of Proto IX (protoporphyrin IX), which then absorbs light energy and produces reactive oxygen species that disrupt membranes by lipid peroxidation. In mammals, the accumulation of Proto IX also occurs in the liver and bile, but these organs are not normally exposed to light intensities like plant leaves. So, there is little opportunity for the Proto IX to produce degrading reactive oxygen species in animals (Shaner, 2003). Peroxidizing herbicides include diphenylether herbicides and oxodiazoles causing accumulation of large amounts of Proto IX, which generates singlet oxygen by light. If this oxygen free radical is not quenched, lipid peroxidation can be initiated (Lydon & Duke, 1988; Matringe & Scalla, 1988; Camadro et al., 1991).

It was observed in 1980 that glyphosate was a highly specific inhibitor of shikimate pathway enzyme 5-enolpyruvylshikimate 3-phosphate synthase (Steinrucken & Amrhein, 1980). Afterwards, sulfonylureas and imidazolinones were introduced as herbicides interfering with biosynthesis of branched-chain amino acids (leucine, isoleucine, and valine). It was shown that both herbicides inhibited the activity of acetolactate synthase (Ray, 1984; Shaner et al., 1984)

Herbicides can also inhibit carotenoid synthesis. As carotenoids are free radical scavengers, they protect chlorophyll from photooxidation (bleaching herbicides). One of the first compounds shown to function in this way was the pyridinazinone SAN 6706 (Bartels & McCullough, 1972). Afterwards, other compounds identified with similar functions include fluridone, difunon, dichlormate, and aminotriazole (Moreland, 1993).

Some times, e.g. glufosinate in suicide attempts, much of the toxicity has been related to the associated surfactant that is included in the formulation of the herbicide rather than to the active herbicide (Koyama et al., 1997).

From all the information here considered, it is obvious that the use of herbicides implies the existence of advantages and disadvantages resulting therefrom, which, in general, are listed in Table 1.

ADVANTAGES	DISADVANTAGES
<ul style="list-style-type: none">– They kill unwanted plants.– They help the crops grow by destroying the weed that is robbing the crops water, nutrients and sunlight.– They can be safely used whereas in some cases manually or mechanically removing weeds can destroy the crop.– They can be used on closely planted crops where other methods cannot be used.– Most of the time one application of the herbicide is enough whereas other methods have to be continually used.– They are easy to use.– They work fast.– Herbicides are relatively cheap, and cheaper than manual weeding.– Non-selective herbicides can effectively clear fields, where houses and roads can then be built.– They can destroy plants bearing diseases.– Some are biodegradable, and become relatively harmless after decay.	<ul style="list-style-type: none">– Some herbicides are non-biodegradable being harmful for a long period of time.– They are all at least slightly toxic.– They can cause illnesses, and even cause accidental or suicidal death (like paraquat).– They can be carried into streams by runoff rainwater or leached into underground water supplies polluting them.– Herbivores may eat the plants treated with herbicides and then carnivores eat the herbivores. The toxic herbicide would be passed up the food chain increasing in concentration, being harmful for those animals and man.

Table 1. Advantages and disadvantages of herbicides.

Scientists have made phenomenal progresses in understanding the selective action of hundreds of herbicides by studying their absorption and translocation patterns, mechanisms of action in plants, degradative and detoxification mechanisms in plant and soil, interactions

with other pesticides and chemicals, etc. All this has helped in making more effective, economical and safe recommendations for control of numerous weeds (Rao, 2000).

Lately, scientists have already researched on natural product-based herbicides, which are generally considered safer than synthetic herbicides. However, relatively few commercial herbicidal agents have been derived from naturally occurring compounds. One of the indirect and important benefits of the chemical composition and structural characteristics of natural products is that most of these compounds are rapidly degraded in the natural environment. Thus, this accounts for the perception that most natural products are environmentally benign (Dayan et al., 1999).

An alternative approach of natural products-based weed management consists of having crops producing their own phytotoxins to prevent or suppress the growth of competing weeds in its immediate surroundings (allelopathy). Although some species strongly repress the development of other plants, allelopathic crops have had little success so far (Dayan et al., 1999). Weed scientists are now facing new challenges, particularly in the light of emergence of weeds resistant to herbicides and concerns and questions about herbicide residues in food, soil, groundwater and atmosphere (Rao, 2000).

Finally, when scientists were capable of getting genes that encode target site enzymes cloned, modified and expressed in transgenic plants, they could confer herbicide tolerance. The possibility of developing herbicide-resistant crops began a new era in the use of herbicides, stimulating work on the genetics of herbicide resistance.

II. Classification of herbicides

By 1976, the herbicides that interfered with electron transport and phosphorylation in both chloroplasts and mitochondria were separated into five classes: a) electron transport inhibitors, b) uncouplers, c) energy transfer inhibitors, d) inhibitory uncouplers (multiple types of inhibition), and e) electron acceptors (Moreland, 1993).

Electron transport inhibitors include the Hill reaction inhibitors. Uncouplers dissociate electron transport from ATP formation by permeabilization to protons collapsing ΔpH . Energy transfer inhibitors interact with the coupling factor complex. Inhibitory uncouplers are those herbicides that act both as electron transport inhibitors and uncouplers. Electron acceptors are herbicides like bipyridilliums that intercept electron flow from photosystem I.

With isolated mitochondria, the inhibitory uncouplers uncouple phosphorylation at low molecular concentrations and inhibit electron transport at higher concentrations (Moreland, 1993).

The pure electron transport inhibitors also have been called diuron-type and include chlorinated phenylureas, pyridazines, s-triazines, triazinones, uracils, and ureacarbamates. This type of herbicides inhibits the Hill reaction but do not inhibit mitochondrial electron transport. Hence, light was known to be required for the expression of toxicity, i.e., plants maintained in the dark revealed no signs of toxicity (Moreland, 1993).

Inhibitory uncouplers were divided into two groups: dinoseb-type and dicryl-type. Included as dinoseb-type are dinitrophenols, benzimidazoles, benzonitriles, bromophenoxim, and thiadiazoles. Dicryl-type includes arylanilides, dinitroanilines, diphenylethers, bis-carbamates and perfluidone (Moreland & Novitzky, 1988).

Herbicides are designated by common names approved by the Weed Science Society of America or the British Standards Institution (Moreland, 1980), and organic herbicides are classified on the basis of: a) method of application; b) chemical affinity and structure similarity, and; c) mode of action (Rao, 2000).

On the basis of the method of application, herbicides are distributed in two groups: 1) soil-applied and 2) foliage-applied. All herbicides applied at preplanting (surface or incorporation) and preemergence (to crop, weeds, or both) are included in the soil-applied group; those applied at postemergence on the plant parts are included in the foliage-applied group.

Here we also present two general classifications of herbicides according to the mode of action and according to the chemical composition.

According to the mode of action, we present in Table 2 the classification extracted and adapted from a review (Moreland, 1980).

Classes of herbicides	Examples of herbicides
*CHLOROPLAST-ASSOCIATED REACTIONS <i>TYPES OF INHIBITORS</i> Electron transport inhibitors Uncouplers Energy transfer inhibitors Inhibitory uncouplers Electron acceptors Carotenoid synthesis inhibitors	Diuron, atrazine, pyrazon, etc. Perfluidone. 1,2,3-thiadiazolyl-phenylureas. Dinitrophenols, acylanilines, etc. Paraquat, diquat. Amitrole, dichlormate, etc.
*MITOCHONDRIAL ELECTRON TRANSPORT AND PHOSPHORYLATION <i>TYPES OF INHIBITORES</i> Electron transport inhibitors Uncouplers Energy transfer inhibitors Inhibitory uncouplers	Pyriclor. Isipropyl ester of glyphosate. No herbicide. Dinitrophenols, acylanilines, etc.
*MEMBRANE INTERACTIONS Composition alterations Permeability and integrity actions	Dinoben, perfluidone, ioxynil. Paraquat, diclofop-methyl.
*CELL DIVISION Energy relations Microtubules action	Chlorpropham, trifluralin. Prophan, amiprofos-methyl.
*NUCLEIC ACID METABOLISM DNA and RNA action	Trifluralin.
PROTEIN SYNTHESIS Protein synthesis inhibitors	Glyphosate.

Table 2. Classification of herbicides according to the mode of action at the level of the main metabolic systems in the cell.

The other classification, distributing herbicides by groups with chemical affinities (Cremlyn, 1991; Rao, 2000), is presented in Table 3.

Classes of herbicides	Examples of herbicides	Mode of action
ALIPHATICS	¹ Dalapon, ² TCA.	¹ Interference with RNA synthesis; ^{1,2} modifications of protein structure.
AMIDES Acylanilides	Propanil, pentanochlor.	Inhibition of Hill reation in photosynthetic electron transport.
Chloroacetanilides	¹ Allidochlor, ² propachlor, ² alachlor, ² butachlor, ² pretilachlor, ² diethatylether, ² metolachlor, ² metazochlor.	¹ Inhibition of protein synthesis, respiration; ² Inhibition of cell division and protein synthesis.
Benzamides	Benzoylpropethyl, flamprop-isopropyl, fampropmethyl, isoxaben.	Unknown.
Other amides	¹ Naptalam, diphenamid, ² propyzamide, butam, ³ diflufenican.	¹ Alteration of auxin transport; ² interference with cell division; ³ inhibition of carotenoid biosynthesis.
AROMATIC CARBAMATES AND THIOCARBAMATES Arylcarbamates	Propham, chloropropham, barban.	Interference with photosynthesis; alteration of meristematic activity; desorganization of microtubular assembly, inhibiting mitosis.
Thiocarbamates	EPTC, butylate, cycloate, pebulate, diallate, triallate, benthicarb, asulam, nisulam.	Interference with lipid biosynthesis.
ARSENICALS	DSMA, MSMA, cacodylic acid.	They are uncouplers and inhibit respiration.
BENZOTHIADIAZOLES	Bentazon.	Inhibition of photosynthesis and induces lipid peroxation.
BIPYRIDILIUMS	Diquat, paraquat.	Induce lipid peroxidation by reactive oxygen species production.
CARBOXYLIC ACIDS Phenoxyacetic acids	¹ 2,4-D, MCPA, 2,4,5-T, etc.	Inhibition of normal plant growth mimicking auxins (IAA); ¹ Inhibition of oxidative phosphorylation.
Phenoxybutiric acids	2,4-DB, MCPB.	Inhibition of normal plant growth mimicking auxins (IAA).

Classes of herbicides	Examples of herbicides	Mode of action
Benzoic acids	TBA, dicamba.	Inhibition of normal plant growth mimicking auxins (IAA)
Pyridine derivatives	Picloram, clopyralid, triclopyr.	Inhibition of normal plant growth mimicking auxins (IAA).
Propionic acid derivatives	Dchlorprop, mecoprop, fenoprop, haloxyfop, fenoxaprop, fluazifop-P.	Inhibition of normal plant growth mimicking auxins (IAA).
CHLOROALIPHATIC ACIDS	¹ Dalapon, ² TCA.	¹ Interference with RNA synthesis; ^{1,2} modifications of protein structure.
CINEOLES	*Cinmethylin.	Inhibition of asparagine synthetase.
CYCLOHEXANEDIONES	Clethodim, cycloxdim, sethoxydim, tralkoxydim.	Interference with lipid metabolism.
DINITROANILINES	Trifluralin, benfluralin, profluralin, ethalfluralin, fluchloralin, oryzalin, nitratin, butralin, pendimethalin, dipropalin.	Inhibition of cell division.
DIPHENYL ETHERS	Nitrofen, fluorodifen, bifenox, ¹ oxyfluorfen, fomesafen.	Inhibition of Hill reation in photosynthesis and photophosphorylation; ¹ inhibition of protoporphyrinogen oxidase.
IMIDAZOLIDINONES	Buthidazole.	Inhibition of r espiration and photosynthesis.
ISOXAZOLIDINONES	Clomazone.	— —
IMIDAZOLINES	Imazaquin, imazethapyr.	Inhibition of cell division by action on the enzyme acetolactate synthase (ALS) involved in valine and isoleucine biosynthesis.
NITRILES	¹ Dichlobenil, ² bromoxynil, ² ioxynil.	¹ Inhibition of cellulose biosynthesis; ² uncoupling of oxidative phosphorylation and Hill reation inhibition.
ORGANOPHOSPHORUS COMPOUNDS	¹ Glyphosate, bensulide, **tributyl phosphorotrithiolate.	¹ Inhibition of biosynthesis of phenylalanine.

Classes of herbicides	Examples of herbicides	Mode of action
OXADIAZOLES	Oxadiazon.	Causes rapid peroxidative damage.
PHENOLS	Dinoseb, DNOC.	Uncouplers of oxidative phosphorylation.
N-PHENYLPHTHALAMIDES	Flumiclorac.	Inhibition of protoporphyrinogen oxidase.
PHENYL TRIAZINONES	Sulfentrazone.	Inhibition of protoporphyrinogen oxidase.
PHTHALAMATES	Naptalam.	It is an auxin inhibitor.
PYRAZOLIUMS	Difenzoquat.	It affects nucleic acid biosynthesis, photostynthesis, ATP production, K ⁺ absorption and P incorporation in phospholipids and DNA.
PYRIDAZINES	¹ Chloridazon, ² metflurazon, ² norflurazon, ¹ pyridate.	¹ Inhibition of Hill reation in photosynthesis; ² Inhibition of carotenoid biosynthesis.
PYRIDINONES	Fluridone.	Bloks carotenoid biosynthesis.
PYRIMIDINYL THIOBENZOATES	Pyrithiobac.	Inhibition of cell division by action on the enzyme acetolactate synthase (ALS) involved in valine and isoleucine biosynthesis.
QUINOLINE CARBOXYLIC ACIDS	Quinclorac.	Auxin-type herbicide. Accumulates ACC, cyanide and ethylene.
SULPHONYLUREAS	Sulfometuron methyl, DPX-F 6025, DPX-F 5384, chlorsulfuron, DPX-L 5300, bensulfuron methyl.	Inhibition of cell division by action on the enzyme acetolactate synthase (ALS) involved in valine and isoleucine biosynthesis.
TRIAZINONES	Metribuzin.	— —
TRIAZOLOPYRIMIDINE SULFONANILIDES	Flumetsulam.	Inhibition of cell division by action on the enzyme acetolactate synthase (ALS) involved in valine and isoleucine biosynthesis.

Classes of herbicides	Examples of herbicides	Mode of action
TRIAZINES	Simazine, atrazine, prometryne, desmetryn, methoprotryne, terbutryn, cyanazine, eglinazine ethyl, aziprotryne, metrybuzin, met amitron, prometron, terbuthylazine, methroprotryne, aziprotryne.	Inhibition of Hill reation in photosynthetic electron transport.
TRIAZOLES	***Amitrole.	Probable interference with carotenoid biosynthesis leading to photooxidation of chlorophyll.
URACILS (PYRIMIDINES)	Bromacil, terbacil, lenacil, UCC-C4243.	Inhibition of Hill reation in photosynthetic electron transport.
UREAS	Monuron, diuron, linuron, monolinuron, chlorotoluron, isoproturon, difenoxuron, thiazafluron, terbuthiuron, ethidimuron, fluometuron, S3552.	Inhibition of Hill reation in photosynthetic electron transport.
OTHER HETEROCYCLIC HERBICIDES	¹ Endothal, ² ethofumesate, ³ benzolin, ⁴ oxadiazon, ^{4,5} difenzoquat.	¹ Inhibition of lipid and protein biosynthesis; ² interference with plant cuticular wax; ³ interence with cell growth and development; ⁴ inhibition of carbon dioxide fixation and electron transport in photosynthesis; ⁵ alteration of DNA and RNA synthesis.

*Is a member of the herbicide group called cineoles (monoterpenic cyclic ethers) (Romagni et al., 2000)
**Defoliant, induces early leaf abscission through changes in the levels of plant hormones (Matolcsy, 1988)
***Its use in fodder and food crops has been banned worldwide (suspicious of carcinogenicity)

Table 3. Classification of herbicides according to chemical classes.

2. *In vitro* toxicological assays: some biological model-systems

In this section, we will address some of the experimental strategies used by our group to evaluate the toxic potential of pesticides upon interaction with biological systems, using *in vitro* assays. The authors are aware that many other models have been successfully used with the same objectives, some of which will be discussed in confront with authors results in the next section. However, an exhaustive description of *in vitro* model systems used to herbicide toxicological assessment is out of the scope of this chapter.

I. Mitochondria, a key organelle to push cells towards survival or death

Mitochondria are efficient power plants of energy generation for eukaryotic cells. Moreover, mitochondria play a crucial role in several physiological processes and are involved in cell replication, differentiation and apoptosis (Boelsterli, 2003). In this way, mitochondria endowed eukaryotic cells with a broad spectrum of functionalities that prokaryotic cells do not have, but, concomitantly, they brought with them a multiplicity of vulnerable points to host cells. In fact, these organelles are a key target for xenobiotics to induce dysfunction at a cell, organ or organism level and the impairment of energy production is not the only harm of mitochondrial poisoning.

There are several means by which xenobiotics interfere with mitochondrial function. However, as most xenobiotics considered in this chapter are lipophilic molecules, their effects on the activities of mitochondrial matrix soluble enzymes involved in the fatty acid β -oxidation and tricarboxylic acid oxidation pathways will not be directly addressed. The focus of this section will be mainly the xenobiotic-induced alterations of mitochondrial membrane-related processes, such as electron transport, transmembrane potential ($\Delta\Psi$) generation, oxidative phosphorylation, oxygen reactive species generation, permeability transition and the release of pro-apoptotic proteins.

Three important features make mitochondria vulnerable to membrane-active compounds. The first one is the unique lipid composition of the inner membrane (Daum, 1985), with virtually no cholesterol and a high content of cardiolipin (CL), a phospholipid that is common in bacterial membranes but, in eukaryotic cells, only exists in membranes of mitochondria and chloroplasts (Gennis, 1989). Several xenobiotics have a high affinity for CL, which explains their mitochondriotropic effects (Boelsterli, 2003). A very well known example is given by adriamycin-like anthraquinones (Wallace & Starkov, 2000). The accumulation of these xenobiotics at the inner mitochondrial membrane may have different consequences. In the case of adriamycin, for example, it leads to the generation of oxidative stress due to redox cycling of quinone-semiquinone moiety (Boelsterli, 2003). Considering the important roles CL plays in mitochondria (see below), xenobiotic incorporation in specific CL-enriched domains of the inner mitochondrial membrane may predictably have disastrous outcomes for mitochondrial bioenergetics and also for the fate of cells towards survival or death. Cardiolipin is included in the quaternary structure of the complexes II, IV and V (Eble et al., 1990; Fry & Green, 1981; Sedlak et al., 2006) of the mitochondrial electron transport system. Besides those complexes, the complex I also requires cardiolipin for exhibiting optimal activity (Fry & Green, 1981). Furthermore, cytochrome c (cyt c) establishes specific interactions with CL (Rytomaa et al., 1992), which have been suggested to participate in a well coordinated mechanism of programmed cell death, involving cyt c-catalyzed peroxidation of CL, permeabilization of the outer mitochondrial membrane and release of cyt c and other apoptogenic factors from the mitochondria (Gonzalez & Gottlieb, 2007; Basova et al., 2007). As proposed by Basova et al. (2007), CL association with cyt c could be a decisive step in the early stages of apoptosis, turning off the normal functions of this hemoprotein in mitochondrial electron transport and turning on its peroxidase activity. Therefore, xenobiotics, directly interacting with CL or modulating the bilayer properties of the mitochondrial membranes, may have drastic consequences in mitochondrial bioenergetics and/or apoptosis (Gonzalez & Gottlieb, 2007).

The second feature which makes mitochondria vulnerable to xenobiotics is related with the high potential across the inner mitochondrial membrane, typically in the range of -180 to -220 mV. The anisotropic distribution of both charges and pH (the matrix side being

negatively charged and slightly alkaline in opposition to the outer side of the membrane, facing the intermembrane space) confers to mitochondria a high susceptibility to accumulate large amounts of positively charged lipophilic compounds. The accumulation of these compounds into mitochondria, at concentrations that may exceed those of the cytosol by several orders of magnitude, exposes these organelles to potentially toxic compounds at much higher levels than any other cellular compartment.

The third reason why mitochondria are prone to be poisoned by lipophilic compounds concerns with the protein-regulated permeability of the inner mitochondrial membrane, which has a crucial importance for the functional integrity of mitochondria. Therefore, drastic consequences for mitochondrial intactness and bioenergetic functioning can be predicted whenever compounds promote an increase of the passive permeability of mitochondrial inner membrane, impair the activity of transport proteins embedded within it or interfere with the assembling of protein components of the mitochondrial permeability transition pore (MPTP). Xenobiotics that cause the opening of MPTP may also promote the release of apoptosis mediators, inducing apoptosis.

On the other hand, xenobiotics that interact with one or several complexes of the mitochondrial electron transport system, impairing the normal electron flow, may enhance the generation of reactive oxygen species (ROS), leading to an imbalance between prooxidant species and the cellular antioxidants. The complex I (NADH: ubiquinone oxidoreductase) is often a site of interference of xenobiotics acting as electron acceptors in reductase-catalyzed reactions. Redox cycling compounds, as the herbicide paraquat, may be particularly harmful. The generation of ROS, amplified by the cycling process, may lead to oxidative DNA damage and lipid peroxidation (Boelsterli, 2003). Additionally, xenobiotics that promote oxidative stress as well as those that induce a sustained increase of mitochondrial calcium concentration may disrupt mitochondrial function by opening the MPTP. The consequent uncoupling of oxidative phosphorylation, due to the collapse of mitochondrial membrane potential, and the release of pro-apoptotic factors are, in this case, the main cause of cellular damage (Boelsterli, 2003).

Although rat liver mitochondria constitute a common model for toxicity assessment, plant mitochondria can be an alternative model, offering several advantages, namely high activities preserved for much longer periods of time, easier availability and low cost (Vicente & Madeira, 2000). In the context of herbicide toxicity evaluation, the use of plant mitochondria is particularly important since useful plants (crops) are often target of the non-selective toxic effects of those compounds.

II. Unicellular microorganisms, a simple model system to study the complex routes of cell/membrane toxicity

A great number of compounds produced by agrochemical industries, including herbicides, are lipophilic molecules. In the case of biocide compounds, this feature is important to increase their ability to overcome cell membrane barriers. In a toxicokinetic context, the disposition of a foreign molecule in a biological system necessarily involves the passage across cell membranes. Thus, lipophilicity is a major determinant of compound capacity to exert noxious effects on target organisms. However, cellular compartmentalization by phospholipid-based membranes is a feature common to all living organisms and, then, all share a high vulnerability to lipophilic compounds. The selective effects of these compounds on specific organisms may arise from factors that affect compound metabolism, distribution or excretion (toxicokinetics) and from differences in the existence, accessibility or structural

singularities of the molecular target of these compounds (toxicodynamics). The level of concern increases when the interaction of lipophilic compounds with membranes results in unspecific alterations of the physical properties of the lipid bilayer, compromising cell normal functioning. In fact, the lipid bilayer, besides defining a permeability barrier and providing a matrix for embedded proteins, endows the membrane with the capacity of regulating protein function (Lundbaek et al., 2010). Bilayer material properties such as thickness, intrinsic curvature, and the elastic compression and bending moduli, have been shown as having a key role on protein folding, trafficking, distribution in the plane of the membrane and function (McIntosh & Simon, 2006). Therefore xenobiotic-induced bilayer deformations or perturbation of their physical properties may cause drastic effects on a great diversity of vital cell functions. In this case, the interactivity with membrane lipid bilayer is involved in both toxicokinetics and toxicodynamics of the compounds.

In microbial cells, most vital functions are ascribed to the plasma membrane, e.g. signal and energy transductions, regulation of the intracellular environment and transport of solutes (Kaback, 1972; Stock et al., 1990; Trumpower & Gennis, 1994). Not surprisingly, perturbations of membrane structure and function by pollutants will end in more or less severe disturbance of cell growth and viability. Therefore, these simple systems can offer the opportunity to screen the cytotoxic potential of membrane-active compounds. Measurements of growth parameters provided by bacterial cell cultures in liquid media (e.g. specific growth rate, final bacterial yield), the counting of colony forming units in solid growth media and the evaluation of bacterial respiratory activity or other metabolic parameters have been extensively used as short-term screening tests for *in vitro* assessment of the toxicity of several environmental pollutants (Hernando et al., 2003; Sikkema et al., 1995; Monteiro et al., 2005).

On the other hand, bacteria have one, in maximal two, membranes surrounding the cellular content, which can be easily isolated providing a pure membrane fraction. Two experimental approaches may be developed by using bacterial membranes as a biological model system: a) evaluation of the influence of membrane biophysical properties, perturbed by the accumulation of alien lipophilic molecules, on cellular toxic effects; b) identification of bacterial mechanisms of regulation of the membrane lipid composition triggered by xenobiotic incorporation and its impact on counteracting cell toxicity. In fact, alterations of membrane lipid content (the relative proportions of different lipid classes and the acyl chain composition of phospho- and glycolipids) are common strategies for adaptation and responses to stress in bacteria (Jurado et al., 1991; Sikkema et al., 1995; Weber et al., 1996). Since bacterial lipid regulatory system is highly precise and sensitive, studies of the molecular mechanisms of membrane adaptation, together with determinations of growth inhibition and impairment of bacterial metabolic activities, can provide an alternative methodology for toxicity assessment. Thus, microorganisms offer the advantage of allowing the assessment of xenobiotic toxic effects ranging from the molecular to the cell/whole organism level. These data, together with the knowledge of physicochemical characteristics of the compounds, can be useful to establish structure-activity relationships.

In the field of ecotoxicology, two main reasons explain the considerable attention effects of lipophilic pollutants on microorganisms have drawn: firstly, microorganisms are responsible for recycling organic material in soil ecosystems and, then, are valuable indicators of pollutant ecological impact. Thus, toxicological data concerning soil bacteria may be used to define upper limits for concentration of pollutants and to predict environmental toxicity risks; secondly, several of those microorganisms have shown to be

compared with MCPA or dicamba. Paraquat and 2,4-D showed similar efficiency in promoting the decrease of cell energy charge, but paraquat effect on the cell redox state was the highest of all the herbicides tested in potato callus cells. Consistently, paraquat revealed to be the most potent growth inhibitor, too (Peixoto et al., 2008).

As previously referred for paraquat, the activity of the antioxidant enzymes CAT, GST and GR was evaluated upon incubation of the potato callus tissue with different concentrations of the herbicides 2,4-D, MCPA and dicamba (Peixoto et al., 2008; 2009a). Considering the two herbicides of the phenoxyacetic acid group, it was observed that 2,4-D (Peixoto et al., 2008) strongly stimulated the catalase (150%) and superoxide dismutase (about 50%) activities at the highest concentration assayed (50 μ M), whereas MCPA, up to 120 μ M, did not induce any significant change (Peixoto et al., 2009a). Apparently, MCPA does not stimulate production of the superoxide anion and hydrogen peroxide, in contrast with 2,4-D and paraquat (Peixoto et al., 2008). Dicamba promoted an increase of CAT and SOD activities, although at a smaller extent than 2,4-D (Peixoto et al., 2008).

Concerning the GST activity, an enzyme involved in the detoxification of a great deal of pollutants, it was stimulated by both phenoxyacetic acid herbicides (2,4-D and MCPA) and by dicamba, in contrast with paraquat, as previously referred. As GST uses GSH for conjugation with electrophilic compounds, it would be expected GR activity to be also stimulated by the herbicides. In fact, it was observed a stimulation of GR activity in callus tissue cultures incubated with all the herbicides tested, paraquat included (Peixoto et al., 2008; 2009a). Although the experimental approach used in these studies do not enable authors to conclude that GST is involved in 2,4-D, MCPA and dicamba bioinactivation in potato callus tissue, their results concur with data in the literature regarding different herbicides and several plant species for which GST activity plays an important role in detoxification (Hatton et al., 1998). In the case of paraquat, GR activity stimulation was probably induced as a cellular response to an intracellular decrease of GSH used to detoxify the reactive oxygen species generated as a consequence of the herbicide redox cycling (see above). Additionally, GSH oxidation could be also related to other cytotoxic events such as lipid peroxidation and decrease in protein thiols, which have been demonstrated in isolated rat hepatocytes treated with 2,4-D and paraquat (Palmeira et al., 1995b).

In conclusion, data obtained in plant and animal cell cultures concur to suggest similar mechanisms of toxicity by 2,4-D and paraquat in non-target organisms, although with different relative potencies. The decrease of GSH/GSSG ratio is probably the primary event in the cytotoxic process leading to death. In the case of MCPA and 2,4-D, the lack of studies in animal cells precludes any comparison. However, it is probable that MCPA cytotoxicity results primarily from disturbance of membrane physicochemical properties leading to loss of electrolytes and metabolites, decrease in ATP availability and decrease of the cellular redox state. Dicamba biological activity shows some similarities with both 2,4-D and MCPA. It disrupted the membrane integrity as 2,4-D and MCPA but induced an increase of catalase and SOD activities, in contrast with MCPA, although in a lower extent than 2,4-D. At the mitochondrial level, dicamba uncoupled oxidative phosphorylation by inducing H^+ permeabilization, as MCPA, but also inhibited the electron transfer in the mitochondrial redox system, as 2,4-D (see above).

Since all the herbicides tested in potato callus tissue promote significant alterations in plant as well as in animal mitochondria functioning, the bioenergetic impairment may be suggested as one of the mechanisms underlying the cytotoxicity of those compounds in non-target organisms.

III. Effects of paraquat and oxifluorfen on tilapia fish (*Oreochromis niloticus*)

Hundreds of herbicides of different chemical structures, extensively used to control a wide variety of agricultural pests, contaminate aquatic habitats due to leaching and runoff water from treated areas. Fish are among the non-target organisms that can be seriously affected by the ecological imbalance imposed through this excessive charge of chemicals. In freshwater, the presence or absence of fish could be used as a bioindicator of the degree of water pollution. Considering that fisheries are an important source of food provided with protein and lipid of high nutritional value, representing an economic benefit for many countries, herbicide contamination of surface waters derived from agricultural practices is a problem of worldwide importance. On the other hand, fish constitute the vertebrate model most used in the field of ecotoxicology.

Mortality data, although being the definitive demonstration of toxicity for the organism in study, are often left out of ecotoxicological studies as being unable to provide reliable information about the environmental hazard resulting from pollutant contamination. Therefore, rather than mortality, physiological parameters (Tortorelli et al., 1990) and biochemical alterations (Stephensen et al., 2002) have been extensively used to perform an integrated evaluation of the risk pollutants may represent for the wildlife.

Toxicity exerted by many herbicides in aquatic organisms has been associated to an increased generation of ROS (Di Giulio et al., 1989; Livingstone et al., 1990). These toxic effects can be promoted via redox cycling, paraquat being the paradigmatic example, or result from herbicide metabolism by phase I, with production of ROS as by-products. Under physiological conditions, antioxidant defenses are available in fish, like in mammals, to scavenge ROS or to prevent its production (Figueiredo-Fernandes et al., 2006; Peixoto et al., 2006). In the presence of pollutants promoting oxidative stress, organisms can adapt by up-regulation of antioxidant defenses such as GSH-related enzymes, which constitute non-specific biomarkers of exposure to pro-oxidative xenobiotics. Thus, alterations in the activity of antioxidant enzymes have been proposed as early indicators of exposure to pollutants, including herbicides (Di Giulio et al., 1989).

Several field studies for biomonitoring environmental pollutants have shown that alterations of GSH-dependent/producing enzymes as well as GSH content may be useful tools to evaluate the ecotoxicological effects exerted by herbicides in fish. Stephensen et al. (2002) showed that the levels of GSH and GSH-related enzymes in the liver of rainbow trout (*Oncorhynchus mykiss*) constitute suitable biomarkers for oxidative stress in fish developed in laboratory. In this context, tilapia (*Oreochromis niloticus*) has been used as a biological model for toxicological assessment, showing several advantages: high growth rates, easy reproduction, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, as well as good tolerance to a wide variety of husbandry conditions (Fontáinhas-Fernandes, 1998). On the other hand, tilapia constitutes an indicator species in biomonitoring programs, since an increased level of its biotransformation enzymes may reflect the existence of environmental pollutants.

Figueiredo-Fernandes et al. (2006) carried out studies of the effects of paraquat on the hepatic levels of antioxidant enzymes in tilapia. Studies regarding the toxic action of xenobiotics in animals are often performed in liver, since this organ is a main target of chemical injury. This fact results from its role in xenobiotic biotransformation and its unique position within the circulatory system. Therefore, biochemical and histological alterations in fish hepatocytes have been extensively reported as important biomarkers of exposure to toxic compounds, in laboratory as well as in field studies.

The effects of paraquat on oxidative stress enzymes of tilapia hepatocytes were studied in the dependence of temperature and gender (Figueiredo-Fernandes et al., 2006). Males and females of tilapia were kept in tanks at 17 and 27 °C (breeding non-compatible vs breeding compatible temperatures). A qualitative analysis showed that fish mortality induced by paraquat was accompanied by the appearance of extensive areas of hepatocytic necrosis. Preliminary experiments were carried out to select test concentrations of paraquat corresponding to 25% of the LC50, at which hepatocytes showed no signals of necrosis. SOD and GST activities showed sex-dependence, since males present higher values than females at both temperatures assayed. No significant alterations were found in the dependence of temperature. SOD, GR and GST activities were increased by exposition to paraquat. An increase of GR activity induced by paraquat was also demonstrated (Stephensen et al., 2002) in rainbow trout. The authors justify this fact due to the ability of GR to reduce paraquat to its cation radical, causing a chain reaction with paraquat radical promoting oxidation of GSH, which in turn induced an increased activity of GR.

We have also observed alterations of the condition factor (CF) and the hepatosomatic index (HSI) of tilapia exposed to paraquat (Figueiredo-Fernandes et al., 2006). These parameters serve as indicators of the general organism well being. A high condition factor corresponding to a high body weight/length relationship generally means good environmental conditions. Also a high HSI, defined as the ratio of liver weight to body weight, normally reflects a high reserve of energy. However, an increase of both parameters, CF and HSI, was promoted by paraquat in tilapia. An increase of HSI was also registered in different laboratories upon exposition of fish to different pollutants. This alteration was interpreted (Stephensen et al., 2002) as being due to an increased activity of xenobiotic biotransformation enzymes.

Other studies were conducted in tilapia exposed to the herbicide oxyfluorfen (Peixoto et al., 2006). The exposure of tilapia for a period of 14 to 21 days to sub-lethal concentrations (0.3 to 0.6 mg/L) of oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4(trifluoromethyl) benzene], a diphenyl ether herbicide commonly used in agriculture to control broadleaf and grassy weeds with specific recommendations (EPA 1992), resulted in a progressive increase of the activity of catalase and, to a lower extent, of glutathione reductase. These results are consistent with those reported in the literature concerning different aquatic organisms in the presence of pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Otto & Moon, 1995). Regarding glutathione S-transferase (GST), an enzyme engaged in the detoxification of many xenobiotics, by conjugation of their phase I metabolites, it was observed an increase of its activity induced by 7 days exposition to oxyfluorfen, but thereafter the activity decreased to levels lower than the control. Data in literature are also variable, reporting an increase (Otto & Moon, 1995) or a decrease (Pedrajas et al., 1995) of GST activity in fishes exposed to PAHs and PCBs. In contrast to the other enzymes, SOD activity showed a progressive decrease along the 21 days of exposition to the herbicide. These data are supported by other studies using rainbow trout injected with paraquat (Pedrajas et al., 1995) but are opposite to results obtained with other species exposed to other pollutants. In conclusion, fish antioxidant enzymes have a limited potential to be used as pollution biomarkers, since very different responses can be observed depending both on the xenobiotic and on the organism under study.

It was also observed that tilapia exposure to oxyfluorfen promoted changes in the hepatic fatty acid profile, with special incidence on the unsaturated acids (Peixoto et al., 2006),

decreasing the oleic acid content and increasing the nervonic acid (24:1). It was shown that oxyfluorfen also induced alterations of the fatty acid composition in plants (Watanabe et al., 2001), promoting an increase of the monounsaturated chains (C16:1 and C18:1). Disturbance of the fatty acid biosynthesis regulatory network by a potential oxyfluorfen-induced oxidative stress may explain those alterations.

Oxyfluorfen (0.3 and 0.6 mg/L) was also evaluated on the mitochondrial hepatopancreas bioenergetics of tilapia (Peixoto et al., 2009b). Although no significant change was registered in respiration supported by pyruvate/malate (complex I) or succinate (complex II) as detected by oxygen consumption, the mitochondrial $\Delta\Psi$ was dissipated in some extent. Furthermore, the phosphorylation rate decreased, indicating a perturbation of the phosphorylative mitochondrial system, potentially affecting the ATP availability. The controversial results in herbicide-induced oxygen consumption (no effect), $\Delta\Psi$ dissipation and decrease of the phosphorylation rate can putatively be explained as a limited direct effect on the respiratory complexes, referred in literature as “Biochemical Threshold Effect” (Rossignol et al., 2003). The big size of the fishes used in the study could also explain small effects on the mitochondrial respiration, since bigger and fatter fishes could present higher resistance to xenobiotic, due to its accumulation in fat tissue, particularly when the xenobiotic is lipophilic.

In conclusion, oxyfluorfen induces biochemical changes in fish hepatopancreas regarding the antioxidant enzymatic system, fatty acid metabolism and mitochondrial bioenergetics. However, the relationships between those toxicity parameters have not been completely clarified, yet.

4. Concluding remarks

A great deal of work has been done to assess the environmental risks and health hazards resulting from the extensive and intensive use of herbicides. As other pesticides, the herbicides are common contaminants of surface water and soils. When applied to the agricultural areas, they may have different fates: microbial or non-biological degradation, plant uptake or adsorption and transport by surface water far from the site of application. Therefore, organisms in soil and water niches, from microorganisms to plants and animals, may be exposed to a large number of these pollutants and their metabolites. Additionally, the persistence of these compounds in the environment, associated with their lipophilicity, renders them amenable to progressive accumulation in biological tissues (bioaccumulation) and, consequently, prone to be concentrated along the food chains, increasing the concentration towards the top of the chain (biomagnification). On the other hand, water contamination by agricultural run-off and aerial spraying of herbicides may also have dangerous repercussions on public health even in areas far from those primarily affected. Considering all these risks, including those resulting from a daily consume of eventually contaminated food, it is obvious the general public concern with an unrestricted use of herbicides. The acute and chronic toxic effects of herbicides on useful animals and humans have implemented the research focused on the herbicide mechanisms of action.

A great body of evidence has shown that many environmental contaminants affect transversely the most of the organisms of an ecosystem including man, affecting basic cell functioning and structural properties common to all living cells. In the case of lipophilic compounds, where a great number of herbicides are included, membrane structure and function constitute the first potential target for the toxic effects of the incoming compound,

predictably reflected by perturbations in the bioenergetic process, since this physiological event is on the strict dependence of membrane intactness and stability. On the other hand, this process is a basic mechanism required to drive the activity of all living systems. In prokaryotic cells energy transduction, as well as most vital cell functions, is ascribed to the cytoplasmic membrane. Not surprisingly, perturbations of membrane structure and function by toxic compounds will end in severe perturbation and inhibition of cell growth and viability. On the other hand, mitochondria have a crucial role as energy suppliers in eukaryotic cells, being their functioning strictly dependent on membrane intactness and the proper activity of membrane protein complexes. Thus, these organelles are common targets for the toxic action of lipophilic compounds that, upon incorporation into mitochondrial membranes, can act as uncoupling agents, electron-transport inhibitors or energy-transfer inhibitors.

Huge efforts have been done by our research group to develop predictive tests of the ecological hazards resulting from the exposure to pesticides, assessing in laboratory their toxic effects on model biological systems provided by prokaryotic, animal and plant cells. On the other hand, aiming at elucidating the mechanisms underlying the toxic action of pesticides at a cellular or subcellular level, different experimental approaches have been improved. Three kinds of studies have been implemented in our laboratory to address xenobiotic cytotoxicity *in vitro*: 1) a biophysical approach to lipophilic compound/membrane interactions using membrane lipid models, 2) the use of prokaryotic/eukaryotic cells and 3) the use of mitochondria from plant/animal origin, to evaluate and identify the mechanisms underlying the toxic action of xenobiotics *in vitro*.

A great deal of work has been carried out in our laboratory showing that pesticide membrane incorporation leads to alterations of the structural and physical properties of membrane lipid bilayer, induction of lateral phase separation and generation of lipid microdomains, with predictable impact on protein distribution and sorting in different regions of the membrane (Donato et al., 2000; Videira et al., 2002). Consequent repercussions in membrane permeability and the activity of membrane-associated proteins have been also reported (Videira et al., 2001). One of the biochemical mechanisms that have shown a strong correlation with xenobiotic-induced disturbance of membrane lipid structure and organization is the oxido-reductive systems of bacteria and mitochondria of eukaryotic cells, with obvious impact in all cellular energetic processes (Donato et al., 1997b; Videira et al., 2001; Monteiro et al., 2005 and 2008; Pereira et al., 2009).

A remarkable parallelism has been established between pesticide effects on prokaryotic models (namely the micro-organisms *Bacillus stearothermophilus* and *B. subtilis*) and the other model systems. Thus, partitioning and physical effects have been estimated with bacterial membrane lipids similar to those described for synthetic lipid membrane models and native eukaryotic membranes (Donato et al., 1997c; Donato et al., 2000; Martins et al., 2003; 2005); the impairment of the electron transfer in the bacterial respiratory system has been also demonstrated in rat liver mitochondria (Monteiro et al., 2008; Pereira et al., 2009); the inhibition of bacterial growth and loss of animal cell cultures viability have shown the same dependence on the structural characteristics of chemical related compounds, such as organotin compounds (unpublished data). Mitochondrial fractions of eukaryotic cells have also shown to be plausible alternative *in vitro* model systems for an evaluation of pesticide toxicity, which can simultaneously clarify its mechanisms of action. Data obtained from mitochondria assays have shown to be strongly correlated with cytotoxic parameters

provided by more complex biological systems as whole organisms, like fish (Peixoto et al., 2006). These ones have been widely used as models to evaluate the health of aquatic systems and in studies of xenobiotic-induced pathological effects on vertebrates.

The good correlations established with data provided by these different model systems in studies of herbicide toxicity, as described in this chapter, contribute to validate the use of our models in predictive tests of environmental hazards and toxicity assessment. As also emphasized along this chapter, the most remarkable advantage in using models such as bacteria and plant cells or organelles regards the use of a biological material easily and economically obtained, minimizing or avoiding ethical issues associated with the use of mammals.

5. References

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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