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Review

Toxicity of 2,4-Dichlorophenoxyacetic Acid – Molecular Mechanisms

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Abstract

2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide commonly used in agriculture. The residues of 2,4-D are present in air, water, soil and edibles. It constitutes a real hazard for human and animal health as numerous accidents of poisoning deaths caused by this herbicide have been reported. However, the molecular mechanism of its action is still unknown. The point of view concerning the toxic action of 2,4-dichlorophenoxyacetic acid has been evolving from decades and now it is considered that 2,4-D also induces free radical reactions that lead to numerous unbeneficial changes in tissues. The increases of free radical levels cause DNA damage and thus cell death (in apoptotic process). It is also suggested that 2,4-D causes cell apoptosis as the result of change of membrane potential in mitochondria and initiates caspase-dependent reactions. For many years the discussion has been continuing concerning the mutagenicity of 2,4-D and now many documented investigations that have been performed from 2000 unequivocally proved its mutagenic action. The mutagenicity of 2,4-D concerns homologous recombination, A→G mutation, chromosome aberrations, sister chromatid exchange and DNA damage, and also an increase in the frequency of DNA strand breaks. This paper presents literature data (especially the newest that have been published since 2000) that refer to peroxidative capacity of 2,4-D, free radical formation, induction of apoptosis, genotoxic activity and adaptation of the cells and organisms to its action.

Keywords: 2,4-dichlorophenoxyacetic acid, free radical, apoptosis, mutation, conjugates, cell adaptation

General News for 2,4-D

2,4-dichlorophenoxyacetic acid (2,4-D) is a selective herbicide, with highest toxicity to broadleaf plants. 2,4-D is a common herbicide that is used around houses and gardens and also on golf courses, ball fields, parks, and in agriculture and forestry [1, 2]. 2,4-D is present in the following herbicide preparate in Poland: Aminopielik D, M, P and 39, Herbitor 460 SC, Herbatoxol S, Herbatoxol 78 WP, Pielik, Trawit – 1, Chwastox M, etc. 2,4-D is a moderately persistent chemical with a half-life between 20-200 days. Unfortunately, the herbicide does not target only weeds. It can cause low growth rates, reproductive problems, changes in behavior, or death in non-tagged species [3].

2,4-D is easily adsorbed into the human organism from the alimentary tract and skin and is subsequently excreted in the urine in nearly unchanged form [4]. Apart from conjugates of 2,4-D with amino acids or proteins, as well as 2,4-dichlorophenol have been found [5, 6]. 2,4-D was detected in stomach, blood, brain and kidney of 4-day-old rat neonates fed by 2,4-D-exposed mothers [7].

The production and degradation of 2,4-D leads to the creation of many compounds including chlorophenols [8, 9] or dioxins [10, 11] that exert strong toxicity. In the period from 1962 to 1999, 66 cases of phenoxyacetic herbicide poisoning were described, including 22 cases that ended in the patient's death [12]. Persons employed

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in production, commercial distribution, packaging and repackaging as well as other plant protection personnel and those involved in plants spraying are chronically exposed to phenoxyherbicides action [13, 14].

Children living in agricultural communities are heavily exposed to pesticides, whether or not they work in the fields [15, 16]. Farmers' children come in contact with pesticide residues from their parents' clothing, dust tracked into their homes, contaminated soil in areas where they play. They are contaminated by food from fields where 2,4-D is used, also by aerial spraying, contamined well water, and breast milk [17].

Acute toxicity of phenoxyacetic acid derivatives measured as the LD_{50} dose varies between 100 and 1200 mg/kg body mass for various species of experimental animals. Teratogenic, genotoxic [18], neurotoxic [19], immunosuppressive [20], cytotoxic [21, 22] and hepatotoxic effects of 2,4-D have been well documented [23, 24, 25]. 2,4-D causes an array of adverse effects to the nervous system: disruption of the activity of nervous system chemicals and behavioral changes [26, 27, 28]. The researchers also postulated a possible association between exposure to 2,4-D and cancer in humans [29, 30]. The exposure to phenoxyherbicides was associated with an increased risk for Non-Hodgkin's lymphoma [31, 32, 33]. So that on the one hand we have great profits from using herbicides, but on the other we have problems related to their accumulation and health consequences for animals and humans.

The Effect of 2,4-D on Plants and Microorganism

2,4-D has been used from 1942 and still new data conerning molecular mechanisms of its action appear. Depending on the kind of cells (procariotic and eucariotic) and organisms (plants and animals), different mechanisms of its toxic action have been described.

2,4-D reveals toxic activity, but there are microorganisms that use it as a source of carbon and energy. Those organisms are bacteria (eg. *Pseudomonas*, *Chromobacter*, *Flavobacterium* [34, 35], fungi (*Aspergillus*, *Fusarium*, *Penicillum*)[36] and also actinomycetes (*Streptomyces*)[37].

However, phenoxyherbicides and their derivatives in high concentrations may be toxic for microorganisms [38, 39].

It is known that 2,4-D in plants acts similarly to natural auxins such as IAA (indoleacetic acid). The molecular mechanism of action of 2,4-D in plants concerns mostly disrruption of the hormonal equilibrium of the auxin-cytokinin system. 2,4-D inhibits root and shoot growth for both broad-leaved plants and grasses [40].

It has not been fully explained which side effects may be induced by 2,4-D in animals and human organisms. Development of techniques and investigations bring new data that may explain mechanisms of toxic action of disscusing xenobiotics.

At present it is known that 2,4-D can disturb some chemical reactions, it binds enzymes and change their activity, interacts with phospholipids, induces physical interactions in cells' membrane. The compound also causes mutations, induces free radical formation and apoptosis.

Beneath the most often investigated theories of molecular action of 2,4-D are presented: changes induced by ROS formation, changes in cell functions by binding with proteins and lipidsalso changes in DNA structure and apoptosis induction.

Generation of Free Radicals

The mechanism of toxicity of many compounds concerns the formation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, superoxide radical and hydroxyl radical. Discussing compounds are capable of reacting with proteins, nucleic acids, lipids and/or molecules that lead to changes in their structure and finally to cell damage [41, 42]. However, a majority of cells possess defence mechanisms against the potential harmful effects of ROS. These defences include superoxide dismutase, which converts its substrate, superoxide anion, into hydrogen peroxide, and also catalase, which converts hydrogen peroxide into water and oxygen. Extra- and intracellular substances that are antioxidative in nature prevent overproduction of radicals and protect the organism against propagation of peroxidative reactions [43].

Pesticides may induce oxidative stress by generating free radicals, thus causing lipid peroxidation. Increased lipid peroxidation and oxidative stress can affect the activities of protective enzymatic antioxidants that have been shown to be sensitive indicators of increased oxidation reactions.

Lipid Peroxidation

Lipid peroxidation products modify the physiological properties of a cell's membrane. Oxidative changes in membranes are the result of protein polymerization that leads to an increase of erythrocyte membrane rigidity; due to this fact, toxins cause a decline of a membrane's ability to deform [44]. Several studies have reported that ROS can initiate lipid peroxidation and mainly hydroxyl radicals are involved in this process. Chung *et al*. [45] demonstrated that lipid peroxidation (in nucleus cells) is a potential endogenous source for DNA residues modification that play roles in carcinogenesis.

2,4-D can bind to certain phospholipids and disturb physical interactions in membrane, which probably increases the availability of lipids to peroxidation [46]. The induction of lipid peroxidation in pea microsomes may be a result of the production of free radicals (e.g. $^{\bullet}CCl_3$, $Cl_3CO^{\bullet}$, $Cl_3COO^{\bullet}$) formed during metabolism of carbon

tetrachloride in endoplasmatic reticulum with participation of cytochrome P-450 [46]. Duchnowicz *et al*. [47, 48] has shown that 2,4-D caused lipid peroxidation as well as the increase in membrane fluidity at the 16. carbon atom of fatty acids and also hemolysis in human erythrocytes.

Suwalsky et al. [49] showed that 2,4-D disturbs phospholipid bilayer structure, that integrity is essential for the proper condition of cell membrane. The authors postulated that morphological transformation of erythrocytes might be the result of specific oxidative transformation of membrane skeleton.

Damage of Protein

2,4-D is capable of binding itself with proteins. On the one hand it can lead to detoxication of xenobiotic (conjugation with aminoacids and plasma albumins), and on the other it may cause essential disturbances in cell function and, finally, lead to its death (changes in enzymes activity) [50].

Protein damage may be the result of direct impact of 2,4-D or its indirect effect, for example by generation of free radicals (protein peroxidation).

Duchnowicz et al. [51] suggests that damage of erythrocyte membrane proteins may be caused by phenoxyherbicides. They used a 4-Maleimido-TEMPO label that binds covalently to membrane proteins and gives rise of electron spin resonance absorptions. The spectrum erythrocyte membranes consists of two dominant classes of binding sites: strongly (S) and weakly (W) immobilized. Maleimide primarily binds to –SH groups of the membrane spectrin and the cytoplasmic portion of band 3 protein and it is a convenient and sensitive indicator monitor of conformational changes in these membrane proteins. Duchnowicz et al. [51] observed decrease in the W/S (weakly/strongly) ratio in membrane of the human erythrocytes incubated with 2,4-D, that reflect the conformational changes of the labelled molecule during its thermal denaturation.

Hepatotoxicity – Binding with Protein

Covalent modification of liver proteins by reactive metabolites of 2,4-D could mediate some liver damage associated with exposure to 2,4-D, as suggested by mechanistic studies of drug-induced hepatitis [*52*]. Several reports have demonstrated that 2,4-D can bind irreversibly to hepatic proteins in rat and chick [50, 53, 54]. Li *et al*. [*55*] investigated a novel reactive metabolite of 2,4-D – 2,4-dichlorophenoxyacetol-S-acetyl-CoA (2,4-D-CoA). Chemically reactive metabolites of 2,4-D are proposed as mediators of 2,4-D-induced hepatotoxicity. Li *et al*., [55] indicated that 2,4-D-CoA may contribute to 2,4-D-protein adduct formation *in vivo* and therefore associates hepatotoxicity. In mammalian cells *in vitro*, 2,4-D inhibits cell growth, protein and DNA synthesis, and also arrests cells in the G/S phase of the cell cycle [56].

It was also shown that 2,4-D causes growth inhibition of cells, DNA and protein synthesis from *Azospirillum brasilense*. The activity of decarboxylase decrease up to 54% during incubation of 2,4-D. 2,4-D induces increase of the 70 S ribosomes number that bind 50S and 30S subunits (that number is also decreasing). That leads in consequence to protein synthesis inhibition at their first step of formation [57].

Changes in Antioxidative Enzymes Activity, Glutathione and ATP Level

2,4-D induced a decrease in the level of ATP, which led to the fall of the level of adenine energy charge (AEC) [58, 59]. Adenine Energy Charge is a measure of high energy phosphates in cells and it is defined as the amount of instantly accessible energy. The numerical value of AEC defines the physical condition of the organism, tissue or cell. AEC may vary in the range of 0 (only AMP in cell) to 1 (only ATP in cell). The values from 0.8 to 0.9 are typical for healthy and growing organisms [59]. Palmeira *et al*. [58] suggested that 2,4-D can decrease ATP, GSH and NADH levels in rat hepatocytes while conversely increasing the levels of AMP, NAD, LDH and GSSG. Bukowska and Zatorska [*59*] observed a decrease of ATP in human erythrocytes incubated with 2,4-D (*in vitro*).

The prooxidant action of the herbicide 2,4-D was demonstrated by Teixeira *et al*. [60]. Their investigations employed *Saccharomyces cerevisiae* as a eucaryotic experimental model. They observed that the intensity of the electron paramagnetic resonance spectra was dependent on the concentration of herbicide used and time of its action toward yeasts. 2,4-D induces increase of activity of cytosolic catalase and CuZn-superoxide dismutase.

Results reported in literature concerning the effect of 2,4-D on the activity of catalase and superoxide dismutase in different organisms are contradictory. For instance, no effect was registered in catalase and superoxide dismutase activities in 2,4-D stressed cells of *Oreochromis niloticus* [61], while catalase activity was increased in rat erythrocytes (*in vivo*) under the influence of 20 ppm (1ml/100 g body weight) of this herbicide [62] and in kidney and brain tissues of *Cyprinus carpio* [63]. Whereas activity of catalase decrease*s* in human erythrocytes incubated with 2,4-D (*in vitro*) [64]. Bukowska [65] observed also a decrease of activity of superoxide dismutase in human erythrocytes incubated with 2,4-D. The decrease of SOD activity under the influence of 2,4-D was observed also in kidney tissue and increase of its activity was noted for gill tissue of *Cyprinus Carpio* [63]. Moreover, glutathione peroxidase (GSH-Px) activity was increased in kidney tissues of *Cyprinus Carpio* [63] and in human erythrocytes (*in vitro*) [65]. GSH-Px activity may be increased due to increased production and enzyme-inducing effects of H_2O_2 derived from $O_2^{\cdot-}$ [66]. 2,4-D leads to the formation of methemoglobin [48, 67], which is unable to transport oxygen. This conversion is

associated with superoxide anion production [68] and thereby with the formation of products such as hydrogen peroxide or hydroxyl radicals that may be derived from superoxide anion itself.

In relation to chemicals that have this type of activity, a hypothesis has been developed suggesting that the induction of peroxisomal beta-oxidation increases hydrogen peroxide levels and causes oxidative stress in cells. It is known that during this process the formation of free radicals and the depletion of cellular glutathione (GSH) render the cell more susceptible to the development of lipid peroxidation and oxidation of thiols, proteins and DNA. 2,4-D is hepatotoxic and initiates the process of cell death by decreasing cellular GSH [58]. 2,4-D also causes a decrease in the level of reduced glutathione in human erythrocytes [65]. These types of actions may explain an indirect way in which the genetic material could be affected by the 2,4-D [69].

Investigations led by Watahiki *et al*. [70] show that 2,4-D inhibits glutathione S-transferase (GST) activity. Total inhibition of GST by 2,4-D proceeds with a dose of 0,24 mM (90 -100%) and may be the result of competition towards glutathione. The authors suggested that it may occur when 2,4-D effects as a non-substrate ligand that modyfies GST activity or binds *itself* as a substrate with GST and then GSH.

Mutagenicity

2,4-D also caused genetic damage in tests using both cell cultures and laboratory animals. It increased the frequency of a gene mutation in hamster muscle cell cultures, a rise in the frequency of abnormal chromosomes in bone marrow cells of rats and mice, and increased the number of breaks in human DNA and sister chromatid exchange [1, 71]. The mechanisms of genotoxicity and mutagenicity of 2,4-D are poorly understood, and the available genotoxicity data is controversial. Filkowski *et al.* [72] employed the transgenic *Arabidopsis thaliana* 'point mutation' and 'recombination' tests to monitor the genetic effects of the A→G mutation associated with herbicide 2,4-D. They found that 2,4-D had a significant effect on the frequency of homologous recombination.

The highest permissible levels of 2,4-D in drinking water is 100 μg/L [73]. At those concentrations 2,4-D induced homologous recombination (A→G) increased by 2.85 fold. Filkowski et *al.* [72] observed also that 2,4-D did not cause changes in numbers of T → G mutation frequency.

The literature reports of genotoxic potential of this xenobiotic have unfortunately produced inconclusive data. Sister chromatid exchange (SCE) induced by chemicals has been suggested as an appropriate indicator of genotoxic damage, because the SCE rate has shown concordance with endpoints determined by other short-term tests. With respect to mammalian cells (*in vitro*), studies on human lymphocytes showed the increase of chromosomal aberrations and SCEs caused by 2,4-D [74]. Other reports have revealed the presence of chromosomal aberrations only when human lymphocytes were treated with some of the commercial derivatives of 2,4-D [75].

In another study, Chinese hamster fibroblasts showed that herbicide induced an increase of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) mutation rate [76]. HGPRT is a salvage pathway enzyme for purine metabolism (another is specific for adenine). Studies performed in vitro on mouse cells showed an increase in the frequency of chromosomal aberrations by 2,4-D [77], while another investigation failed as no increase in aberration frequency in mouse bone marrow erythrocytes were observed [78]. Finally, three studies reported negative results that were published in 1999 [79, 80, 81]. These reports measured *in vitro* gene mutations and chromosomal aberrations, as well as *in vivo* unscheduled DNA synthesis and micronuclei.

The investigations of recent years seem to be more consistent (Table 1). Madrigal-Bujaidar [69] established that 2,4-D had a moderate genotoxic effect in mice that were treated *in vivo* with high doses (200 mg/kg) of this compound and suggested a minor hazard for humans.

Holland *et al*., [82] showed that both pure and commercial 2,4-D increased the number of micronuclei at the highest non-toxic dose (0.3 mM). The woole blood exposure to 2,4-D produced a minimal increase in micronucleus frequency (MN) and even smaller one in isolated lymphocyte cultures. Induction of 2,4-D took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). The lymphocyte RI was more strongly affected by commercial preparation of 2,4-D containing 9.4% of the chemically pure compound, than with an equal concentration of 2,4-dichloro-phenoxyacetic acid. This suggests that other ingredients present in the commercial pesticide preparation may be responsible and/or may enhance the toxic effect. However, the mitotic index did not show any significant change between commercial or pure 2,4-D either [82].

Zeljezic and Garaj-Vrhovac [83] reported that 2,4-D and their pesticide preparation Deherban A® caused an increase in chromatid and chromosome breaks, number of micronuclei and number of nuclear buds. The incoherent genotoxicity results may be attributable to different methodologies and treatment protocols. For example, the selection of compositionally different 2,4-D salts and acids or solvents in each experiment could lead to different absorption and metabolism rates. The same way, doses, type of cells and organisms and also employed methods seem to be important. Generally, the recent years reports (beyond 2000 with using the newest and more sensitive methods) confirmed genotoxic proprieties of 2,4-D and those could be considered as the ultimate.

2,4-D-Induced Apoptosis of Cells

Toxic concentrations of 2,4-D lead to apoptosis of cerebellar granule cells [90]. 2,4-D also destroys the vascu-

Table 1. Comparision of the results of genotoxic and mutagenic potential of 2,4-D.

Author	Dose	Cell and organism type	Type of change
Ateeq *et al*. [84]	1 mg/L 25 mg/L	*Allium cepa* *Catfish Clarias batrachus*	Clastogenicity, frequency of chromosome aberrations and mitotic index Micronuclei Erythrocyte alterations
Filkowski *et al.* [72]	3-100 mg/L	*Arabidopsis thaliana* – plant	Homologous recombination A→G mutation Lowest concentration cause positive effect
Kaya *et al.* [85]	221 mg/L	*Drosophila*	Somatic mutation and recombination viewed as wing spots
Madrigal-Bujaidar *et al*. [69]	200 mg/kg	Bone marrow and germ cells of mice	Increase in sister chromatid exchange
Holland *et al*. [82]	1 mg/L 2.2-22 mg/L	Human lymphocytes	Replicative index Micronuclei formation
Zeljezic and Garaj-Vrhovac [83]	0.4-4 µg/ml	Human lymphocytes	Increase in chromatid and chromosome breaks, number of micronuclei and number of nuclear buds
Amer and Aly [86]		mice	Chromosomal aberrations
Abul Farah *et al*. [87]	25-75 µg/ml	fish *Channa punctatus*	Micronucleus induction
Mičić *et al.* [88]	20-100 µg/g	mussel	Increase in sister chromatid exchange
González *et al.* [83]	2-10 µg/ml	*Chinese Hamster* ovary cells	Induced sister chromatid exchange and DNA damage

lar structure of the thymus and releases thymocytes with fragmented DNA that could be indicative for apoptosis induced by the herbicide [91].

Kaioumowa *et al*. [92] also tested the effect of the dimetylammonium salt of 2,4-D on apoptosis in Jurkat T cells and human lymphocytes; these authors detected significant numbers of apoptotic cells at concentrations from 2 to 4 mM. Apoptosis induced by DMA-2,4-D was dose- and time-dependent, and dependent on Fas**,** TNF (Tumor Necrosis Factor) receptor 1 or the aromatic hydrocarbon receptor (AhR); however, DMA-2,4-D provoked disruption of the mitochondrial transmembrane potential and activation of caspases. While an inhibitor of caspase-9, as well as caspase-9 and caspase-3 inhibitors in combination, strongly blocked DMA-2,4-D induced apoptosis, an inhibitor of caspase-3 had a moderate inhibitory effect. Unlike Fas-mediated apoptosis, the initiator caspase-8, was not involved in DMA-2,4-D – induced apoptosis. DMA-2,4-D kills human lymphocytes by initiating apoptosis *via* a direct effect on mitochondria. The activation of caspases occurs downstream of mitochondrial damage, and the dysfunction of mitochondria appears to be sufficient for triggering all downstream events leading to apoptosis.

In contrast to the above-mentioned investigations, Lin and Garry [93] could not detect any apoptosis in MCF-7 breast cancer cells after application of 2,4-D. This study underlines the necessity to evaluate several parameters of cell death and at least several time points [94]. In contrast to the findings of Del Bino et al., [95] in adherent CF-7 cells, the TUNEL assay proved to be the most sensitive method in the present investigations, since a high number of strand breaks were detected at 4 mM 2,4-D.

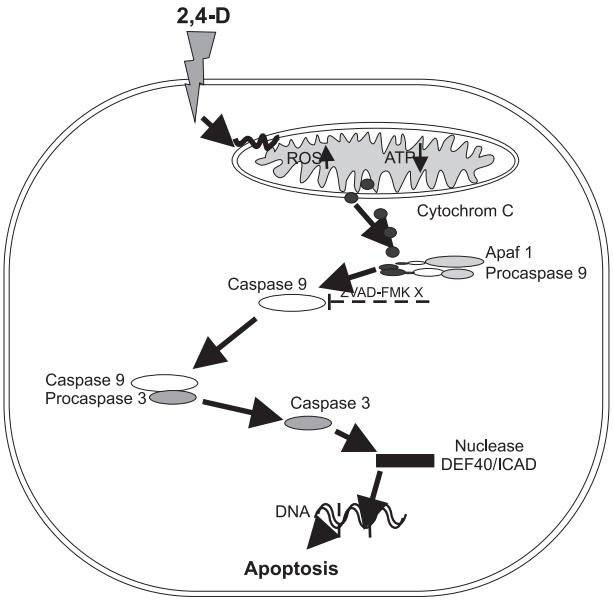

Fig. 1. 2,4-D indirect disruption of the mitochondrial transmembrane potential, inhibition of oxidative phosphorylation and decreasing level of ATP in mitochondria. Then opening of permeability transition pores was observed and the release of cytochrome C, which activates caspases 3. Cytochrome C induces formation of a complex between Apaf 1 and caspase-3 that activates caspase 9 and further activates the nuclease DFF40/ICAD, which initiates DNA fragmentation.

Tuschl and Schwab [96] investigations suggested that 2,4-D exerts its cytotoxic effects by the induction of apoptosis *via* a direct effect on the mitochondrial membrane potential ($\Delta\psi$). Studies of Di Paolo *et al*., [97] indicated

the effect of 2,4-D on apoptosis by an inhibition of mitochondrial function. 2,4-D formed an adduct with a 52 kD protein in the inner mitochondrial membrane and the authors related the covalent binding of 2,4-D to this protein with alterations of mitochondrial function.

Effects of 2,4-D formulations on the oxidative function of mitochondrial particles were also described by Oakes and Pollack [98]. Ultrastructural changes of rat liver mitochondria and lysosomes after 2,4-D treatment were also observed by Sulik *et al.* [99]. 2,4-D have the potential to inhibit the oxidative phosphorylation of mitochondria, and therefore the production of energy in the body [98, 58].

The recent reports confirmed the apoptosis induction by 2,4-D. Mičić *et al*. [88] treated *Mytilus galloprovincialis* (*in vivo*) with 2,4-D and observed morphological changes characteristic for apoptotic cells using fluorescence microscopy.

Induction of apoptosis caused by 2,4-D may be related to indirect interaction of 2,4-dichlorophenoxyacetic acid with mitochondrial membrane as it suggested by Di Paolo *et al.*, [50]. On the basis of studies described above it is possible to suggest that apoptosis development in cell occurs under the influence of 2,4-D [based on data: 92, 21, 54, 98].

Adaptation of Cells and Organisms to 2,4-D

2,4-D may be connected with aminoacids (especially among plants) asparaginic acid and glutaminic acid and alanine, and also isoleucyne, phenylalanine and tryptophane. Among animals and plants 2,4-D connects proteins to form complexes [99, 100]. Those metabolites undergo faster biotransformation when compared with substrate.

Cserháti et al. [101] reported that phenoxyacetic acid derivatives interacted with human and bovine serum albumins. The lipophilicity of derivatives exerts a significant impact on their capacity to bind to serum albumins. They support the hypothesis that the binding of phenoxyacetic acid derivatives to albumins may involve hydrophobic forces occurring between the corresponding apolar substructures of these derivatives and the amino acid side chains.

2,4-D is metabolized to 2,4-DCP by human cytochrome P450 3A4 (CYP3A4), the major form of monooxygenase enzyme in human liver [102]. The essential role is the connection of 2,4-D with GSH and the activity of GST plays an essential role in the defence from hepatotoxicity [55].

ATPase is one of the most important enzymes located in membrane. Changes in its activity influence the ratio of transport of various substances across membrane.

The stimulation of the activity of the H(+)-ATPase present in the vacuolar membrane (V-ATPase) of Saccharomyces cerevisiae was described in response to a moderate stress induced by 2,4-dichlorophenoxyacetic acid (2,4-D). This in vivo activation (up to 5-fold) took place essentially during the adaptation period, procceding cell division under herbicide stress, in coordination with a marked activation of plasma membrane H(+)-ATPase (PM-ATPase) (up to 30-fold) and the decrease of intracellular and vacuolar pH values, suggesting that activation may be triggered by acidification. Single deletion of VMA1 and genes encoding other V-ATPase subunits led to a more extended period of adaptation and to slower growth under 2,4-D stress [103].

Duchnowicz et al. [53] observed an increase in the activity of the Na^+, K^+ ATPase at concentration of 1mM of 2,4-D but higher concentrations (2-4 mM) caused a decrease in activity of this enzyme. They claimed that reasons of the toxic effect of higher concentrations of 2,4-D is the decrease in ATPase activity in membrane and thus changes in ion transport, and disruption of both sides of the membrane. The decrease of ATPase activity in human skin fibroblasts was also observed by Casorbi and Foret [104].

Conclusion

The following conclusions can be stated on the base of the above data.

1. The impact of 2,4-D provokes generation of free radicals, increases the lipid peroxidation process, depletion of ATP, NADPH and GSH concentration and also modulates the activity of antioxidant system.
2. Numerous mutagenic changes occur in cellular DNA such as homologous recombination, A→G mutation chromosome aberrations, sister chromatid exchange and DNA damage and increase in the frequency of DNA – strand breaks *detection.*
3. The disturbance of mitochondrial membrane are caused directly by 2,4-D. 2,4-D is capable of developing the process of apoptosis dependant on caspases activity.
5. Cell and organism adaptation to 2,4-D develop numerous defensive processes: detoxication by monooxygenases, conjugation with aminoacids, proteins, glucose, glucuronic acid and changes in ATPase activity.

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