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Herbicide 2,4-D: A Review of Toxicity on Non-Target Organisms

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Abstract The intensive use of pesticides has increased exponentially in Brazil and worldwide due to the need to meet the food demands of a growing population. If the management/monitoring of the use of pesticides is adequately performed, it would not compromise the expected benefits or have negative effects on the environment as a whole. In order to examine the information available on herbicide use in Brazil and worldwide, this paper presents a review of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its chemical properties, action on target organisms, environmental fate, and toxicity to non-target organisms. This herbicide is a synthetic auxin used to control broad-leaved weeds, and the action in target organisms is well known. Although 2,4-D has been widely used worldwide, many studies have shown that this herbicide induces alterations in non-target organisms. Therefore, ecotoxicology studies are important to assess the risk the herbicides can be to different ecosystems. Thus, it is advised to use this herbicide and other pesticides with caution.

Keywords Pesticides · Mutagenesis · Synthetic auxin · Agent Orange

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1 Introduction

Pollution can be classified as coming from point or non-point sources (Holt 2000). Point source pollution includes treatment plants of industrial effluents, domestic sewage, accidental spills, and mining. The emissions of point sources of pollution, usually as direct discharge of contaminants into water bodies, are more easily detected and controlled. On the other hand, non-point emissions are difficult to control, vary in time and space, and can involve pathways that result in partial accumulation of contaminants before reaching water bodies. A typical example of non-point source pollution is the use of pesticides in the soil (Costa and Olivi 2008).

Emissions of contaminants into the air, soil, and mainly water are associated with natural processes and mainly human activities. World population growth and increasing food demands have led to higher pesticide use in crops to prevent or control pests in order to increase yields (Caldas and Souza 2000). The first compounds used to manage pests or diseases were sulfur, lime, and some arsenic salts (Sanches et al. 2003).

The intensification of pesticide use in recent decades and their adverse effects on humans and the environment have led to the regulation of their use and production in several countries to minimize negative impacts to ecosystems. Each country created multidisciplinary committees and agencies with legal and managerial aspects to assess the level of danger and the risks that these products offer to humans and the environment (Zagatto and Bertoletti 2006). Pesticides are biocidal products used in agriculture to exterminate a pest. In

this diversified group of chemical compounds are fungicides, insecticides, and herbicides considered extremely aggressive to the environment and to human health (Grisolia 2005).

After the Second World War, the number of new compounds and their extensive use in agriculture increased dramatically. With the rise of intensive monocrop systems and the decrease in diversity, several pests have evolved, which are then controlled with chemicals (Amarante 2002). Given that pesticides, such as herbicides, have contaminated the environment due to widespread use, assessment of the toxicity of these compounds is essential (Marin-Morales et al. 2013). These compounds have contaminated aquatic systems, and therefore monitoring groundwater, particularly near agricultural areas and sources of primary drinking water, is especially important (Sanches et al. 2003).

Despite the increase in crop yields, herbicide use can have serious ecological effects, contaminating receiving waters with toxic compounds and inhibiting biological treatment systems, even at low concentrations (MarrónMontiel et al. 2006). The application of pesticides is not often uniform, even when using modern equipment. Because of this, and also because of the different stages in which pests can be found, the doses can be lethal or sublethal. Thus, the pest population that received sublethal doses can present a significant amount of DNA mutations due to stress induced by pesticide application, leading to pesticide resistance (Gressel 2011).

Considering the problem of indiscriminate and increasing use of pesticides, which offers serious risks to the environment and biodiversity, this paper aims to provide an overview on the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its chemical properties, action on target organisms, environmental fate, and toxicity in animals and higher plants bioindicators, evaluated by histological, histochemical, and genetic biomarkers.

2 Chemical Properties, Action on Target Organisms, and Environmental Fate of the Herbicide 2,4-D

The basic formulation of 2,4-D is an acid, but 2,4-D is produced as an inorganic salt, amine, or ester through various production processes, and it is used in many commercial products. The molecular formula of 2,4-D is $C_8H_6Cl_2O_3$ (Fig. 1), with a molecular weight of 221.04,

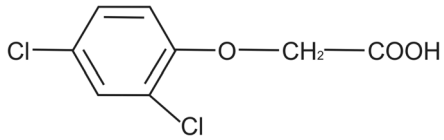

Fig. 1 Chemical structure of 2,4-dichlorophenoxyacetic acid

melting point of 140.5 °C, boiling point of 130 °C, and water solubility of 900 mg/L at 25 °C (acid) (Kennepohl and Munro 2001).

According to Wang et al. (1994), the octanol–water partition coefficient (K_{ow}) of 2,4-D has a large difference in amounts partitioned to water phase between presence at the ionic state and at the molecular state. In a higher pH solution, the K_{ow} is 0.027, and in a lower pH solution the K_{ow} is 18.23, implying that the pH value might affect the bioaccumulation process of 2,4-D.

Many derivatives of chlorinated phenoxyacetic acids are classified as plant growth regulators or hormones that act as synthetic auxins. These derivatives are widely used as herbicides and include 2,4-D (Kumari and Vaidyanath 1989; Filkowski et al. 2003). These herbicides have a similar effect to natural auxin indoleacetic acid (IAA) in most plants; however, they have long duration due to their high stability in the plant, so they are more effective than IAA. Auxin herbicides have direct action because they induce ethylene biosynthesis through the synthesis of ACC (1-aminocyclopropane-1-carboxylic acid). Additionally, in the indirect pathway of action, ethylene stimulates production of abscisic acid (ABA). ABA inhibits cell division and expansion, and ethylene promotes leaf senescence. Inhibition of growth, tissue damage, and cell and plant death are the consequences (Grossmann 2003).

Synthetic auxins, such as 2,4-D, persist for long periods in the plant and consequently are effective and lethal. The mode of action of auxin herbicides is dose dependent, and its effect also depends on the sensitivity of tissues and species (Pazmiño et al. 2012). While the concentration of natural auxins and their effects are strictly controlled, auxin herbicides bypass the natural regulatory mechanisms of susceptible plants and cause an uncontrolled response to auxin. In low doses, auxin herbicides have hormone properties similar to those of natural auxins. However, higher concentrations cause several growth abnormalities in sensitive dicotyledons (Kelley and Riechers 2007). The mode of action of 2,4-D is characterized by an increase in plasticity of the cell wall and abnormal increase of the synthesis of proteins and ethylene, resulting in uncontrolled cell division and

damage to the vascular tissue of plants (USEPA 2005). Therefore, because of its chemical resemblance to auxins, 2,4-D overstimulates growth, culminating in the death of the target plant (Oruç et al. 2004; Benli et al. 2007).

2,4-D is not considered persistent in the terrestrial environment because it is just adsorbed to soil particles when there is a high rate of organic carbon (Walters 1999). However, 2,4-D is not easily biodegradable in aquatic environments (Chingombe et al. 2006) and is frequently detected in water bodies as a free anion. The decomposition of this anionic form occurs mainly by microbial degradation. Biodegradation in water depends on several factors, including temperature and the availability of oxygen and nutrients (Walters 1999).

The 1988 2,4-D Standard Registration established the environmental fate relating to the degradation of 2,4-D to 2,4-D esters and 2,4-D amine salts to 2,4-D acid. Data indicate that 2-4-D esters are rapidly hydrolyzed in aquatic environments and alkaline wet soils. However, 2,4-D ester may be persistent in acid aquatic environments and dry soils. In most environmental conditions, rapid degradation of 2,4-D esters and amine salts to 2,4-D acid occurs (USEPA 2005).

According to Kennepohl and Munro (2001), the carcinogenic effect of 2,4-D has not been demonstrated. This information was corroborated by the United States Environmental Protection Agency (USEPA 2005), which classified the herbicide as a Group D herbicide (non-classifiable as a human carcinogen) due to the lack of evidence as a carcinogen.

3 Use of the Herbicide 2,4-D in Brazil and Worldwide

The development of 2,4-D and other phenoxyacetic herbicides transformed agriculture in much of the world and should be classified as a major contribution of science (Zimdahl 2010). The application of auxin herbicides began a new phase in agricultural production due to its systemic mobility in the plant and its selective action, mainly against dicotyledonous weeds (Grossmann 2003). 2,4-D has been widely used worldwide (WHO 1984) in a large scale in the control of broad-leaved weeds since the beginning of the 1940s (Grisolia 2005; Itoh et al. 2013). In the past, 2,4-D mixed with 2,4,5-T was used for weed control (Wolfe 1984). More than 10 million gallons of this mixture, known as Agent Orange, in a 1:1 ratio (Mortelmans et al. 1984) was used in the Vietnam War to defoliate trees (Wolfe 1984).

Although 2,4-D is one of the most successful widely used herbicides, its intensive use has resulted in resistant weeds and many contamination issues when present in concentrations higher than those recommended (Teixeira et al. 2007). The herbicide 2,4-D was introduced in the 1940s, and after more than 50 years of use, it is still the most widely used throughout the world. It is mainly applied in wheat, sorghum, corn, rice, sugarcane, soybeans, and pastures (Mičić et al. 2004).

According to the Brazilian Ministry of Agriculture, Livestock and Farm Supplies (Ministério da Agricultura, Pecuária e Abastecimento) (MAPA 2017), there are 24 commercial formulations that include 2,4-D as an active ingredient, either alone or in combination with two other herbicides, aminopyralid and picloram. The reports of the Brazilian Institute for Environment (Instituto do Meio Ambiente e Recursos Naturais Renováveis) (IBAMA 2016) show that between 2009 and 2012 the 2,4-D was the third/fourth active ingredient of most pesticides sold in Brazil, and after 2013–2014 it was the second best-selling active ingredient in the country; on that basis, it can be noted that 2,4-D was the best-selling selective herbicide in the country between 2009 and 2014.

The concentrations of 2,4-D recommended for the control of weeds predominant in Brazilian agricultural cultivars are presented in Table 1.

4 Toxicity of 2,4-D to Different Organisms

Pesticides are different from other chemicals because they are applied in the environment to control unwanted living species. Therefore, they have to be biologically active and for this reason are characterized by different degrees of toxicity. Since this toxicity is not always specific to the target organisms, the use of pesticides can present risks to human health, survival of non-target species, and the environment (Colosio and Moretto 2008). In view of the use of these substances on a large scale, investigation of the toxicity in various environments is necessary and prudent since it can affect different organisms.

When animals experience acute exposure to different compounds, by inhalation or by the presence of the toxicant in the environment in which the organism lives,

Table 1 Dosage of 2,4-D used in Brazilian crops for weed control

Crop	Dose of 2,4-D (kg/ha)	Target plant
Wheat	0.32–1.005	*Bidens pilosa* *Commelina benghalensis* *Euphorbia heterophylla* *Galinsonga parviflora* *Ipomoea grandifolia* *Ipomoea purpurea* *Raphanus raphanistrum* *Sida rhombifolia* *Sonchus oleraceus*
Corn	0.403–1.209	*Acanthospermum hispidum* *Aeschinomene rudis* *Alternanthera tenella* *Amaranthus deflexus* *Amaranthus viridis* *B. pilosa* *C. benghalensis* *E. heterophylla* *Emilia sonchifolia* *G. parviflora* *I. grandifolia* *I. purpurea* *Portulaca oleracea* *R. raphanistrum* *Richardia brasiliensis* *S. rhombifolia*
Soy	0.67–1.209	*B. pilosa* *C. benghalensis* *E. heterophylla* *I. grandifolia* *I. purpurea* *R. raphanistrum* *R. brasiliensis* *S. rhombifolia* *Spermacoce latifolia*
Rice	0.403–1.209	*A. hispidum* *A. rudis* *A. tenella* *A. deflexus* *A. viridis* *B. pilosa* *B. pilosa* *C. benghalensis* *E. heterophylla* *G. parviflora* *I. grandifolia* *I. purpurea* *P. oleracea* *R. raphanistrum* *R. brasiliensis* *S. rhombifolia*
Irrigated rice	0.201–1.209	*A. hispidum* *Aeschynomene denticulata* *A. rudis* *B. pilosa* *Ipomoea aristolochiaefolia* *Ricinus communis*
Sugarcane	0.403–2.345	*A. hispidum* *A. rudis* *A. tenella* *A. viridis* *B. pilosa* *C. bengalensis* *E. heterophylla* *E. sonchifolia* *G. parviflora* *I. grandifolia* *I. purpurea* *P. oleracea* *R. brasiliensis* *R. raphanistrum* *S. rhombifolia*
Pasture	0.806–1.612	*A. deflexus* *B. pilosa* *Conyza bonariensis* *Croton glandulosus* *Cyperus rotundus* *E. heterophylla* *Momordoca charantia* *P. oleracea* *S. rhombifolia* *Sida cordifolia* *Sida glaziovii* *Solanum americanum* *Solanum brasiliensis* *Solanum palinacanthum*
Coffee	0.806–2.821	*A. hispidum* *Ageratum conyzoides* *A. deflexus* *Amaranthus hybridus* *Amaranthus spinosus* *A. viridis* *Brassica rapa* *C. benghalensis* *E. heterophylla* *G. parviflora* *I. aristolochiaefolia* *I. purpurea* *Leonorus sibiricus* *P. oleracea*

Table adapted from the guidelines of manufacturers of the DMA 806 BR, Dow AgroSciences; Aminol 806, Adama; 2,4-D Nortox, Nortox S.A.

such as fish (water) and earthworms (soil), the dose absorbed by the animals is generally unknown; thus, the concentration of the toxicant in the environment capable of causing 50% death of the individuals is expressed as lethal concentration 50 (LC_{50}) (Klaassen 2013, p. 34). The traditional acute toxicity test is usually performed to determine the 50% lethal dose (LD_{50}), that is, the absorbed dose of a given compound required for the death of 50% of the exposed organisms. The LD_{50}, therefore, is based on statistical calculations, which

represent a theoretical Gauss curve, with a confidence limit of 95%, of the dose–response relation of an exposed population, which is rarely found in practice (OGA et al. 2014, p. 30). Acute toxicity tests provide information necessary for the experimental design of different bioassays in vitro and in vivo.

The lethal doses and concentrations of 2,4-D vary among species. *Eisenia foetida* have a 14-day LC_{50} of 350 mg/kg soil (WHO 1997). Daphnia (*Daphnia magna*) have an LC_{50} of greater than 100 mg/L (Heggstrom 2009). The LC_{50} value for Nile tilapia (*Oreochromis niloticus* L.) adults was found to be 86.90 mg/L (Sarikaya and Selvi 2005). The LC_{50} ranged from 250 to greater than 600 mg/L for fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), and rainbow trout (*Salmo gairdneri*). The single-dose dermal LD_{50} exceeded 2000 mg/kg in rabbits (Gorzinski et al. 1987). The LD_{50} of a single oral dose in rats ranged from 553 to 1090 mg/kg (Gorzinski et al. 1987). Dogs are more sensitive to an oral LD_{50} of 100 mg/kg (Bovey and Young 1980; Garabrant and Philbert 2002).

Pesticides have been the focus of ecotoxicological studies, as they contaminate the atmosphere, water, and soil; persist in the environment; enter ecological chains; and have adverse toxic effects on organisms from bacteria to humans. Evaluation of the impact of pesticide use on non-target organisms is conducted with different tests. Small mammals, birds, soil microorganisms, pollinator insects (bees and wasps), and aquatic organisms (fish, zooplankton, phytoplankton, and microcrustaceans) are considered representative organisms for these different tests (Grisolia 2005).

The use of herbicides in agricultural crops can cause health problems due to their persistence in the environment and accumulation throughout the food chain. Food is the main route for exposure of humans to these chemicals, and fish and shellfish have been recognized as major vectors for contaminant transfer to humans (Ateeq et al. 2006). 2,4-D can cause several metabolic alterations and tissue necrosis in non-target organisms, including important members of the food chain (Gallagher and Di Giulio 1991).

2,4-D is quickly absorbed in the gastrointestinal tract after oral exposure; the agrochemical residues can be observed in plasma levels from 10 min to 24 h after exposure, depending on the dose and the chemical form of 2,4-D (Knopp and Schiller 1992). The absorption of 2,4-D ester has been reported to be slower than 2,4-D acid and salt (Erne 1966), but their excretion rates are similar (Knopp and Schiller 1992).

According to Bécaert et al. (2006), 2,4-D has a negative effect on the soil by directly inhibiting or removing a source of enzyme production (bacteria). This inhibiting effect results in the loss of functional stability of urease, β-glucosidase, and sulfatase, affecting soil stability due to changes in the functional mechanisms of resistance and recovery. In laboratory tests with *Eisenia fetida* (Annelidae), 2,4-D had serious effects on development and reproduction. The toxic effects observed after exposure of animals to 2,4-D-contaminated soils were more severe than those observed for animals exposed to the herbicide glyphosate in the same concentration (Correia and Moreira 2010).

Hoy (1985) exposed *Scytonotus simplex* (millipede) to three different doses of 2,4-D in three different ways in the soil (applied evenly in the substrate, applied in half of the substrate, and added to half of the food provided) and made comparisons to a control group. In the control group, mortality was not observed until the 10th day, while in treatment groups mortality was observed within 2 days of the experiment and progressively increased during the first 10 days. Comparisons among the three methods of application of 2,4-D suggest that this species of millipede avoids surfaces treated with 2,4-D and that the spatial distribution of 2,4-D residues in the field can influence the effect of the herbicide on the animal. A uniform distribution, contaminating a wide portion of the soil, seems more harmful than an irregular one.

According to Gallagher and Di Giulio (1991), this herbicide is usually considered non-toxic to fish in low concentrations. However, Fonseca et al. (2008) reported that 2,4-D affects the brain and activity of the enzyme acetylcholinesterase in the muscles and some metabolic parameters of the blood and tissues of *Leporinus obtusidens*, possibly due to the stress caused by the toxicity of this herbicide. Sarikaya and Yilmaz (2003) also described 2,4-D as highly toxic to fish, with serious effects to humans and animals, since it accumulates in tissues and causes acute poisoning (WHO 1984).

Ateeq et al. (2006) conducted a histological analysis of the gonads and liver of fish of the species *Clarias batrachus* to examine DNA fragmentation, and they confirmed the genotoxic effect of 2,4-D. In the histological analysis of the gonads, in the ovaries, this herbicide induced apoptosis, indicated by the presence of atretic oocytes, nuclear blebbing, karyolysis, hypertrophy, and

vacuolation, in addition to mitochondrial deformities, electron-dense cytoplasm, damage to the plasma membrane, vacuolation, and heterochromatinization. In the testicular tissue, the tubules were relatively smaller in size and contained mainly spermatids. Some cells in the seminiferous tubules had signs of necrosis characterized by dissolution of the nucleus and nuclear membrane. Leydig cells were slightly hypertrophied, which may be a response to an increase in the functional demand, confirming the genotoxic effect of 2,4-D.

Cattaneo et al. (2008) reported that fish of the species *Rhamdia quelen* exposed to 2,4-D exhibited some behavioral alterations, such as lethargy and erratic swimming. The histological analysis of the liver revealed alterations after exposure, such as abnormal arrangement of hepatic cords, rupture of the cell membrane, and vacuolation in hepatocytes. The fish species *Geophagus brasiliensis* subjected to concentrations of 1, 5, 10, 20, 40, and 80 mg/L of 2,4-D for 96 h presented changes in mortality rates, oxygen consumption, and ammonia excretion. Mortality rates were directly proportional to the concentrations of the herbicide, reaching 100% at a concentration of 80 mg/L. Oxygen consumption rates change significantly in higher concentrations, which may result in changes in energy metabolism. The ammonia excretion rates were inversely proportional to the concentrations exposed, revealing changes in the animal's metabolism that may result in the excretion of the toxic substances absorbed. The mean neutral red retention team was significantly lower in comparison to the control, and the decrease of the dye retention team was directly related to the increase of the 2,4-D concentration (Barbieri 2009).

Tayeb et al. (2010) assessed the toxicity of 2,4-D administered to rats via oral gavage and observed prominent alterations in the liver tissue. After 4 weeks, animals treated with 15 mg/kg of 2,4-D exhibited ruptured hepatic cords in several areas. Focal necrosis was also observed, as well as vacuolation in hepatocytes and vessel dilation. These alterations were more prominent after treatment with a 75 mg/kg dose. Necrosis was more frequently observed in the group treated with this concentration, in addition to increase in glycogen charge. The treatment with 150 mg/kg of 2,4-D induced other histopathological alterations, such as pyknotic nuclei, congestion of hepatocytes, significant increase of cells undergoing necrosis, vacuolated hepatocytes, and loss of the typical polyhedral shape. Ruptured hepatic cords were also observed in several areas, demonstrating subacute toxicity to the liver caused by 2,4-D.

In order to investigate the effects of 2,4-D on testicular morphology and function, dwarf goats received three different treatments with the herbicide. The results revealed alterations in the seminiferous tubules, such as hyperemia and stromal edema, and detachment of the basal membrane from the surrounding fibromuscular layer. A reduction in the number of Sertoli cells, with vascular alterations of the intertubular stroma, was present in all treated groups, characterizing lower reproduction rates. Thus, exposure to 2,4-D negatively affected sperm counts of individuals, which might cause sperm dysfunction in humans and other species exposed to this toxic compound when applied to agricultural crops (Obidike et al. 2012). However, epidemiological data reported in some studies do not provide sufficient evidence that the herbicide is toxic to human sperm cells (Burns and Swaen 2012). In addition, other studies have reported that 2,4-D induced chromosome aberrations, also confirming its genotoxic/mutagenic effect (Tukula and Jalal 1985; Mustonem et al. 1986; Kale et al. 1995). Pesticides are bioactive molecules that tend to form electrophilic metabolites capable of reacting and combining with biological macromolecules, thus potentially predisposing the genetic material to changes through covalent bonds (Rodrigues 2002).

Taking into account the wide use of 2,4-D, we can infer how harmful the presence of this herbicide may be to non-target organisms. This compound, although useful for agriculture, may in the long term cause a change in soil since studies have shown disturbance of soil fauna, such as earthworms and millipedes, when exposed to 2,4-D, thereby altering the quality of the soil. Although this herbicide is applied to the soil, its drain occurs for the different types of water bodies, thus damaging aquatic animals. The consequences of its presence in the aquatic environment can interfere in the whole food chain since there are reports of the accumulation of this substance in the tissues of fish. Although there are no studies demonstrating its chronic toxicity in humans, the exposure of other mammals reveals how harmful the herbicide is and its ability to interfere with the physiology of animals. In view of this, a concern is raised about the correct use of this

compound in cultivated areas since its effects reach beyond the proposed target.

Bioassays with plants have been considered adequate for the assessment of genotoxicity and mutagenicity, mainly due to ethical reasons. Besides that, the assays with these bioindicators are validated and recommended, and their protocols are standardized by different environmental institutions, including the United Nations Environment Programme, the US Environmental Protection Agency, and the World Health Organization. Many higher plants are frequently used to monitor environmental pollutants (Leme and Marin-Morales 2009; Iqbal 2016; de Souza et al. 2016). According to Ma et al. (1995), plants are direct biological receptors of pollutants, and they constitute an important tool for genetic tests and environmental monitoring.

Croker (1953) reported that 2,4-D caused alterations during mitosis in *Allium cepa*. Alterations were dose dependent and classified as structural or physiological. The latter, such as adherence, chromosome condensation, and delay in the formation of the mitotic fuse, were observed after 2 h of treatment. Structural alterations include chromatid breaks, observed after 2 to 4 h of exposure to higher concentrations. Chromosome breaks were observed in groups exposed to all concentrations. Therefore, 2,4-D affects the nucleic acid cycle, suggesting that its effect on the chromosome structure is analogous to that of radiations.

Fiskesjö et al. (1981) studied the effects of 2,4-D by *Allium* test. They found that the mitotic index decreased in all concentrations tested and noticed a swelling in the roots (C-tumor) at a concentration of 1 ppm. Polyploid cells and giant cells were also seen. Pavlica et al. (1991) also examined the effects of two different concentrations of 2,4-D on *A. cepa*, 45 and 450 μM; they observed that after 2 and 24 h of treatment + 24 h of tap water, the concentration of 45 μM (μg/mL) decreased the mitotic activity almost three times lower than compared to the control group. On the other hand, the concentration of 450 μM stimulated cell division; the induction of chromosomal aberrations was much higher in the concentration of 450 μM. 2,4-D had a prominent effect on the mitotic fuse. Most of the abnormalities found were C-mitosis, disturbances in the anaphase, and problems in the reconstitution of the nucleus. The herbicide caused chromosome alterations during metaphase, anaphase, and telophase, except for micronuclei that were observed mainly in interphase. Chromosome bridges during anaphase and telophase were found after 2 and 24 h of treatment with 450 μM, and laggards were also observed, although in low frequency. The most frequently observed alteration was micronuclei in the groups exposed to the two concentrations. Chromosome adherence was also frequently observed and accompanied by instability of the mitotic fuse and chromosome. Fragmentation of the chromatin in interphase nuclei was observed in the group exposed to the highest concentration, as chromatin bodies.

According to Ateeq et al. (2002), onion bulbs exposed to different concentrations of 2,4-D, especially 25–100 ppm, exhibited prominent alterations associated with general growth and cell shape, in addition to a mitotic index near zero. In doses ranging from 5 to 20 ppm, a "Crochet hook," C-tumors, and swelling were observed in roots. In the group exposed to 1–4 ppm used for the chromosome aberration test, this parameter could not be calculated effectively since the roots were short (3–5 mm).

Assessment of the genotoxic effects of Avenoxan®, a commercial formula of 2,4-D in *A. cepa* and *Allium sativum*, revealed that the decrease in mitotic index in the two species had a dose–response effect. All concentrations induced abnormalities during mitotic division compared to the negative control in both species. The abnormalities were C-mitosis, adherences, anaphases and telophases with a bridge, lost chromosomes, multipolar anaphase, micronucleus, and breaks, the most common being C-mitosis (Gul et al. 2006).

Genotoxicity studies on 2,4-D in transgenic plants (*Arabidopsis thaliana*) demonstrated that the increase in concentration of the herbicide from 0–3 to 0–30 μg/L increased the frequency of transitions A → G when compared with the control group. The increase from 100 to 300 μg/L in the concentration of 2,4-D resulted in a decrease in the frequency of recombination. Interestingly, no effects of 2,4-D were observed on the frequency of transversions T → G. The increase in the frequency of recombination in low concentrations was associated with the increase of the levels of point mutations A → G. Thus, in general, 2,4-D in lower concentrations had a strong effect on recombination. However, this trend was not observed in higher concentrations (Filkowski et al. 2003).

A study by Grabinska-Sotaa et al. (2003) on the toxicity of derivatives of synthetic auxins to broadleaved plants and grains demonstrated that phenoxyacetic herbicides inhibited root growth. However, grasses in general were less sensitive to 2,4-D

Table 2 Summary of studies of the toxicity of 2,4-D to different organisms

Organism	Dosage of 2,4-D	Author	Effects in organisms
Bacteria	36 mg/kg of soil	Bécaert et al. (2006)	Negative effect on soil, maybe for right inhibition or remotion of bacteria
Eisenia foetida	1, 10, 100, 500, 1000 mg/kg of soil	Correia and Moreira (2010)	High mortality rate, severe effect on the development and reproduction
Scytonotus simplex	Four doses and different forms of application of 2,4-D	Hoy (1985)	High mortality rate and influence of spatial distribution of 2,4-D residues in the field
Leporinus obtusidens	1, 10 mg/L	Fonseca et al. (2008)	Effect in the brain and in the activity of the muscle enzyme acetylcholinesterase (AChE) and some metabolic parameters of blood and tissue
Cyprinus carpio	Varied doses of 2,4-D	Sarikaya and Yilmaz (2003)	High toxicity to fish
Clarias batrachus	75 ppm	Ateeq et al. (2006)	Genotoxic effect and histopathological alterations in male and female gonads
Rhamdia quelen	1 to 780 mg/L	Cattaneo et al. (2008)	Behavior alterations and histopathological alterations
Geophagus brasiliensis	1, 5, 10, 20, 40, and 80 mg/L	Barbieri (2009)	Changes in mortality rates, oxygen consumption, and ammonia excretion
Mouse	Oral treatment at doses of 15, 75, and 150 mg/kg	Tayeb et al. (2010)	Marked alterations of hepatic tissue
West African Dwarf (WAD) goats (male)	75, 100, and 125 mg/kg body weight	Obidike et al. (2012)	Histopathological alterations in testicular tissue and loss of effectivity of animal reproduction
Allium cepa	25, 125, 250, 500, 1000, 2500, 5000 ppm	Croker (1953)	Structural and physiological alterations in mitosis
Allium cepa	0.001, 0.01, 0.1, 1 ppm	Fiskesjö et al. (1981)	Mitotic index decreased, C-tumor, polyploid cells, and giant cells
Allium cepa	4, 5, 450 μM	Pavlica et al. (1991)	Strong effect on the mitotic fuse
Allium cepa	1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 75, 100 ppm	Ateeq et al. (2002)	Alterations in general growth and in cell shape, C-tumors, and swelling in roots
Arabidopsis thaliana	0, 5, 10, 30, 100, 200, 300 μg/L	Filkowski et al. (2003)	Strong effect on the recombination in minor concentrations tested
Sinapis alba, *Lepidium sativum*, *Avena sativa*, *Triticum aestivum*	0.3, 1.5, 3, 30, 150, 300, 3000, 0.072, 0.72, 7.2, 36, 72, 7200 mg/L	Grabinska-Sotaa et al. (2003)	Inhibition of root growth
Hordeum vulgare	1 or 2 L/ha	Geras'kin et al. (2005)	Cytogenetic aberrations
Allium cepa, *Allium sativum*	0.1, 0.2, 0.4%	Gul et al. (2006)	Decrease in mitotic index, induces C-mitosis, adhesions, anaphases and telophases with bridge, lost chromosomes, multipolar anaphase, micronucleus, and breaks
Triticum aestivum	50, 100, 200, 400, 800, 1200 ppm	Kumar et al. (2010)	Reduction the mitotic index and induction of chromosomal abnormalities
Phaseolus vulgaris	0.1, 0.2, 0.3 ppm	Cenkci et al. (2010)	Reduction in root growth, increased soluble protein and the number of bands in RAPD tests

derivatives, and therefore the toxicity of herbicide derivatives was higher for broad-leaved plants than for grasses. In a study on the cytogenetic effects of 2,4-D in 50% water (1–2 L/ha) on *Hordeum vulgare* (barley), Geras'kin et al. (2005) reported that the minimum dose increased the occurrence of cytogenetic aberrations nearly fourfold. Although significant, this effect was not dose dependent, as the frequency of cells with aberrations did not change significantly when the herbicide dose was doubled.

Kumar et al. (2010) reported that 2,4-D reduced the mitotic index of wheat plants, with a dose-dependent response. The variety HUW 468 exhibited the highest frequency of abnormal cells at the concentration of 1200 ppm of the herbicide. The induction of chromosome abnormalities was also dose dependent in all treatments. The most common aberrations observed were adherence and bridges, but also multipolarity, in addition to laggards in response to the maximum dose of the herbicide in all varieties.

A study that evaluated the genotoxicity of 2,4-D in bean seedlings using the comet assay and RAPD (random amplified polymorphic DNA) reported that genomic template stability and root growth were reduced in the treatments compared with the negative control. An increase in total soluble proteins was observed in seedlings exposed to 2,4-D, contrary to the decrease observed in the positive control group. Damage to DNA assessed with the comet assay was substantially higher than that of the negative control in all treatment groups. Patterns of RADP also exhibited significant differences between the negative control and the treatment groups, with disappearance of a normal band and/or appearance of an additional band. Thus, the number of bands increased with the increase in the concentration of the auxin herbicide, demonstrating a dose-dependent effect (Cenkci et al. 2010).

In the studies reviewed here about the action of 2,4-D in plant bioindicators, it is observed that although this herbicide has its main mode of action in plants, based on physiological processes, since it is a synthetic auxin, 2,4-D may also interact with plant DNA; in all the studies reviewed, it was able to induce some level of change in the genetic material. The studies present changes in mitotic index and induction of chromosomal aberrations, mutations, and micronuclei, which are widely recognized genetic biomarkers of the action of different compounds in these bioindicators. The studies presented above are summarized in Table 2 for better visualization and comparison of data.

In this way, it is worth noting that different types of organisms are currently used as bioindicators, that is, sentinel species that are used as indicators to evaluate the environmental quality of the possible effects of natural risks or anthropogenic origin. Thus, the studies reviewed, using different animals and higher plants, to investigate the effects of 2,4-D, and analysis of the results showed that although the herbicide is effective when used in broad-leaved plants, it can endanger the different ecosystems.

5 Final Considerations

Even in low concentrations, 2,4-D may have cytotoxic, genotoxic, and mutagenic effects on plants, as well as histological, physiological, and behavioral alterations in animals. Thus, the studies presented here reinforce the warning that pesticides, although important for agriculture, need to be used more cautiously, applied only in the indicated amounts and, as far as possible, replaced by other methods less harmful to the environment and biodiversity.

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