Chapter 19

# Characterization, Modes of Action and Effects of Trifluralin: A Review

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55169

## 1. Introduction

The use of chemicals to control human diseases, plagues and weeds in agriculture started in the late 19th century, but only after the Second World War did this practice follow rather scientific criteria [1]. According to targets against which they are designated, the chemicals used in agriculture are called insecticides, fungicides, herbicides, nematicides, among others [2].

All pesticides have the common priority of stopping a metabolic process essential to undesirable organisms, for which they are toxic. These chemicals act directly upon the organisms, eliminating or controlling them, such as interfering in their reproductive process [3].

Among agricultural pesticides, herbicides comprise the most employed group in agriculture. The main function of these chemicals is to control weeds, weed competition reduces productivity, without significantly impacting crop yield. Weeds tend to compete with crops by extracting essential elements from the soil, water, intercepting light and $CO_2$, interfering in the culture development and affecting agricultural production practices including harvest [4]. Herbicides are also used for eliminating plants from both road, railways, and riversides [3].

The mechanism of action of some herbicides on organisms is not completely understood [5]. Lack of detailed information about the action of herbicides on the biological environment may cause damage to human health [1], [6] and [7].

Herbicides may be classified according to different criteria related to their properties, characteristics, use, efficiency, permanence in the environment and mechanism of action. As for their chemical features, herbicides may be classified as carbamates, amides, diphenyl ethers, amino phosphates, and dinitroanilines, among others [8].

Classification of herbicides based on their mechanism of action has changed over time, both according to the discovery of new herbicides and the elucidation of site of action of the herbicide on plants. The internationally accepted classification is the one proposed by the Herbicide Resistance Action Committee (HRAC). In it, the herbicides are classified in alphabetical order in accordance with their sites of action and chemical classes (Table 1). Herbicides having unknown site of action are grouped under Z until identification. The (numeric) Weed Science Society of America (WSSA) classification system is also listed in Table 1 [5].

| HRAC | SITES OF ACTION | CHEMICAL GROUP | WSSA |
|---|---|---|---|
| A | Inhibition of Acetyl-CoA Carboxylase (ACCase) | Aryloxyphenoxypropionates (FOPs) | 1 |
| | | Ciclohexanodiones (DIMs) | 1 |
| | | Phenylpyrazolones (DENs) | 1 |
| B | Inhibition of Acetolactate Synthase (ALS) (or acetohydrxy acid synthase AHAS) | Sulfonylureas | 2 |
| | | Imidazolinones | 2 |
| | | Triazolopyrimidines | 2 |
| | | Pirimidinil(tio)benzoates | 2 |
| | | Sulfonylaminocarbonyl-triazolinones | 2 |
| C1 | Inhibition of Phtosynthesis in photosystem II | Triazines | 5 |
| | | Triazinones | 5 |
| | | Triazolinones | 5 |
| | | Uracils | 5 |
| | | Pyridazinone | 5 |
| | | Phenyl Carbamates | 5 |
| C2 | Inhibition of Phtosynthesis in photosystem II | Ureas | 7 |
| | | Amides | 7 |
| C3 | Inhibition of Phtosynthesis in photosystem II | Nitriles | 6 |
| | | Benzotiadiazinones | 6 |
| | | Phenyl-pyridazines | 6 |
| D | Inhibition of Phtosynthesis in photosystem I | Bipiridiliuns | 22 |
| E | Inhibition of Protoporphyrinogen Oxidase (PPO) | Diphenyl ethers | 14 |
| | | Phenylpyrazoles | 14 |
| | | N-phenylftalimidas | 14 |
| | | Thiadiazoles | 14 |
| | | Oxadiazoles | 14 |
| | | Triazolinones | 14 |
| | | Oxazolidinediones | 14 |
| | | Pyirimidinediones | 14 |
| | | Others | 14 |
| F1 | Inhibition of carotenoid biosynthesis in naphytoenedesaturase (PDS) | Pyridazinones | 12 |
| | | Pyridine Carboxamides | 12 |
| | | Others | 12 |
| F2 | Inhibition of carotenoid biosynthesis in 4-hydroxyphenyl-pyruvate-dioxygenase (4HPPD) | Triacetones | 27 |
| | | Isoxazoles | 27 |
| | | Pyrazoles | 27 |

| HRAC | SITES OF ACTION | CHEMICAL GROUP | WSSA |
|---|---|---|---|
| | | Others | 27 |
| F3 | Inhibition of carotenoid biosynthesis (unknown target) | Triazoles | 11 |
| | | Isoxazolidinones | 13 |
| | | Diphenyl ethers | 11 |
| G | Inhibition of EPSP synthase | Glycines | 9 |
| H | Inhibition of glutamine synthase | Phosphinic acid | 10 |
| I | Inhibition of DHP (dihydropteroate synthase) | Carbamates | 18 |
| K1 | Inhibition of microtubule assembly | Dinitroanilines | 3 |
| | | Phosphoramidates | 53 |
| | | Pyridines | 3 |
| | | Benzamides | 3 |
| | | Benzoic acid | 3 |
| K2 | Inhibition of mitosis | Carbamates | 23 |
| K3 | Inhibition of cell cycle | Chloroacetamides | 15 |
| | | Acetamides | 15 |
| | | Tetrazolinones | 15 |
| | | Others | 15 |
| L | Inhibition of cell wall (cellulose) synthesis | Nitriles | 20 |
| | | Benzamides | 21 |
| | | Triazolocarboxamides | 27 |
| | | Quinolinocarboxylic acid | 26/27 |
| M | Decouplers (cell membrane disruptors) | Dinitrophenols | 24 |
| N | Inhibition of lipid synthesis (different from ACCase inhibitors) | Tiocarbamates | 8 |
| | | Phosphoroditioates | 8 |
| | | Benzofurans | 16 |
| | | Chlorocarbonic acid | 26 |
| P | Auxin mimics | Phenoxicarboxylic acid | 4 |
| | | Benzoic acid | 4 |
| | | Pyridinecarboxylic acid | 4 |
| | | Quinolinocarboxylic acid | 4 |
| | | Others | 4 |
| Q | Auxin transport inhibitors | Ftalamates | 19 |
| | | Semicarbazones | 19 |
| R | ... | ... | |
| S | ... | ... | |
| . | ... | ... | |
| Z | Unknown | Arylamino Propionic acid | 25 |
| | | Pirazoliuns | 26 |
| | | Organoarsenicals | 17 |
| | | Others | |

**WSSA.** Weed Science Society of America; **HRAC.** Herbicide Resistance Action Committee.

**Table 1.** Herbicide Classification in accordance with their mechanism of action.

## 2. Trifluralin identification and characterisitcs

Trifluralin belongs to the dinitroaniline group which has the aniline structure as a basis, containing $NO_2$ molecules at 2 and 6 or 3 and 5 positions of the benzene ring. This group has more than ten different herbicides, among which are trifluralin, dinitramine, oryzalin and pendimethalin [8].

Trifluralin has been used in agriculture since 1963 [9]. This herbicide is registered separately or in mixtures, and used in the following crops: *Glycine max*, citrus, *Coffea arábica* under formation, *Gossypium hirsutum*, *Arachis hypogaea*, *Phaseolus vulgaris*, *Allium sativum*, *Ricinus communis*, *Manihot esculenta*, *Helianthus annuus*, *Solanum melongena*, *Daucus carota*, *Abelmoschus esculentus*, *Brassica oleracea*, *Brassica oleracea capitata, Brassica oleracea botrytis*, *Capsicum annuum*, *Lycopersicon esculentum*, and ornamental plants [10].

Trifluralin is available either in emulsifiable concentrate or in crystalline solid both formulations of the yellow-orange color. It is not quite soluble in water (0.3 to 0.6 mg/L solubility at 25°C) [9], it is mildly volatile (1.1. $10^{-4}$ mmHg pressure vapor at 25°C), its density is 1.36 g/cm$^3$ at 22°C, it is considered alkaline and long-lasting in the environment (120-240 days) [8]. Trifluralin has a high affinity to soil [11], is relatively immobile and has a half-life of 3 to 18 weeks, depending on the soil and the geographical location [12].

Trifluralin chemical composition is $\alpha,\alpha,\alpha$–trifluoro–2–6-dinitro–N–N– dipropyl–p–toluidine [13]. The chemical structure formula is shown in Figure 1.

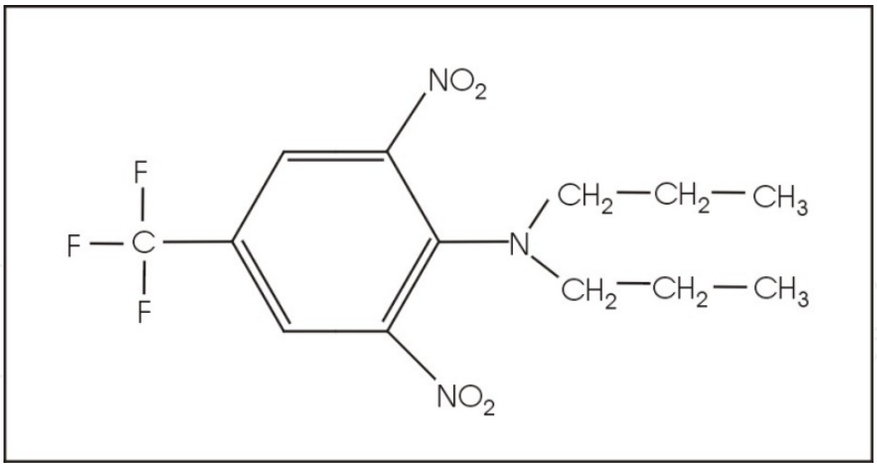


**Figure 1.** Trifluralin chemical structure formule.

Trifluralin commercial products contain nitrosodipropylamine, a carcinogenic contaminant (NDPA) [14]. This compound reacts with $0^6$-guanine DNA and may cause mutation [15]. On account of concerns about this characteristic, the *Environmental Protection Agency* (EPA) demanded that industries make sure products containing trifluralin active principle had nitrosodipropylamine 0.5 ppm concentrations at the most [14].

http://dx.doi.org/10.5772/55169

USEPA (1999) [16] classifies trifluralin as group C: possibly carcinogenic to humans, based on evidences with animals, not with humans.

## 3. Trifluralin behavior in the environment

### 3.1. Behavior in soil

Trifluralin is strongly adsorbed by organic matter colloids and not much by clay ones. In organic matter rich soils, adsorption prevents absorption of the product by plant roots. Therefore, the use of this herbicide under such conditions is not advisable [10]. Leaching, as well as soil lateral movement is quite reduced compared to some pesticides [17]. Its main characteristic is soil persistence resulting from low mobility, which can cause damage to crops following its application [12].

Such herbicides as trifluralin, applied in pre-emergence, act better when soil humidity is between high and elevated. Therefore, the herbicide may at least be partially solubilized and distributed in the first layers of the soil surface, which will protect it from losses [8].

This herbicide degradation in soil occurs through chemical, microbial pathways and photolysis. Chemical degradation promotes dealkylation of the amino group, reduction from the nitro to the amino group, partial oxidation from the trifluoromethyl to the carboxyl group and, subsequently, degradation into smaller fragments (Figure 2).

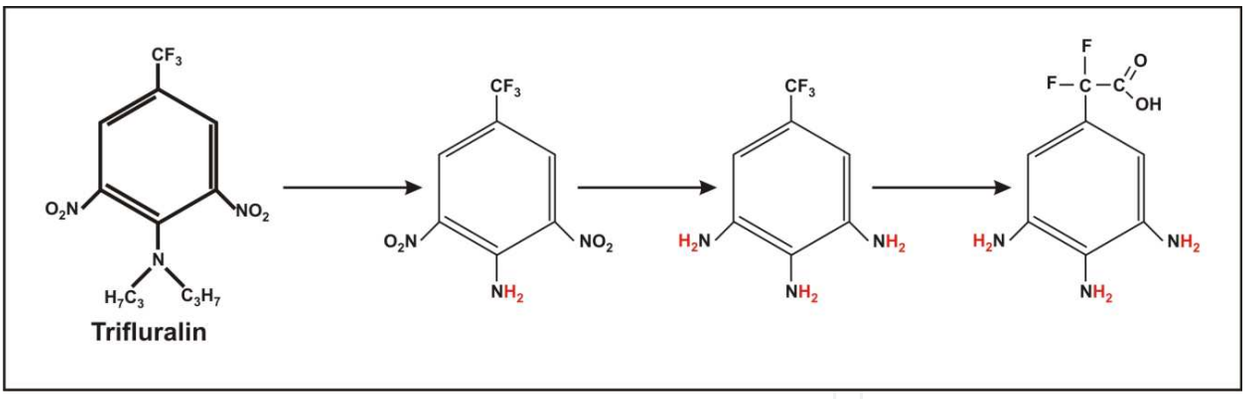


**Figure 2.** Possible sequence of events that occur during trifluralin chemical degradation.

Microbial degradation may occur under aerobic and anaerobic conditions (Figure 3). However, it is observed that degradation occurs mainly under anaerobic conditions, as the ones observed in poorly drained soils, when there is subsequent rainfall. Under anaerobic conditions, within the same time period, 98% of trifluralin degrades, whereas under aerobic conditions only 25% of the product decomposes. Among the fungi capable of decomposing trifluralin are *Sclerotiumrolfsii*, *Aspergillusniger*, *Fusarium*sp and *Tricoderma*sp [10]. According to Carter and Camper [18], trifluralin may also be degraded by *Pseudomonas* sp.

Trifluralin is also sensitive to degradation by ultraviolet rays, and its volatility is one of the main factors of product loss in the soil as well [19] and [20]. Trifluralin photodecomposition generally involves three processes: propylamine oxidative dealkylation, cyclization and nitro group reduction (Figure 4) [21].

The first product of trifluralin photolysis, according to Dimou et al. [21] and illustrated in Figure 3, seems to be a mono-dealkylate deriving from the main compound, originating compound 1. Dealkylation is attributed to the free radical oxidation. Another intermediate of photodegradation appears to be formed by cyclization reactions. The compounds 4 and 5 are apparently formed by reaction among trifluralinpropylamine $\alpha$ carbon and the $NO_2$ group of compound 1, ant they are identified as 2– ethyl -7nitro-1-propyl-5 (trifluoromethyl)-1*H*-benzimidazole and 2-ethyl-4 nitro-6- (trifluoromethyl)-1*H*-enzimidazole, respectively. The benzimidazoledealkylate (compound 4) is the most stable photoproduct, which can last in the environment longer, making its detection possible. This product may be formed by the reaction of compound 5 dealkylation.

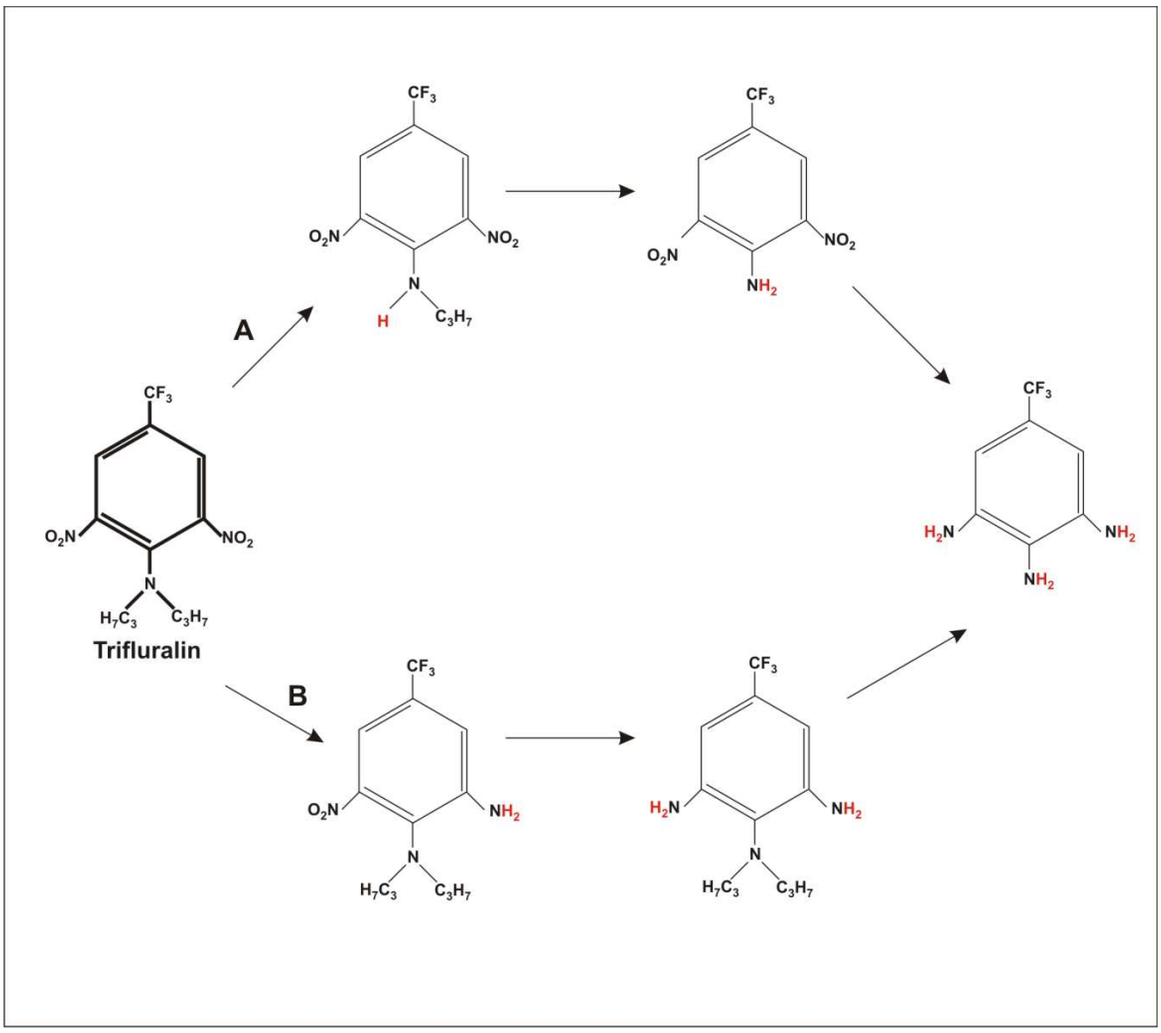


**Figure 3.** Trifluralin microbial degradation by aerobic (A) and anaerobic (B) pathways. Source: Audus [22].

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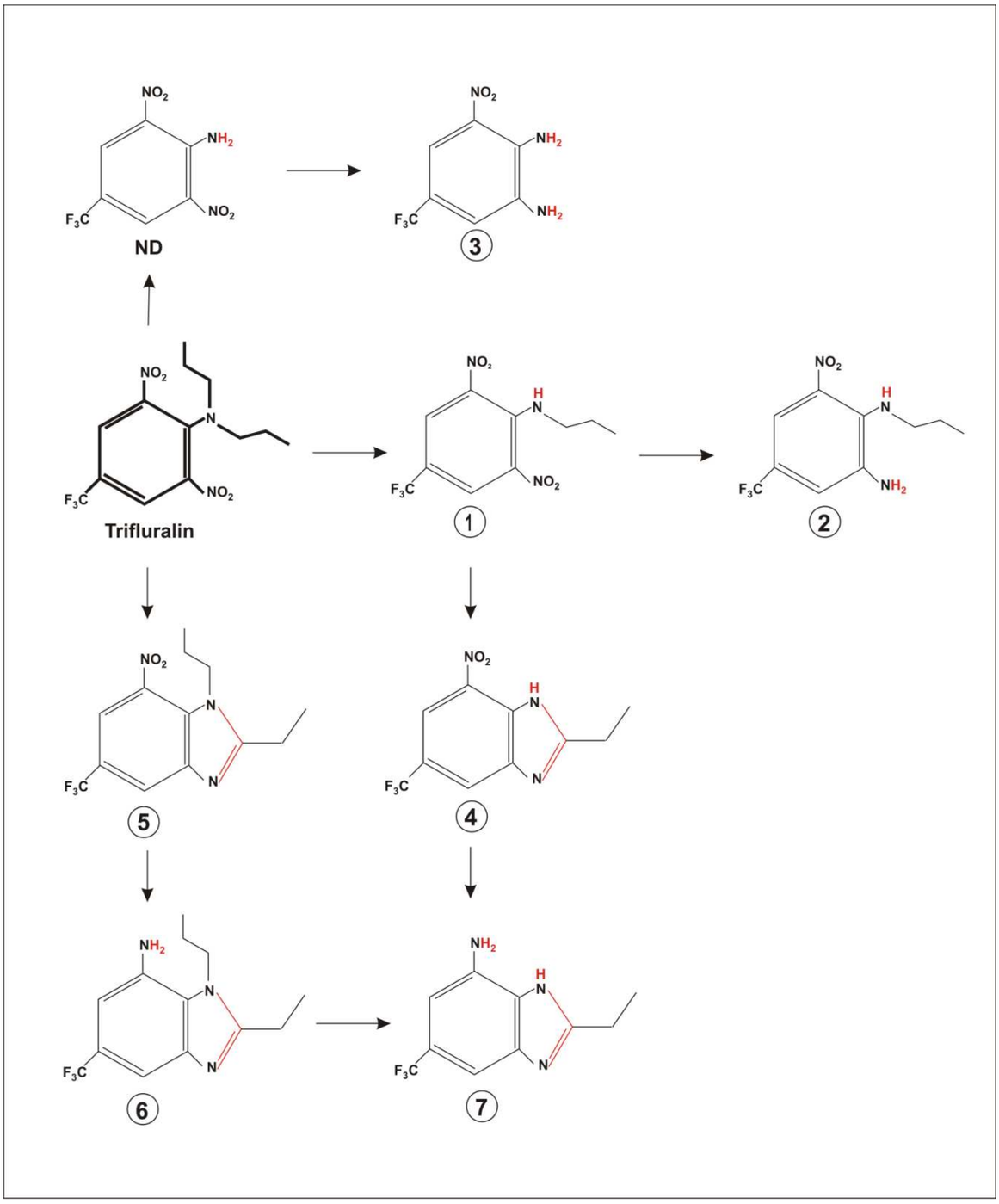


**Figure 4.** Trifluralin photodegradation. *ND=Substance not detected in the source. Modified scheme by Dimou et al. [21].

Compounds 4 and 5 can be reduced in water by not so clear mechanisms [23], straight from the aryl hydroxylamine formation [24] to form compound 7 and 6, respectively. According to the same author these products have also been formed during trifluralin chemical degradation. Compound 2 and 3 are formed from $NO_2$ to $NH_2$ group reduction of compound 1 and 2,6-dinitro-4- (trifluoromethyl) benzenamine (compound ND), respectively. These compounds

have also been identified during trifluralin chemical degradation [24], showing that this pathway also happens in other processes, besides photodegradation [21].

Trifluralin average persistence in soil for the recommended doses under field conditions is of 1.8 ppm residue after 180 days following application [25]. However, according to the same author, this persistence may vary in accordance with the kind of soil and climatic conditions.

### 3.2. Herbicide behavior in water

Water contamination with trifluralin may occur by sediment leaching while equipment is being cleaned, or due to accidental spills. Nevertheless, only 0.5% of the quantity applied to the soil in field conditions is leached and may consequently contaminate water sources. This percentage means a rather low water contamination, representing smaller concentrations than 1.0 $\mu g\ L^{-1}$. As a consequence, trifluralin is not commonly detected in surface water [9] and [26].

While Zimmerman et al. [26], Dayama and Coupe [27], Thurman et al. [28] and were carrying out analyses in the Mississippi River, they detected extremely low levels of trifluralin (lower than 0.1 g/L). Once this herbicide is widely used, the authors ascertain that low concentrations of it detected in surface water may be attributed to its low mobility in soil and low solubility in water (lower than 1 mg/L). USEPA [29] and the European Community legislation [30] established limits of 2μg/ L and 0.1μg/ Ltrifluralin in drinking water, respectively. According to Dimou et al. [21], trifluralin degradation in water is influenced by the presence of nitrate ions, which accelerate photolysis reaction. Products derived from this reaction have either low or no toxicity, when compared to the whole product.

### 3.3. Herbicide behavior in the air

Grover et al. [31] ascertain that trifluralin is quickly dissipated in the atmosphere. Depending on the season of the year, about 25% of the product applied is volatilized, but only 2-3 $\mu g/m^3$ at the most of trifluralin is found in the air, soon after its application, to less than $100ng/m^3$ a few hours later [32]. According to the United States Environment Protection Agency (1993) [33], an average 0.27 $ng/m^3$ concentration of herbicide, varying from 0 to 3.4 $ng/m^3$, was found in the Canadian atmosphere between 1988 and 1989.

Mongar and Miller [34] state that low concentrations of this herbicide found in the atmosphere are due to both trifluralin quick reaction with the hydroxyl radical (OH) and the photolysis reaction, which promotes the product degradation.Nonetheless, Waite et al. [32] verified that of the five most used herbicides on the Canadian prairies, trifluralin was the most frequently found in the air (79% of samples).

### 3.4. Herbicide behavior in plants

Trifluralin is a pre-emergence herbicide which must be incorporated into the soil and applied soon after sowing, when the plant seeds are beginning the germination process [36]. The herbicide absorption occurs mainly by the hypocotyl, then by the seedling radicles, at the beginning of germination [10].

Trifluralin's main mechanism of action is the inhibition of cell mitosis. This herbicide typically acts on the meristems and tissues of underground organs, such as roots, epicotyls, hypocotyls, plumules, rhizomes, bulbs and seeds [8].

The inhibition of radicle development by trifluralin action, both on main root growth and the emission of secondary roots, is quite evident in some dicotyledons. Thickening of the hypocotyls also commonly occurs [8], as well as swollen root tips [36]. According to Almeida [25], trifluralin induces several biochemical changes in higher plants, including alterations of carbohydrate, lipid, nitrogen concentrations and, especially, nucleic acid alterations. Therefore, the product affects cell division in meristematic tissues, thus inhibiting seed germination and the formation of new radicle and hypocotyl cells.

Bayer et al. [37] report that trifluralin promotes a decrease in the zone of meristematic tissues and the interruption of mitosis in the roots of wheat, cotton and onions. The onion cells treated with trifluralin showed to be small, dense and multinucleated, abnormal, weak and aberrant [38].Studies conducted by Fernandes [39] using *Allium cepa* showed that the toxicity of trifluralin residual concentrations might induce changes in that plant. The author observed that the herbicide promoted plant growth inhibition, higher turgidity, weakness and thickness of the roots, in relation to the control treatment.

Plants grown in soils treated with trifluralin exhibited residues on the roots only. No residue was found on the leaves, fruit and seeds [25]. These results indicate that trifluralin is not transported by sap into other plat tissues.

## 4. Trifluralin mechanisms of action

Plant growth and development depend on mitosis in their meristematic regions. Cell division is a process that requires different cell organelles, structures and the products of many genes to be working correctly. Dinitroanilines, the family to which trifluralin, phosphoride amides and N-phenyl carbonates belong, are microtubule-depolymerizing chemical compounds [5], [40], [41], [42] and[43]. According to Senseman [36], the herbicide-trifluralin complex inhibits microtubule polymerization, leading to physical misconfiguration and loss of function. As a consequence, the mitotic spindle does not form, causing misalignment and chromosome separation during mitosis. In addition to that, the so-called spindle apparatus is not formed.

Microtubules are subcellular structure filaments, basically made up of heterodimeric tubulin protein (Figure 5A) [44]. They have important cellular functions, which are directly related to mitosis and indirectly related to organism development. These structures are involved in several cellular processes such as chromosome migration, cellular structure maintenance, cellulose microfibril orientation and organization, cell wall formation, intracellular movement, as well as cellular differentiation [42] and [45]. Most sets of cell microtubules are labile and their functions depend on this lability.The mitotic spindle is one of the most extraordinary examples, whose formation is brought about after disorganization of cytoplasmic microtubule at the beginning of mitosis. For this reason, the mitotic spindle is targeted by various specific

anti-mitotic drugs, which interfere in the exchange of tubulin subunits between the microtubules and the pool of free tubulins [46].

*In-vitro* analyses of *Chlamydomonas reinhardii* showed that trifluralin specifically binds tubulins, demonstrating that it is the first subcellular target of dinitroaniline action [47]. Trifluralinsub-micromolar concentrations totally blocked cytokinesis and inhibit nuclear division in *Toxoplasma gondii* by interfering in intracellular spindle and in other cytoskeletal components [48].

According to Anthony and Hussey [47], the herbicide-tubulin complex is related to the suppression of microtubule growth. With minus-end specific microtubule depolymerization, the tubules progressively start to get shorter, eventually leading to total loss of microtubule (Figure 5B). The author still states that cortical microtubules are among the most resistant to trifluralin action and microtubule spindles and fragments are among the most sensitive to the herbicide action.

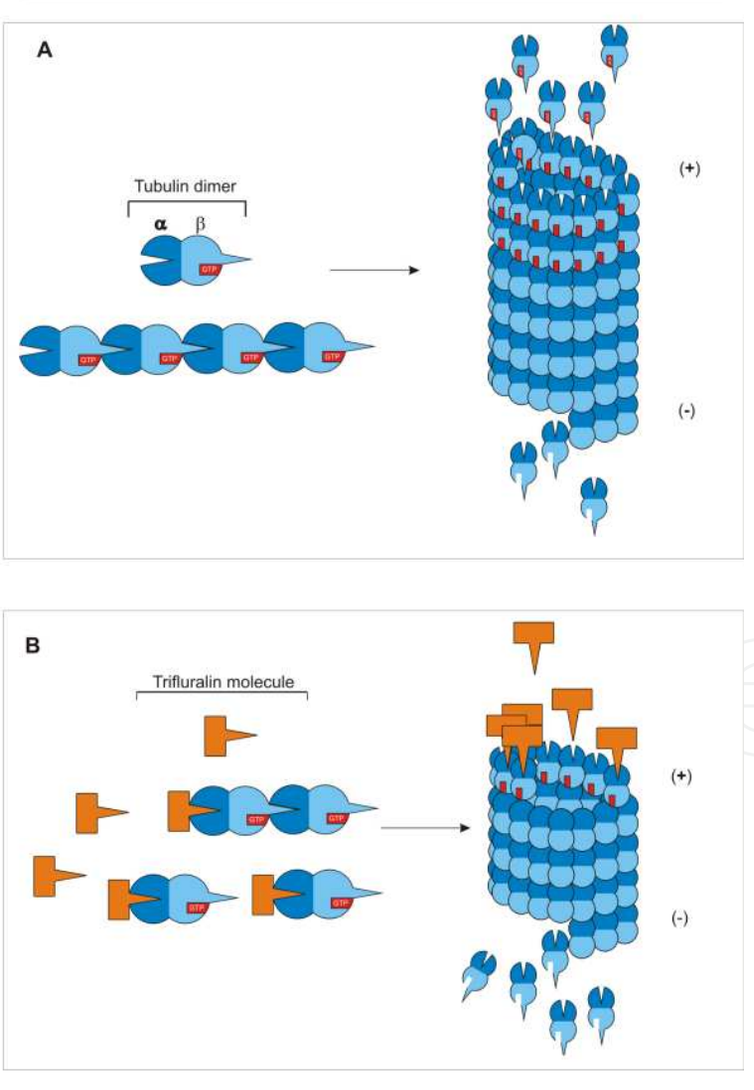


**Figure 5. A.** tubulin dimers forming the microtubule; **B.** herbicide-tubulin complex preventing microtubule polymerization.

Anthony et al. [49] ascertained that, as a rule, the tubulin sequence is the most preserved among the different organisms; and this preservation is related to the basic functions of microtubules. Mahresh and Larry [50], however, believe that, depending on the organism, dinitroaniline herbicides have different affinities to tubulins, since they do not interact with vertebrate tubulins, although they interact with plant and *Chlamydomonas* tubulins. This situation is reinforced with data from Anthony and Hussey [47], Baird et al. [51], Breviário and Nick [52] and Yemets and Blume [53], who ascertain that dinitroaniline herbicides are compounds with higher specificity for binding plant tubulins than to those of vertebrates.

Studies on plant resistance to dinitroanilines showed that some plant species own a natural mutation which bring about a change in base pairs, and consequently in their genetic code. One of these alterations of base causes a change in the amino acids of the tubulin protein. Threonine, a normal amino acid at position 239, is changed into isoleucine, stopping group $NO_2$ of the dinitroaniline herbicides from binding the tubulin molecule, thus preventing its mechanism of action (Figure 6) [47].

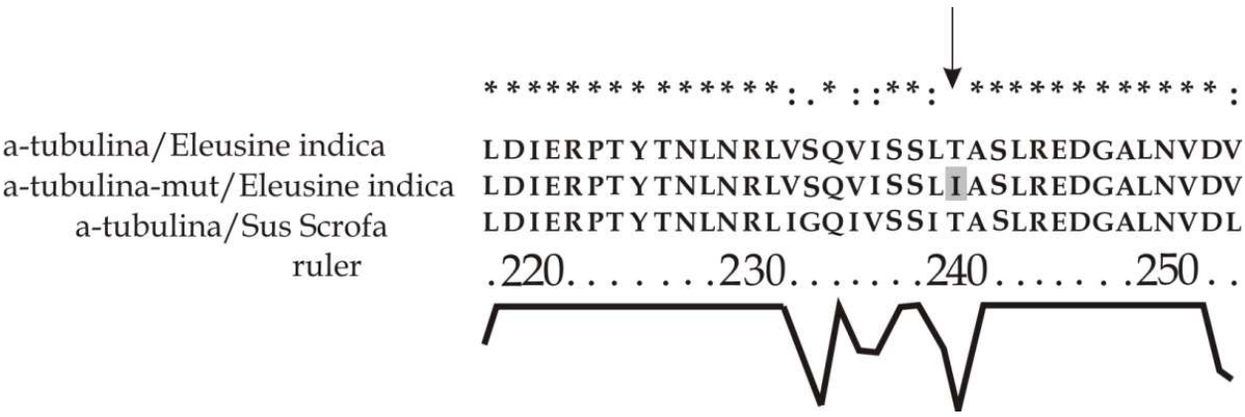


**Figure 6.** Alignment of amino acid sequence of α-tubulins, evidencing the position of substitution in the mutating tubulin from *Eleusine indica* (Thr 239 into Ile- represented in black and indicated with an arrow). Modified from Blume et al. [54].

From these pieces of information, it would be intuitive to hypothesize the idea that the smallest affinity of trifluralin to vertebrates should be owing to the fact they do not have the amino acid at position 239, seemingly the herbicide target site. Nevertheless, it can be seen in Figure 7 that the threonine amino acid at position 239 of the $\alpha$-tubulin protein is present in plants, parasites and vertebrates, including man.

However, Hashim et al. [58] found mutations in the $\alpha$-tubulin gene expression which changed the amino acid synthesis at a different position than that found by Anthony and Hussey [47]. According to Hashim et al. [58], *Alopecurus aequalis* plants that underwent mutations, which altered the amino acid synthesis at positions 202, 136 and 125 of the $\alpha$-tubulin, also brought about resistance to trifluralin.

Sree et al. [59], Hansen et al. [60] and Vidakovié-Cifrek et al. [61], ascertain that trifluralin can inhibit microtubule polymerization by binding tubulin. However, it can also cause changes in

the ion calcium concentration in cytoplasm and influence polymerization and depolymerization regulation of microtubules. According to Hertel et al. [62], changes in the quantity of free $Ca^{2+}$ in cytoplasm, due to trifluralin action, can alter calcium-dependant biochemical and physiological processes, in addition to causing problems to microtubules, either in animals or in plants. Vidakovié-Cifrek et al. [61] report that trifluralin may increase the concentration of $Ca^{2+}$ ions in cytoplasm, influencing onion root mitosis.

Due to trifluralin chemical structure, this herbicide tends to receive two electrons, which significantly increases its toxicity, since the group $NH_2$ hydrogen of trifluralin tends to bind the polar group of cellular membranes and cause disorganization to its structure, eventually bringing function disorders [63]. This disorganization in the membrane structure seems to interfere mainly in the permeability of plasma and mitochondrial membranes. Trifluralin changes the permeability of membranes because it promotes a collapse in their electric potential, making $Ca^{+2}$ efflux of the mitochondrial inner membranes and $Ca^{+2}$ go from the outer to the inner surface of the cell membrane via uniporters, thus increasing the concentrations of such ions in the inner cytoplasmic membrane.

Since low levels of calcium are needed for polymerization, Hepler [64] ascertains that mitotic spindles may undergo disorders due to the high levels of this ion. Low concentrations of free calcium in the cytoplasm (0.1-0.2 μM) are essential to prevent phosphorus precipitation, compete with $Mg^{2+}$ for binding sites and act as a secondary messenger [65].

According to Alberts et al. [46], $Ca^{+2}$ is important for regulating mitochondrial enzyme activity, and it is imported from the cytosol through an H+ electrostatic gradient. It is also believed that this process is important to remove $Ca^{+2}$ from the cytosol when cytosolic $Ca^{+2}$ levels get dangerously high.

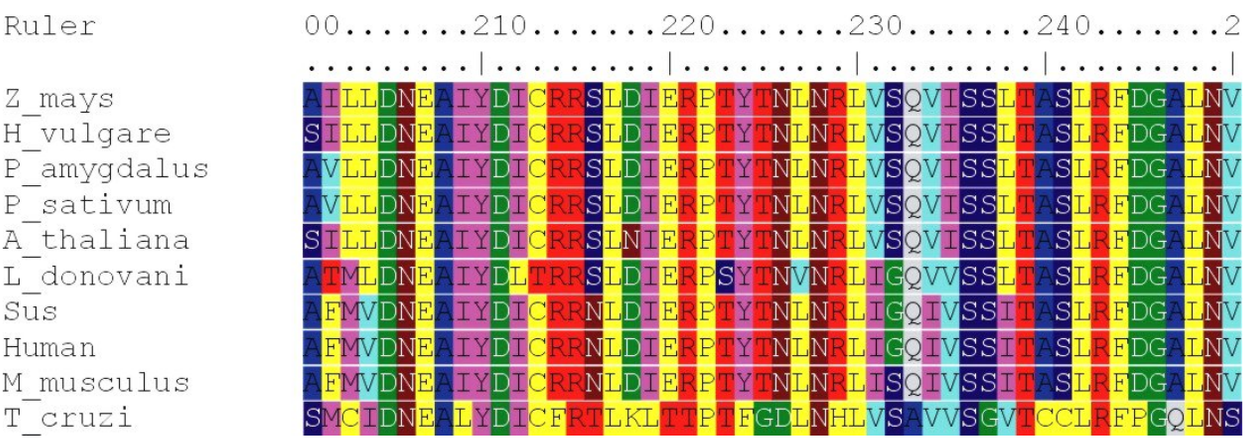


**Figure 7.** Comparisons among sequences of α-tubulin amino acids of species *Zea mays* (vegetable), *Hordeumvulgare* (vegetable), *Arabidopsis thaliana* (vegetable) *Prunus amygdalus* (vegetable), *Pisum sativum* (vegetable),Leishmania d*onovani* (parasite), *Trypanosoma cruzi* (vegetable),*Mus musculus* (vertebrate), *Sus scrofa* (vertebrate) and *Homo sapiens*. The sequences were obtained from the data base at NCBI (National Center of Biotechnology Information) in accordance with the codes P14641, Y08490, P29511, P33629, U12589, U09612, M97956, P05213, P02550 and P04687, respectively [55]. The sequences were aligned by means of the ClustalW program [56], using default parameters. The alignment was then analyzed using the MPALign program [57].

Another important factor to be considered is the derivate generation through pesticide biodegradation [66] and [67]. One of the byproducts of trifluralin biodegradation is an aniline: 2,6dinitroaniline (Figure 8) [68].

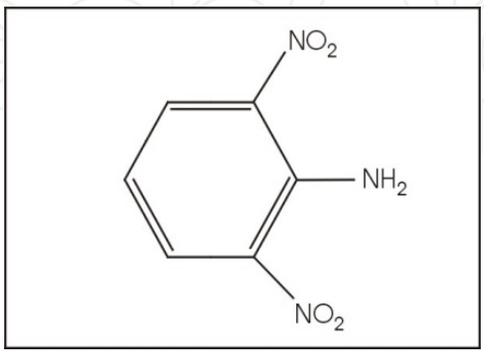


**Figure 8.** Chemical structure of 2,6 dinitroaniline.

Anilines are compounds that cause a variety of toxic effects depending on the structural changes they undergo. Several studies demonstrate that anilines and halogens can induce metahemoglobin formation and also be toxic to the kidneys and the liver, either treated *in vitro* or *in vivo* [69] and [70]. Aminophenols, the primary products of aniline metabolism, are compounds related to neurotoxicity induction [69].

## 5. Trifluralin toxic effect

Although many researchers and international governmental agencies have investigated and published trifluralin toxic effects on different fields, whether they are related to either acute or chronic toxicity, cytotoxicity, genotoxicity, mutagenicity and carcinogenicity, the results shown are confusing and often contradictory.

According to the W.H.O (World Health Organization) [70], trifluralin causes hemoglobin oxidation (by forming metahemoglobin), red blood cell destruction, besides being toxic to the kidneys and the liver, and stimulating depression in the central nervous system. It may cause vomiting, diarrhea, weakness, profuse sweating, loss of sight, memory and concentration, and dermatitis as well. This herbicide is considered to be neurotoxic and gastrointestinal irritant. It can lead to death because of ventricular fibrillation [71], although several authors [10], [72], [73], [74], [75] and [76], ascertain that trifluralin is a low toxicity substance.

Trifluralin lethal concentrations and doses for vertebrates and invertebrates are shown in Table 2.

| Treatment | Species | Group | Popular Name | Toxicity |
|---|---|---|---|---|
| Cl50 (48h) | *Lepomis macrochirus* | Fish | Bluegill | 19 µg $L^{-1}$ |
| CL50 (48h) | *Mola mola* | Fish | Ocean sunfish | 19µg $L^{-1}$ |
| CL50 (48h) | *Cyprinus carpio* | Fish | Common carp | 1.0mg $L^{-1}$ |
| CL50 (96h) | *Oncorhynchus mykiss* | Fish | Rainbow trout | 0,21mg$L^{-1}$ |
| CL50 (96h) | *Oncorhynchus mykiss*, *Lepomis macrochirus, Mola mola* | (Young)fish | Rainbow trout, Bluegill, Ocean sunfish | 10-90µg $L^{-1}$ |
| CL50 (48h) | *Daphnia magma* | Micro-crustacean | - | 0,56 mg$L^{-1}$ |
| CL50 (96h) | *Procambarus clarkia* | Crustacean | Lobster | 12mg$L^{-1}$ |
| DL50 (oral) | *Apis mellifera* | Insect | Honey bee | 0,011mg bee-1 |
| DL50 (oral) | *Mus musculus* | Mammal | Laboratory mice | >500 mg $kg^{-1}$ |
| DL50 (oral) | *Ratus norvegicus* | Mammal | Laboratory mice | > 10.000 mg $kg^{-1}$ |
| DL50 (oral) | - | Mammal | Dog | > 200 mg $kg^{-1}$ |
| DL50 (oral) | - | Mammal | Rabbit | > 200 mg $kg^{-1}$ |
| DL50 (oral) | - | Bird | Hen | > 200 mg $kg^{-1}$ |

Data extracted from Gangolli [77].

**Table 2.** TrifluralinCL50 and DL50 for different organisms

Meister [78] conducted tests with animals and verified that trifluralin does not have any toxic effect on them when they are exposed to the product either through ingestion, inhalation or when in contact with the skin. Nauseas and severe gastrointestinal discomfort may occur after trifluralin ingestion. When placed in the rabbit eyes, it produced a mild irritation, which was reverted within seven days. In humans, it may induce skin allergies and, when inhaled, it may irritate the throat and the lungs.

Table 3 shows some information regarding trifluralin chronic, sub-acute and sub- chronic toxicity to different organisms.

| Treatment | Species | Group | Popular Name | Toxicity | Symptoms |
|---|---|---|---|---|---|
| LOEC | *Amphiprion percula* | Fish | clownfish | 5µg $L^{-1}$ | - |
| NOEL | *Amphiprion percula* | Fish | clownfish | 2µ $L^{-1}$ | - |
| CE50 (10 days) | *Chlorococcum* sp | Protozoa | - | 2,5 mg $L^{-1}$ | - |
| Sub-acute(dermis -14 days) | Oryctolagus caniculus | Mammal | Rabbit | 2mL $Kg^{-1}$ | diarrhea and mild erythema |
| Sub-chronic(ingestion - 3 months) | *Ratus norvegicus* | Mammal | Mouse | 25, 50 e 100 mg $kg^{-1}$ $dia^{-1}$ | no effects produced on either survival or appearance * |

*Liver weight of the animals submitted to the 50 and 100mg $Kg^{-1}$ diet somehow showed to be higher, when compared to the control animals. Data extracted from Gangolli [77].

**Table 3.** Data on trifluralin sub-acute, chronic and sub-chronic toxicity.

According to the Occupational Health Service [79], prolonged skin contact with trifluralin may cause allergic dermatitis. The WSSA [80] states that administering trifluralin to dogs while washing them for two years does not cause toxic effects. However, in trifluralin chronic assays conducted with 60 animals (F344 mice), which received 0.813, 3250 and 6500 ppm dietary does for two years, damage to their liver and kidneys were observed [81].

Worthing [71] states that trifluralin is highly toxic and neurotoxic. The author ascertains that the herbicide is capable of accumulating in the adipose tissue and inhibiting the immunologic function of the thymus. Trifluralin is regarded as possibly teratogenic and fetal toxicity.It has the property of altering the endocrine and reproductive system, and it reduces the quantity of semen, besides increasing the number of abnormal sperm.

In studies conducted by Ovidi et al. [82], they tested trifluralin concentration of 1.53 mg/ml and observed that the herbicide exerts a specific effect on the reproductive system in plants, by direct action on the formation of the pollinic tubes, since it causes complete microtubule depolymerization. The authors even suggest that pollinic microtubule cytoskeleton may be used as bioindicators for studies on toxicity induced by aneugenic agents such as trifluralin.

As a general rule, the effects of pesticides may be diversified, such as the direct reaction with nuclear DNA; incorporation of DNA during cellular replication; interference in mitosis or meiosis, resulting from incorrect cell division [83].

Genotoxic effects may lead to DNA breaks, causing loss of genetic material and mutations which lead to cell death or result in carcinogenesis. Genotoxicity is assessed by different tests, carry out with several organisms and provide safe, precise information regarding their potential to damage the DNA.There are a number of reports evaluating trifluralin for genotoxicity, immunotoxicity, and reproductive toxicity, although the results are not entirely consistent, trifluralin does not appear to be strongly genotoxic [84].

Chromosome aberration tests have shown evidences of trifluralin mutagenicity for different plant species [85], [86], [87], [88], [89] and [90]. Könen and Çavas [91], Peña [92] and Canevari [93] ascertained that the herbicide is capable of inducing significant microtubule rates in *Oreochromis niloticus*. Kaya et al. [94] also ascertained that the herbicide may be considered genotoxic to *Drosophila melanogaster*, since it exhibited positive outcomes for the Somatic Mutation and Recombination Test (SMART). Tests conducted in the bone marrow of mice exposed to trifluralin showed that it is potentially genotoxic [95] and it is also capable of influencing serum concentration of reproductive and metabolic hormones, especially thyroxin [96]. Nonetheless, tests performed on bacteria [14], on *Drosophyla melanogaster* conducted by Bryant and Murnik [97] and Foureman [98], on cells taken from the bone marrow of mice conducted by Nehéz et al. [99], Pilinkaya [100], Gebel et al., [95], and on cell culture conducted by IARC [101] and Ribas et al. [35 and 102] demonstrated contradictory results. According to Chan and Fong [103], Bhattacharya et al. [104] and Esteves et al. [105], due to its characteristics, mechanisms of action and, especially its reduced effects on human cells, trifluralin can be regarded as a promising substance for fighting Leishmaniasis. There is also research that confirms the use of trifluralin as a powerful antiparasitic to treat *Trypanosoma* [106] and [107], *Toxoplasma* [48] and *Plasmodium* [108].

Studies carried out by Peña [92] and Canevari [93] indicate that low trifluralin concentrations may induce mutagenic effects. These authors observed significant presence of micronuclei in erythrocytes of fish submitted to acute treatments with this herbicide. When the micronuclei diameters were measured by Canevari [93], data indicated that they could be derived from losses of whole chromosomes, thus proving the aneugenic effect of the herbicide due to the pesticide interference in the mitotic spindle.

*Allium cepa*meristematic cells treated with trifluralin also presented problems during mitosis, such as polyploidies, C-metaphases, multipolar anaphases, anaphase-telophase chromatin bridges, chromosome delay and loss of genetic material [89]. (Figure 9).

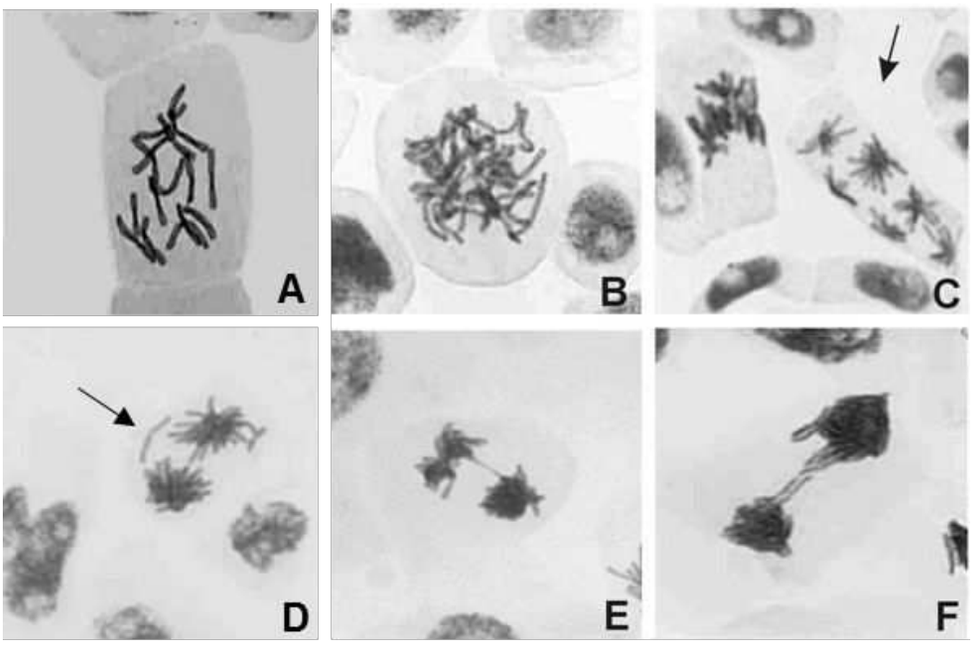


**Figure 9.** Meristematic cells of *Allium cepa* treated with trifluralin. **A.** C-metaphase; **B.**polyploid cell; **C.** multipolar cell; **D**. loss of genetic material; **E.**chromosome bridge; **F.**telophase with chromosome delay.

According to Fernandes et al. [88], in the bioassays with root meristems of *Allium cepa* treated with trifluralin, a large amount of interphase cells with more than one nucleus and cells with micronuclei and a mini cell were observed (Figure 10).

Lignowski and Scott [85] observed C-metaphases, micronuclei, amoeboid nuclei and polyploidies in root meristems of wheat and onion submitted to trifluralin action. Due to the occurrence of irregular metaphases, they concluded that the mitotic spindle might have been broken owing to the herbicide action on it.

Bioassays performed with trifluralin, using *Pisum sativum* as test material revealed a positive action of the herbicide with the increase in chromosome alterations, C-mitosis and anti-mitosis effects [87].

Fernandes et al. [89] ascertained that, among the root meristems of *Allium cepa* under division, trifluralin promotes a significant increase in the irregular metaphase rate. These data corroborate the statement of Lignowski and Scott [85], Lee et al. [109], Dow et al. [110], Werbovetz et al. [111] and Ovidi et al. [82], who characterized trifluralin as a powerful microtubule inhibitor, which is therefore capable of accumulating a large amount of meristematic cells in metaphase.

Genotoxicity tests using the comet assay in human lymphocyte cultures showed that trifluralin produced a significant increase in the length of the comet's tail. This increase is due to DNA breaks, since there is an induction of nucleotide excision repair, resulting from damage caused by the herbicide action [103]. As for the frequency of comet-bearing cells, the author observed that, after 48 hours of exposure to the herbicide, few tailed nucleoides were found. These results proved to be statistically significant, though.

According to Ribas et al. [35], trifluralin has a genotoxic effect on human cell cultures because it causes a decrease in cell proliferation. The same author ascertains that this herbicide has not revealed carcinogenic effects, since it caused little induction exchange between sister chromatids.The micronucleus test conducted by Ribas et al. [35], used for detecting aneugenic activity, has also produced a negative response, which contradicts studies carried out by several other authors [88], [89], [91], [92], [97], [112], among others) who ascertain that trifluralin brings about chromosome aberrations and nuclear alterations resulting from problems in the mitotic spindle

According to Kang et al. [113], trifluralin is not associated with bladder, kidney, liver, leukemia, colorectal or hematopoietic-lymphatic cancers. The authors only suggest a possible connection between trifluralin exposure and the risk of colon cancer in human beings, but the inconsistency per exposure level and a small number of colon cancers indicate that this could be an incidental finding.

Data from the National Cancer Institute (NCI) [114] report that mice subjected to trifluralin chronic exposure, at low concentrations, had an increase in hepatocellular carcinoma and higher incidence of alveolar bronchial adenomas. An increase in bladder cancer was also verified in mice exposed to low trifluralin concentrations. It was observed that, when male mice were submitted to high doses of trifluralin, they presented higher incidence of follicular cell and thyroid gland tumors [115]. Trifluralin has been reported to cause a significant increase

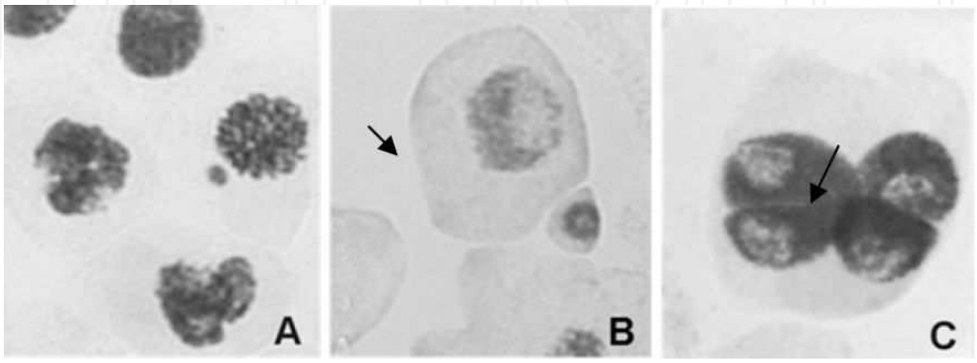


**Figure 10.** Meristematic cells of *Allium* cepa treated with trifluralin. **A.** cell with micronucleus; **B.** cell with micronucleus and an adjacent mini cell; **C.**polynucleated cell.

in thyroid follicular cell tumors in male Fischer 344 rats only at the highest dietary dose of 6500ppm in a 2-year chronic study [115].

## 6. Final considerations

The increase in agricultural productivity has occurred thanks to several factors, among which are improvements in genetics, agricultural machinery and the use of substances that allow control of weeds in agriculture.

The use of pesticides has generated discussions and controversy among the scientific community and its users, registering advantageous and disadvantageous recommendations in different ways. Among contrary recommendations to the use of pesticides, we can point out lack of detailed studies on the action of such chemicals on the exposed organisms, making it impossible to associate their action with the emergence of eventual problems. In the soil, trifluralin is moderately persistent, which might jeopardize organisms that are eventually exposed to it. Trifluralin is a substance that has a microtubule-depolymerizing activity, which prevents cell division, a fact that might compromise organism development.

Existing reports characterize trifluralin as a highly acute toxic substance to fish, but there are not enough descriptions of its chronic toxicity and cytotoxic effect. Studies mainly related to its genotoxic, mutagenic and carcinogenic potential are mostly inconclusive or even contradictory. There is little information about the toxicity of products derived from trifluralin degradation and its effects on the organisms.

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