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Neurotoxic Effects of Triazole Fungicides on Nigrostriatal Dopaminergic Neurotransmission

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1. Introduction

Azole or conazole fungicides represent a large group of substances widely used agriculturally for the protection of crop plants and pharmaceutically in the treatment of various fungal diseases. They are synthetic compounds that can be classified into *imidazole* or *triazole*, depending on the number of nitrogen atoms in the five-membered ring. Some fungicides from the imidazoles group include, among others, the ketoconazole, miconazole and clotrimazole. Within the group of triazoles we can cite the itraconazole, fluconazole, flusinazole, triadimefon and flutriafol.

In turn, the triazole can be divided into two groups: *triazole antifungal drugs*, that include the fluconazole, voriconazole, isavuconazole, itraconazole, etc., and *triazole plant protection* fungicides, with tebuconazole, triadimefon, triadimenol, paclobutazol and flutriafol as the fungicides most commonly used. Structural formulas of triazole ring and some triazole fungicides are shown in the Fig. 1.

Azole fungicides exert their antifungal activity binding the half of the azole ring to the heme protein and by subsequent inhibition of cytochrome P450 51 (Cyp51), the enzyme that facilitates the 14-alpha-demethylation of lanosterol to ergosterol in mushrooms. Ergosterol is a component of fungal cell membranes, serving the same function that cholesterol serves in animal cells. Ergosterol is necessary to maintain the membrane fluidity and the integrity of the wall of fungal cells (Ghannoum & Rice, 1999).

Triazole fungicides have great importance in agriculture and medicine, being commonly used in different ways and in large quantities throughout the world. The intensive use of these compounds can generate a lot of residues that may potentially lead to substantial environmental contamination. So, triazole residues or triazole metabolites may occur in the environment and should be considered as a risk from food, drinking water and non-occupational exposure.

Despite this large scale use and the risk of exposure of human populations, there are few studies on potential toxic effects of this group of pesticides on biological systems. Most available data are published by regulatory agencies (e.g., Food and Agriculture Organization, Environmental Protection Agency), and the toxicity evaluations have been performed only according to regulatory submission requirements.

In this way, some data available by Environmental Protection Agency - USA (EPA, 2006), show that after an oral administration, the triazole fungicides are quickly absorbed and widely distributed in all evaluated tissues. After this absorption and distribution, the

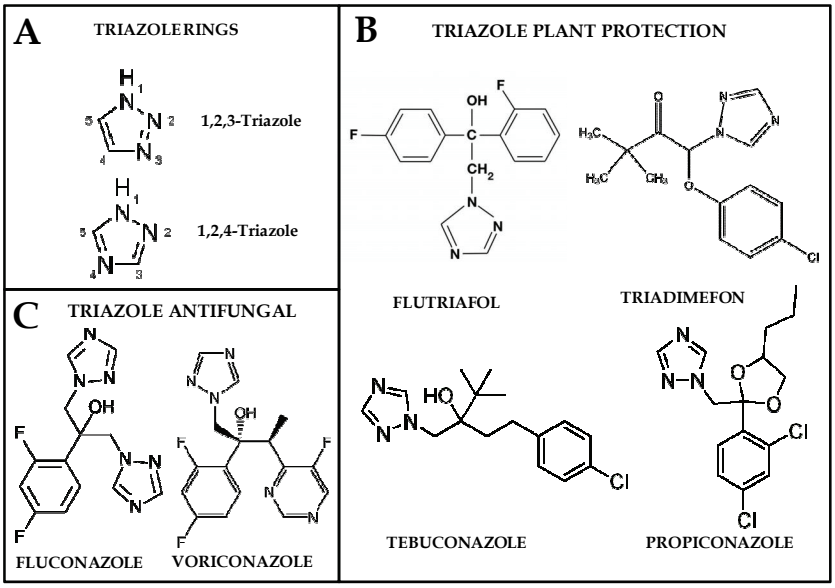

Fig. 1. Structural formulas of some triazole fungicides. A) The two isomers of triazole ring, which characterizes this group. B) and C) Examples of triazole plant protection fungicides and triazole antifungal drugs, respectively.

triazoles are metabolized giving origin to a variety of compounds whose toxic effects are currently being studied. Also according to the EPA (2006), among these compounds, the 1,2,4-triazole, triazole alanine and triazole acetic acid are the most common metabolites of the triazoles found in rat and mouse tissues. Excretion occurs mostly via urine, largely as unchanged parent (80-95%). With an estimated half-life of 8-10 hours, excretion is largely completed within 48 hours of administration of a single dose.

Studies published by EPA (2006) propose that triazoles present various deleterious effects on mammalian biological systems, especially on the nervous system. So, the EPA report shows evidences that exposure to triazoles, in general, causes neurotoxicity, including: neuropathological lesions in rat and mouse brain; neuropathological lesions in rat peripheral nervous system; and decreases in brain weight in several studies in both rats and mice.

However, as described above, in spite of their large use, comprehensive data assessing the effects of triazoles economically relevant on mammalian biological systems, mainly on nervous system, have been quite limited, and in the case of certain triazole fungicides (e.g., flutriafol), almost completely lacking. Because of this, in this chapter we propose to review some effects and possible mechanisms of action of two important triazoles: triadimefon and flutriafol.

Flutriafol ([RS]-2,4′-difluoro-α-[1H-1,2,4-triazol-1-ylmethyl] benzhydryl alcohol) is an economically important agricultural chemical that has proved its effectiveness in controlling several diseases affecting a wide range of crops. It is extremely persistent in the environment and it is accumulated in soil after repeated annual applications. Its residues also present high potential of mobility in the soil.

Flutriafol would likely also be a groundwater contaminant (EPA, 1991). Although the final destination and the behavior of this fungicide in the water have not been precisely evaluated, its use in large quantities and their application on cereals, could be indicate a probably water contamination. This contamination could occur due to the leaching process, taking this pesticide to the interior of the watercourses.

The toxicological effects of flutriafol on biological systems are little described. However, some studies in rats have associated its exposure to a decrease in body weight, ocular damages, decrease in fetal bone formation, hepatotoxicity with alterations of liver volume and hypertrophy, respiratory system irritation, and the suspicion of possible reproductive toxicity with a decrease in female fertility, since it is also considered to be an endocrine disruptor (Zarn et al., 2003).

Despite the fact that the acute oral LD_{50} of flutriafol has been established about 1200 mg/kg in rats, some works also demonstrate that sub-acute administration of low doses of flutriafol (10, 50, and 125 mg/kg) to pregnant rats produces a significant dose-related reduction of fetal ossification in the treated groups. At the dose of 10 mg/kg, incomplete ossification of some skull bones can be noted, and at doses of 50 and 125 mg/kg, the incidence of fetuses with extra ribs is increased (PSD, 1996). Such data could indicate that exposure to low concentrations of flutriafol can cause adverse effects in rats, although neurotoxic effects have not been described until recently.

Another important triazole fungicide, from a toxicological point of view, is the triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazole-1-yl)-2-butanone], a broad spectrum, systemic triazole fungicide registered for use on fruits and grains.

Triadimefon is remarkable for the neurobehavioral effects that it induces in rodents. So, it was observed that exposition to low doses of triadimefon (50-100 mg/kg) increased the frequency of locomotion and rearing in rats. Also, exposition to higher doses (200 mg/kg) of triadimefon induced highly stereotyped behaviors and self-mutilation (Crofton et al., 1988, 1989; Perkins et al., 1991; Moser et al., 1995; Walker et al., 1990). It is known that changes in motor activity may occur as a result of neurochemical changes, specifically in the dopaminergic neurotransmission in the nigro-striatal pathway. So, to verify the hypothesis that the triadimefon-induced behavioral effects can be due to an action on dopaminergic system, Crofton et al. (1989) evaluated the effects of combined treatment of triadimefon with either an inhibitor of dopamine synthesis or a dopamine vesicle depletor (reserpine). These authors observed that reserpine partially blocked the increases in motor activity produced by triadimefon, confirming that the fungicide produces its effects acting on the dopaminergic terminal.

Those were the initial studies that confirmed the effects of triadimefon on the dopaminergic system in rodents. Currently, the stimulatory effect on motor behavior of this fungicide has been well characterized and it is well known that this effect is produced by neurochemical changes in dopaminergic neurotransmission in the nigro-striatal pathway.

Based on this information, and considering the commercial importance of the triazole fungicides flutriafol and triadimefon, its persistence in the environment and the correlation between exposure to triazole fungicides and dopaminergic system alterations, the objective of this chapter was to review the effects of these fungicides on the dopaminergic nigro-striatal system. The first part of this chapter includes a review of the physiology of dopaminergic neurotransmission in rat striatum. The second part of this review deals with the *in vitro* and *in vivo* effects of both fungicides on dopaminergic neurotransmission.

2. Dopaminergic neurotransmission

Dopamine is a neurotransmitter related to cognition, motor functions, motivation and reward, emotions, attention, and learning. In the nigrostriatal system, dopamine has a fundamental function in the control of complex movements. Because this, the functions performed by dopamine in the striatum are of great clinical importance and several neurological diseases like Parkinson and Huntington occur as a result of dysfunction in this region. For example, if there is a sharp decline in dopamine levels or its actions are blocked, a syndrome with hypokinesia and muscle rigidity, characteristic of Parkinson's disease, is observed. This is produced because dopaminergic neurons, a smaller proportion of brain neurons, have a large functional significance.

2.1 Synthesis of dopamine

From a biochemical point of view, dopamine is a biogenic amine which belongs to the group of catecholamines. Dopamine is synthesized in the terminal of dopaminergic neurons from tyrosine, a non-essential amino acid, which may come from the diet or synthesized in the liver from phenylalanine. The blood tyrosine reaches the brain through the blood-brain barrier using a specific transport system for neutral amino acids.

Dopamine is synthesized in two enzymatic steps shown in the Fig. 2: the first step is the hydroxylation of tyrosine to form L-dihydroxyphenylalanine (L-DOPA) by the action of cytosolic enzyme tyrosine hydroxylase (EC 1.14.16.2). This stage is the limiting step in the biosynthetic pathway of catecholamines, so that tyrosine hydroxylase is subject to strict control by end product inhibition. The next step in the synthesis is the decarboxylation of L-DOPA to form dopamine by the action of the enzyme DOPA-decarboxylase (EC 4.1.1.28). This enzyme is not a limiting factor in the biosynthesis of catecholamines and it is not specific for the L-DOPA but can decarboxylate other amino acids.

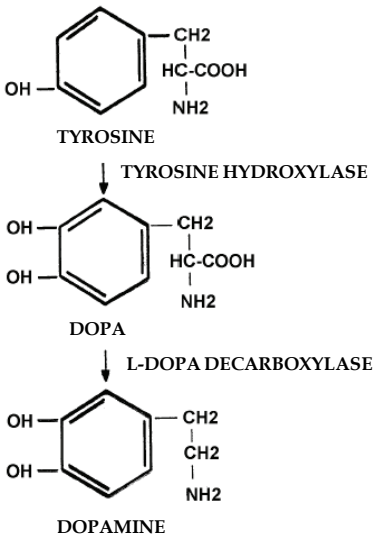

Fig. 2. Scheme of the dopamine biosynthesis.

2.2 Release of dopamine

After its synthesis, dopamine can be stored in synaptic vesicles in the dopaminergic axon terminal. Storage of dopamine is done through a contra-transport mechanism by which dopamine is specifically transported using a membrane transporter into synaptic vesicles following a hydrogen gradient generated by an ATPase.

However, not all dopamine is stored in synaptic vesicles into the dopaminergic terminal. Several experimental evidences indicate the existence of two pools of intracellular dopamine: a *vesicular pool*, consisting mainly of newly synthesized dopamine and a *cytoplasmic pool*, consisting of reuptaked dopamine and newly synthesized dopamine that is not stored in vesicles.

When an action potential reaches the dopaminergic terminal, the change in the membrane potential opens voltage-dependent calcium channels and the entry of these ions into the terminal. The calcium ions induce the migration of the synaptic vesicles to the active zones, its anchorage to the terminal membrane, and the exocytotic dopamine release. Dopamine can also be released by a calcium-independent process, through the dopamine transporter (DAT). After its release, dopamine diffuses into the synaptic space and interacts with specific receptors that can be pre- or post-synaptic.

After the release of dopamine to the synaptic cleft and its interaction with specific receptors, the action of the neurotransmitter may be finished by different mechanisms, as shown in Fig. 3.

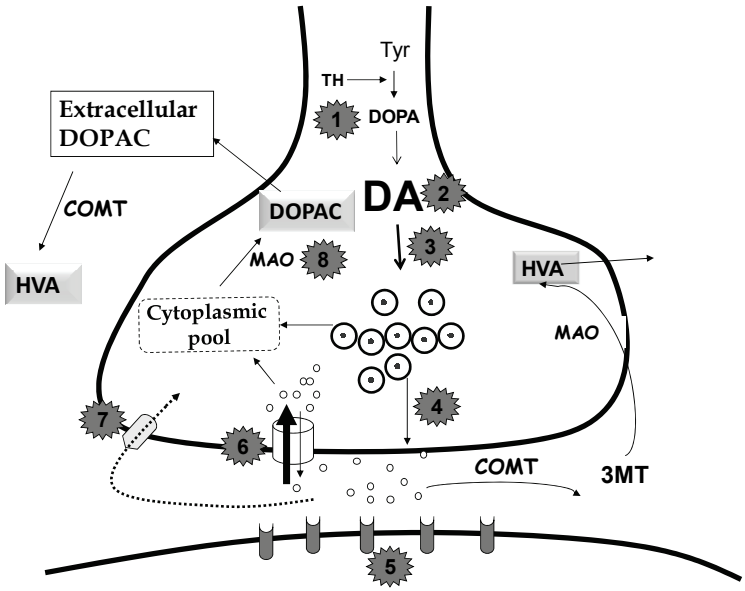

Fig. 3. Dopaminergic synapse. Tyrosine hydroxylase (TH) converts tyrosine to DOPA (1) and DOPA decarboxylase converts DOPA into dopamine (2). Dopamine can be stored in synaptic vesicles (3) and then released (4). Once released, dopamine can bind to post-synaptic receptors (5), be metabolized, reuptaked (6) or join autoreceptors (7). Within the axon terminal, dopamine may be metabolized by mitochondrial monoamine oxydase (MAO).

2.3 Reuptake of dopamine: the dopamine transporter (DAT)

Once released into the cleft synaptic, the synaptic actions of the dopamine can be finalized through three main mechanisms: 1) reuptake or transport of dopamine back to inside of the presynaptic terminal; 2) enzymatic breakdown (see next section) and; 3) diffusion into the cleft synaptic.

The reuptake of dopamine is realized directly by transport of this substance back into the axon terminal together with sodium and chloride ions. This reuptake is carried through the presynaptic membrane by a membrane transporter, the DAT.

The DAT is a glycoprotein of 58-77 kDa with 12 transmembrane domains and a large extracellular loop with N and C terminals to the cytosolic side. This protein can be divided into three regions according to their functional roles. The first five transmembrane domains are related to functions common to all carriers of the same type as, for example, the ionic dependence of transport.

Under certain conditions (for example, altering the composition of the extracellular medium), the DAT can reverse the direction of dopamine transport, going to release dopamine to the extracellular medium rather than reuptake it.

2.3 Catabolism of dopamine

The enzymatic breakdown (catabolism) of dopamine is achieved through the action of two enzymes: monoamine oxydase (MAO) and catechol-O-methyl-transferase (COMT), which differ in their activity and location (Fig. 4).

MAO is an intraneuronal enzyme located in the external mitochondrial membrane and it acts on reuptaked dopamine. COMT is an extraneuronal enzyme that acts on released dopamine.

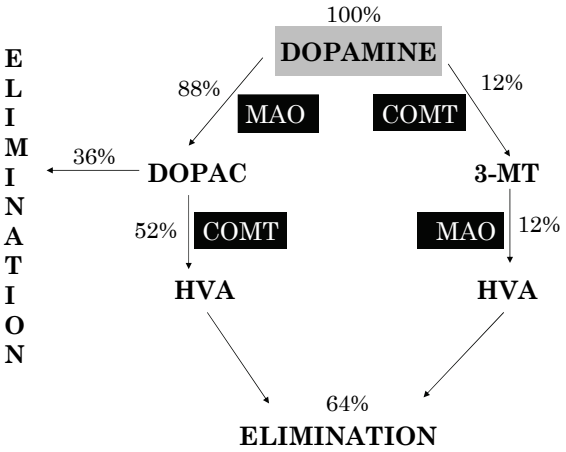

Fig. 4. Scheme of the catabolism (degradation) of dopamine. The numbers indicate the transformation rates in percentage (Adapted from Westerink, 1985).

In presence of molecular oxygen, MAO coverts dopamine into their corresponding aldehydes. The aldehydes generated are rapidly oxidized, producing acidic metabolites, as

the dihydroxyphenylacetic acid (DOPAC), or reduced, resulting in neutral metabolites as methoxyhydroxyphenylethanol (MOPET) and dihydroxyphenylethanol (DOPET). There are two MAO isoenzymes: MAO-A, mainly involved in catecholamine metabolism, and MAO-B, related to the metabolism of another neurotransmitter, the serotonin.
COMT catalyzes reactions of methylation of catechols and acidic and neutral metabolites, transforming most of the dopamine released in 3-methoxytyramine (3-MT). In turn, 3-MT is subsequently converted into homovanillic acid (HVA) by the action of MAO. HVA can also be produced from DOPAC by the action of COMT.
Under *in vivo* normal conditions, the majority of dopamine is degraded to its acidic metabolites DOPAC and HVA. Taking into account the site of DOPAC and HVA synthesis, we could consider DOPAC as an index of intraneuronal degradation of dopamine, while HVA would be an index of extraneuronal degradation. However, as HVA also is produced from DOPAC, it could be considered as a secondary metabolite of dopamine.
The ability to measure levels of dopamine and its metabolites provides an index of activity of both enzymes and of dopamine neurons in general.

3. Effects of triazole fungicides on nigrostriatal dopaminergic system

It has been described that triazole fungicides triadimefon (and its metabolite triadimenol) and flutriafol produce changes in dopaminergic neurotransmission (Walker & Mailman, 1996; Gagnaire & Micillino, 2006; Santana et al., 2009). As previously described, the effects produced by triazole fungicides on the dopaminergic system began to be described at the end of 1980s and early 1990s, with initial observations of neurotoxic effects of triadimefon on the motor activity. Several posterior studies, demonstrated that these behavioral effects produced by triadimefon were produced by changes in dopaminergic neurotransmission. In this session we will show some experimental results that demonstrate the effects and possible mechanisms of action of flutriafol and triadimefon on the nigro-striatal dopaminergic system of rodents.

3.1 Behavioral and *in vitro* effects of triadimefon

Triadimefon increases locomotion and induces stereotyped behavior in rodents (Crofton et al., 1988, Moser & MacPhail, 1989). Some possible targets for the alterations in dopaminergic neurotransmission induced by triadimefon are the mechanisms of synthesis, release, reuptake, and degradation of dopamine in nerve endings.
To determine the possible neurochemical mechanism of action of tiradimefon to induce behavioral change, Crofton et al. (1989) evaluated the effects of combined treatment of triadimefon with either a tyrosine hydroxylase inhibitor (D,L-alpha-methyl-p-tyrosine methyl ester, alpha MPT) or a depletor of catecholamine stores (reserpine). These authors observed that alpha-MPT did not block the increased motor activity produced by triadimefon, while reserpine reversed this effect. Based in this result, the authors suggested that increased motor activity produced by triadimefon was not mediated through release of newly synthesized catecholamines, but rather on dopamine released or reuptaked.
It has also been shown that the hyperactivity induced by triadimefon could be mediated through an action of this fungicide on dopamine post-synaptic receptors. So, dopamine D1 (SCH23390) and D2 (remoxipride) antagonists blocked, in different proportions,

triadimefon-induced locomotion (MacPhail et al., 1993). However, Walker et al. (1990) demonstrated that triadimefon does not bind directly to these dopamine receptors, being probably the activation of dopamine system produced in an indirect form. These authors also hypothesize that triadimefon could be act in a similar form to psychomotor stimulants such cocaine, amphetamine and methylphenidate, that exert their behavioral effects by increasing the concentration of dopamine in the synaptic cleft. To test this hypothesis Walker and Mailman (1996) examined the effects of triadimefon on dopamine uptake, dopamine efflux, and binding to the DAT.

Walker and Mailman (1996) observed that both triadimefon, and its main metabolite triadimenol, inhibited the *in vitro* uptake of [^{3}H]dopamine in striatal synaptosomes from rat striatum, increasing the concentrations of this neurotransmitter in the extracellular medium. The authors also observed that the two fungicides appear to act in a similar form to other well-characterized inhibitors of dopamine uptake bounding with high affinity to the DAT (Walker & Mailman, 1996). These data about the mechanism of action of triadimefon and triadimenol suggest that in high concentrations (high levels of exposure) these fungicides can produce similar effects to those caused by psychomotor stimulants.

Psychomotor stimulants (e.g. cocaine, amphetamine) are known to cause behavioral sensitization, a phenomenon defined as a progressive increase in the locomotor or stereotypic response to drug treatment (development phase) with an even further enhancement of this response following a drug challenge after a withdrawal period (expression phase). The effects produced by triadimefon on the dopaminergic system seem to indicate that exposure to this fungicide could lead to behavioral sensitization.

The possibility that exposition to triadimefon could lead to behavioral sensitization, was tested in a long-term study by Reeves et al. (2003; 2004). In these works, the authors observed that intermittent intraperitoneal injections of triadimefon appear to induce behavioral sensitization in mice. This behavioral effect was associated with neurochemical long-term changes in both the mesolimbic and nigrostriatal dopaminergic system.

3.2 *In vivo* effects of triadimefon and flutriafol on dopamine release from rat striatum

The data described in prevous section refer to effects of triadimefon observed in *in vitro* experimental preparations, such as cell cultures and isolated preparations of nervous tissue, among others. In the present section we will describe the effects of triadimefon and flutriafol on *in vivo* release of dopamine in the striatum of rats. The data reviewed here were obtained by microdialysis technique which allows monitoring the *in vivo* release of dopamine in awake and freely-moving rats using cerebral microdialysis (see Bergquist et al., 2002, for review).

The microdialysis technique coupled to high affinity liquid chromatography with electrochemical detection (HPLC-EC) was used by Gagnaire and Micillino (2006), to examine the effects of administration of triadimefon on *in vivo* dopamine release from rat striatum. These researchers observed that systemic administration of this fungicide produced a gradual and sustained increase in striatal dopamine levels. In this study, the authors report that i.p. administration of 100 or 200 mg/kg of triadimefon increased dopamine extracellular levels to 170 and 398%, respectively, measured by *in vivo* brain microdialysis (Fig. 5A).

The effects of flutriafol on *in vivo* dopamine release were studied by Santana et al. (2009). These authors demonstrated that *in situ* administration of different concentrations of this fungicide into rat striatum also induced changes in dopaminergic neurotransmission. So, intrastriatal administration of 1, 6 and 12 mM flutriafol through the microdialysis probe produced significant concentration-dependent increases in dopamine levels to 218 ± 51%, 1376 ± 245% and 3093 ± 345%, respectively (Fig. 5B).

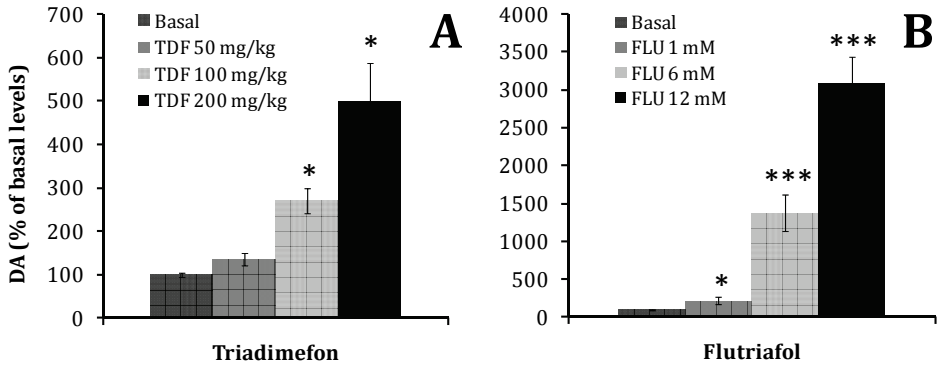

Fig. 5. Maximal increases (mean ± SEM) in dopamine levels in striatal microdialysis samples taken after systemic administration of triadimefon 50, 100 or 200 mg/kg (A) and intrastriatal infusion of flutriafol 1, 6 or 12 mM (B). The maximal increases produced by triadimefon and flutriafol were observed at 240 and 40 min after the beginning of infusion, respectively. The results (n=4- 8/group) were expressed as a percentage of the basal levels (100%). Basal levels were considered as the mean of substance concentrations in the three samples before triadimefon (or vehicle) or flutriafol perfusion. Significant differences: *P<0.05; *P<0.01; and *P<0.001 with respect to the control group or basal (Source: Gagnaire & Micilino, 2006; Santana et al., 2009).

So, administration of triadimefon and flutriafol produced significant dose-dependent increases on *in vivo* dopamine release from rat striatum. In accordance with these results, at least two general mechanisms can be proposed to explain the increases in extracellular DA levels observed: triadimefon and flutriafol could be implicated in inducing the neurotransmitter release from synaptic vesicles or producing dopamine reuptake inhibition. To check which mechanism would be implicated in inducing dopamine release by these fungicides, different drugs with known actions were used on dopaminergic terminals, as well as modified perfusion medium administered together with the fungicides into the striatum.

To evaluate the mechanism by what triadimefon induces *in vivo* dopamine release, Gagnaire and Micilino (2006) used the following experimental approach: a) compared the effects of this fungicide to those of amphetamine, a releaser of dopamine from its storage sites; b) investigated whether the increase in the dopamine levels induced by triadimefon was sensitive to tetrodotoxin (TTX), a blocker of voltage sensitive sodium channel and; c) compared the release dopamine profile with that of GBR 12909, a classical inhibitor of DAT. The results obtained are summarized in the Fig. 6.

Systemic administration of triadimefon 200 mg/kg induced significant increases in extracellular dopamine levels which reached 741% of basal levels. When triadimefon was administered to TTX (5 µM) pretreated rats, it had no effect on the dopamine release, i.e., TTX completely inhibited the increase in dopamine induced by triadimefon. On the other hand, Gagnaire and Micilino (2006) observed that systemic administration of amphetamine (2 mg/kg) produced a sharp increase in dopamine release which was not inhibited by intrastriatal infusion of TTX (Fig. 6). Finally, in the same study, these authors compared the dopamine release profile which that of GBR 12909. This DAT inhibitor was systemically administered (10 mg/kg) and its effects on the extracellular dopamine levels were evaluated in experimental conditions similar to those for the study of the effects of triadimefon. In the same way that the triadimefon, the acute treatment with GBR 12909 produced a gradual and sustained increase in extracellular dopamine levels which reached 356% of the control values (Gagnaire & Micilino, 2006).

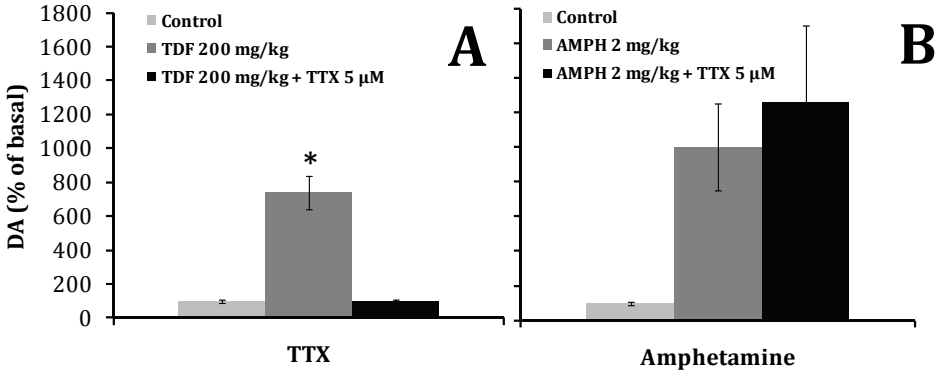

Fig. 6. Representative histograms of possible mechanism of action of triadimefon on the *in vivo* dopamine release. The samples of dopamine were obtained by *in vivo* brain microdialysis and extracellular levels were quantified by HPLC-EC. Triadimefon (200 mg/kg) or amphetamine (2 mg/kg) was administered via i.p. 90 min after the beginning of the perfusion of TTX. A) Administration of triadimefon induced a maximal increase of 741% on dopamine extracellular levels and TTX completely inhibited this increase in dopamine release. B) Administration of amphetamine induced a maximal increase of 1000% on dopamine extracellular levels; when TTX was administered in amphetamine pretreated animals the maximal increase in dopamine levels was of 1260%. There was no statistically significant difference between the two groups. The results are shown as the mean ± S.E.M., expressed as a percentage of basal levels (100%) of control group. Basal levels were considered as the mean of dopamine concentrations in the three samples before treatment administration. Significant differences were *P<0.05, respect to the basal levels (Source: Gagnaire & Micilino, 2006).

Taken together, these results by Gagnaire and Micilino (2006) show that the triadimefon-induced dopamine release from rat striatum appear to be action potential dependent since infusion of TTX completely inhibited this increase. The results also suggest that triadimefon does not act as a dopamine releaser like amphetamine and appear to act in a similar way to GBR 12909, since the two substances induced increases in dopamine release with similar profiles.

In other study Santana et al. (2009) investigated the neurochemical mechanism by what flutriafol produce increases in extracellular dopamine levels in striatum of rats. To evaluate if flutriafol-induced dopamine release was due to an increased dopamine exocytotic release and/or a change in the activity of DAT, Santana et al. (2009) investigated the effects of flutriafol under Ca^{++}-free or Na^{+}-free conditions, and after pretreatment with reserpine and TTX. The results are summarized in the Fig. 7.

The involvement of vesicular release in the increase of striatal dopamine levels induced by flutriafol was investigated by measuring the effect of coadministration of 6 mM of this fungicide, diluted in a Ca^{++} or Na^{+}-free Ringer medium, on dopamine extracellular levels. When flutriafol was perfused in either Ca^{++}-free or Na^{+}-free Ringer, the dopamine levels reduced 92% and 70%, respectively, when compared with the effect of flutriafol only (Fig. 7). These results showed that the striatal output of dopamine induced by flutriafol were Ca^{++}- and Na^{+}-dependent, although the lack of these ions in the medium did not completely block the dopamine release induced by flutriafol. Perfusion of flutriafol in TTX-treated or reserpine-pretreated animals, reduced the levels of dopamine by 73% and 86%, respectively, also compared with effect of flutriafol infusion (Fig. 7). These results seem to indicate that dopamine release evoked by flutriafol is dependent of dopamine stored in the synaptic vesicles and on depolarization mediated by voltage-sensitive sodium channels.

In another set of experiments, Santana et al. (2009) studied the involvement of the DAT in the flutriafol-induced dopamine release by measuring the effect of coadministration of 6 mM flutriafol and nomifensine on dopamine release. When nomifensine or flutriafol were administered, the dopamine release increased over 16 and 14 times with respect to the basal levels, respectively (Fig. 8). This effect of nomifensine is due to the fact that it acts as an inhibitor of the dopamine uptake (Meiergerd & Schenk, 1994; Wieczorek & Kruk, 1994), increasing the extracellular dopamine levels. When flutriafol was coinfused with nomifensine, the increase was over 32 times, showing an additive effect between flutriafol and nomifensine. This addictive effect could mean that both substances act through different mechanisms at the dopaminergic terminal: nomifensine acting on DAT and flutriafol acting on exocytotic release, corroborating the results of the other experimental groups.

In this way, we can observe that the neurochemical mechanism of action of triadimefon and flutriafol to induce *in vivo* dopamine release from rat striatum appear to be different. So, opposed to the data described for triadimefon, the results obtained by Santana et al. (2009) showed that flutriafol-induced dopamine release *in vivo* is not due to an action of this fungicide on DAT, but through an exocytotic release. Considering the difference observed in the biochemical mechanisms of action of two pesticides belonging to the same class, some comparisons between these two studies can be considered. There is a great difference in the chemical structures between the compounds. So, triadimefon might have in its structure a site able to link to DAT, thus changing the transporter activity.

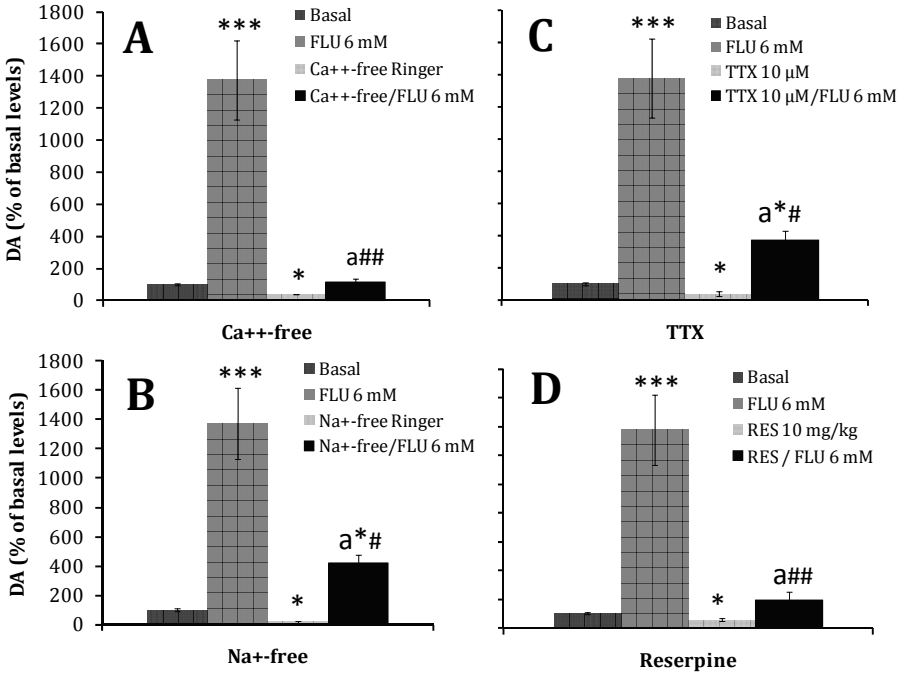

Fig. 7. Representative histograms of mechanism of action of flutriafol on the *in vivo* dopamine release. The samples of dopamine were obtained by *in vivo* brain microdialysis and extracellular levels were quantified by HPLC-EC. Intrastriatal infusion of 6 mM flutriafol during 1 h induced a maximal increase of 1376 ± 245% on dopamine extracellular levels. To determine the mechanism of action, flutriafol was administered together with TTX, reserpine (RES) or in a Na^+-free and Ca^{++}-free Ringer solution. These different treatment, administered during 1 h, produced significant decreases in dopamine release in striatum: TTX (10 µM): 37±15%; Na^+-free Ringer solution: 20±7%; Ca^{++}-free Ringer: 40±1%; and reserpine (10 mg/kg, i.p., administered 1 h before the beginning of experiment): 58±7% of decrease respect to basal levels. A) When flutriafol was infused in the Ca^{++}-free Ringer solution the maximal increase in dopamine level observed was 113 ± 25%, relative to basal levels; B) Administration of flutriafol diluted in the Na^+-free Ringer solution increased dopamine levels to 418 ± 57%, respect to basal; C) In TTX pretreated animals the administration of flutriafol induced an increase of 368 ± 63%, respect to basal and; D) Intrastriatal infusion of flutriafol in reserpine pretreated animals produced a maximal increase of 191 ± 63%, of basal. These increases observed were 92%, 70%, 73%, and 87% lower than that observed for flutriafol in non-pretreated rats, respectively. The values specified represent the maximum increases observed 30 min after the start of the flutriafol infusion. The results are shown as the mean ± S.E.M., expressed as a percentage of basal levels (100%). Basal levels were considered as the mean of dopamine concentrations in the three samples before treatment administration. Significant differences were *P<0.05, **P<0.01, and ***P<0.001, respect to the basal levels, and #P<0.05, ##P<0.01, respect to 6 mM flutriafol control group (Source: Santana et al., 2009).

Another important fact, which would explain the discrepancy between these results, is the difference in the experimental design used in both studies. Gaignaire and Micillino (2006) compared the dopamine release profile induced by i.p. administration of triadimefon with those obtained from different compounds administered into the striatum, while in study of Santana et al. (2009) all the drugs (including flutriafol) and modified mediums were administered *in situ* through microdialysis probe, according to the classically described experimental protocols for this technique.

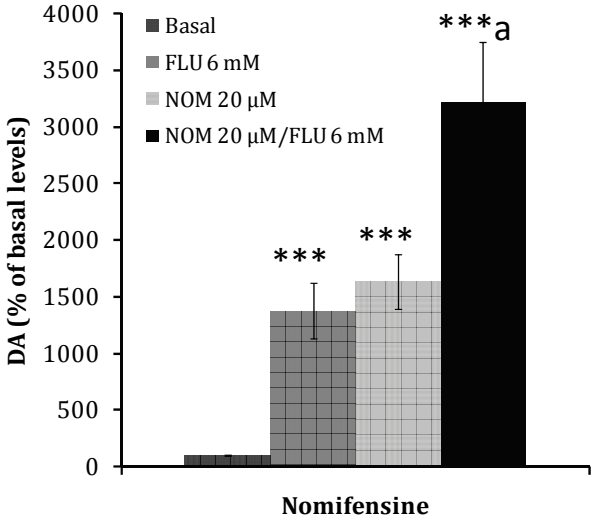

Fig. 8. Representative histograms of the effects of 6 mM flutriafol infusion in nomifensine (NOM) pretreated rats on the dopamine extracellular levels from rat striatum. The results are shown as the mean ± S.E.M., expressed as a percentage of basal levels (100%). Basal levels were considered as the mean of dopamine concentrations in the three samples under treatment administration. Significant differences were *P<0.05, **P<0.01, and ***P<0.001, respect to the basal levels and [a]P<0.05, respect to 6 mM flutriafol control group.

Additionally, Gagnaire and Micillino (2006) reported that intrastriatal administration of TTX in triadimefon i.p. pretreated animals produced an increase in dopamine release that was significantly lower than those observed when the pesticide was administered in non

pretreated animals, similar by the results obtained by Santana et al. (2009). Nevertheless, the former authors only affirm that the fungicide does not act as a stimulant of dopamine release, but it would promote an increase in extracellular dopamine levels caused by inhibition of DAT. Gagnaire and Micillino (2006) also observed that the effect produced by triadimefon resembles that one provoked by amphetamine (with a similar release profile), indicating that this fungicide would either block dopamine uptake or induce vesicular release from the storage sites, without the occurrence of a TTX-provoked inhibition.

7. Conclusion

Triadimefon and flutriafol are two economically important fungicides that belongs the class of triazole, an economically important group of fungicides largely used in agriculture to plant protection and in the treatment of fungal diseases. Because its intensive use, these compounds represent a potential risk for the contamination of environment and human populations. Despite this potential risk, there are little studies about toxicological effects of these fungicides on the biological systems of mammals. The triazole fungicide more studied is the triadimefon, being its neurotoxic effects on nervous system is well characterized. Triadimefon increases locomotion and induces stereotyped behavior in rodents; these effects are associated with increased dopamine turnover in nigrostriatal and mesolimbic brain dopamine pathways. Additionally, triadimefon exposure may generate behavioral sensitization, an effect also mediated by the dopaminergic system. More recently, was demonstrated that systemic administration of different doses of triadimefon to rats produced a gradual and sustained increase on *in vivo* dopamine release from striatum, that was dose-dependent. The effects of other triazol, the flutriafol, on *in vivo* dopamine release in rat striatum also were studied. Just as triadimefon, also flutriafol, administered *in situ*, induced concentration-dependent dopamine release from striatum. However, although triadimefon and flutriafol belong to the same chemical group, their mechanisms of action appear to be different. In the case of triadimefon, the increases in the extracellular dopamine levels possibly occur through an inhibition of DAT while flutriafol induces dopamine release via a vesicular, Ca^{++}, Na^{+} and TTX-dependent mechanism, being independent of DAT.

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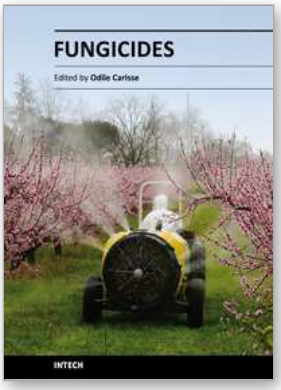

Fungicides

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Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.

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