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Photochemical and Ga_2O_3-photoassisted decomposition of the insecticide Fipronil in aqueous media with UVC radiation

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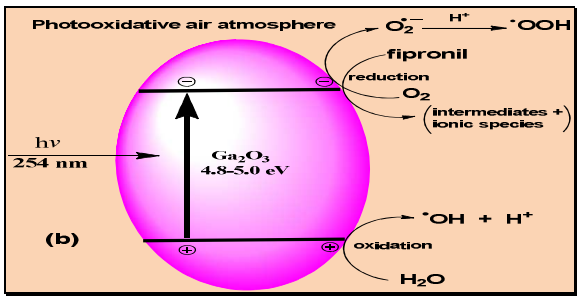
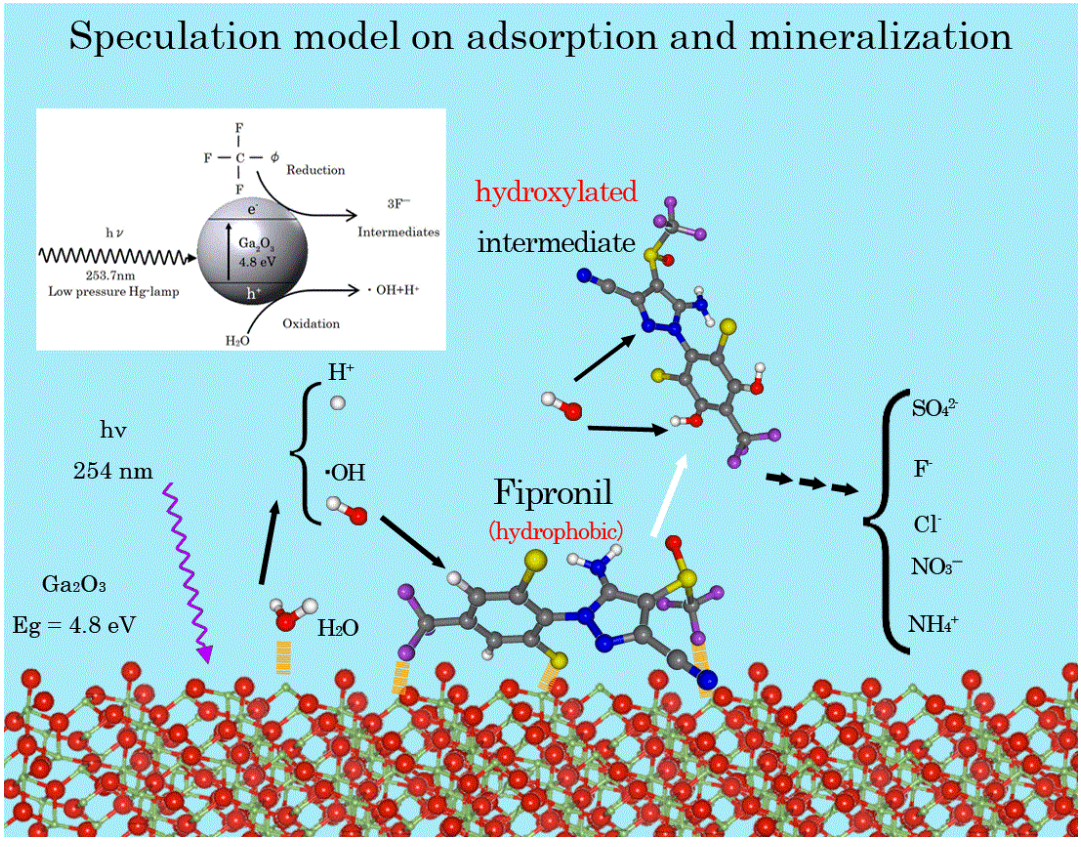


Fipronil is degraded photolytically and photocatalytically (β-Ga_2O_3 and TiO_2) in aqueous media under UVC illumination under reductive and oxidative conditions.



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Photochemical and Ga_2O_3-photoassisted decomposition of the insecticide Fipronil in aqueous media with UVC radiation

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Abstract

The insecticide Fipronil is a phenylpyrazole-based and one of the relatively new widely used generation of insecticides whose biochemical action differs from the more traditional insecticides such as the organo-phosphates and -carbamates. Although several studies have been reported to determine the fate of Fipronil under environmental field conditions, including irradiation with UVB/UVA natural or simulated sunlight (wavelength > 290–300 nm), which resulted in the formation of products that retained the basic phenylpyrazole structure, the present article examines the photodegradation of Fipronil on irradiation of aqueous solutions under both reducing (nitrogen) and oxidative (air oxygen) atmospheric conditions at 254 nm (UVC; low-pressure Hg lamp), and for comparison purposes in the presence of the wide band-gap (4.8–5.0 eV) gallium sesquioxide (β-Ga_2O_3) semiconductor (and for comparison also TiO_2). The fate of the insecticide under the such conditions was ascertained by absorption spectroscopy in the UVC spectral region, by HPLC techniques for the desulfonation, defluorination, dechlorination and formation of both nitrate and ammonium ions, in addition to ESI-TOF-MS mass spectral methods to identify some of the possible intermediates that may have formed following the breakup of the phenyl and pyrazole rings. Mass spectra indicated that within 30 min filoprin was converted to Fipronil-desulfinyl (loss of S=O fragment; photoproduct (**III**)) and in less than 1 hour of irradiation a significant quantity of the insecticide's photoproduct (**III**) had also decomposed as no significant mass peaks were seen above m/z = 319. The largest yields of SO_4^{2-} (79%), F^- (44%), Cl^- (33%), NO_3^- (34%) and NH_4^+ (15%) were obtained under air-equilibrated conditions in the presence of the metal oxide β-Ga_2O_3 (Ga_2O_3/O_2), followed by air O_2, Ga_2O_3/N_2 and N_2 atmospheric conditions. Frontier electron densities and partial charges on all the atoms of Fipronil were calculated (hf/6-31g* configuration; Gaussian 09 software) to infer a possible, albeit not detailed, pathway(s) for the direct photolysis and when the metal oxides were involved in the photodegradation. In addition, Ames tests were carried out on the intermediate products of the photodegradation of Fipronil after 3 hrs and 24 hrs of UVC illumination; none of the intermediates displayed mutagenic activity.

Keywords **:** Fipronil; Ames test; Gallium sesquioxide; Photodegradation; Insecticide

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1. Introduction

The increasing number and quantities of pesticides (a general term that also includes insecticides) used domestically and in agriculture have recently been the subject of some importance because of their often indiscriminate use and their potential for environmental contamination. Insecticides are used to control insects by either killing them or by preventing them from engaging in undesirable or destructive behaviors. They are commonly used in agricultural, public health, and industrial applications, and also find niches in household and commercial usage as for example in the control of roaches and termites. Traditionally, the most commonly used insecticides have been the organo-phosphates, -carbamates and pyrethroids. Insecticides account for about 12% of total pesticides applied to surveyed crops, with corn and cotton accounting for the largest shares of insecticide use in the United States [**1**]. Their persistence in the environment is of some concern owing to the photodegraded residues that may be even more toxic than the original insecticides [**2**]. For instance, the metabolite Fipronil-sulfone (**I**), a primary biological metabolite of insecticide Fipronil [**3**], and Fipronil-sulfide (**V**) are more toxic to fresh water invertebrates, as is the other primary environmental metabolite (photoproduct) Fipronil-desulfinyl (**III**) about 9-to-10 times more acutely toxic to mammals than the parent compound, Fipronil [**4, 5**]; their half-lives can run from a few weeks in aerobic soils, transforming Fipronil to Fipronil-sulfone (**I**), to a couple of years for Fipronil-desulfinyl (**III**) to degrade depending on the nature of the soil (**Figure 1**) [**1, 6**].

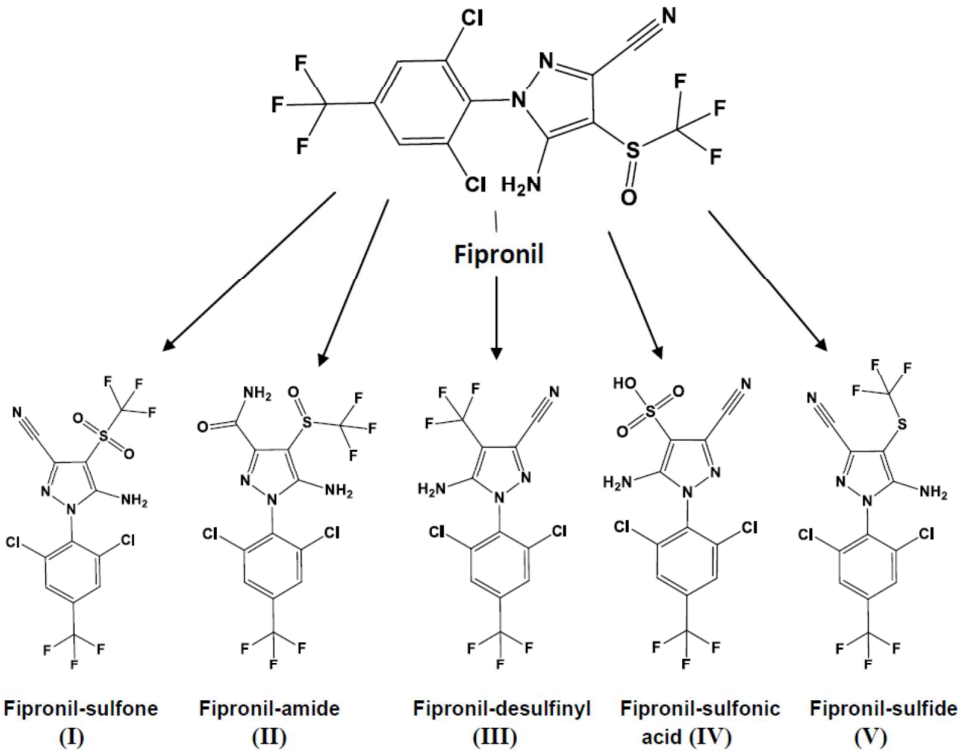

Figure 1. – Structure of the insecticide Fipronil and of its five photoproducts (metabolites) **I** to **V found in the natural environment**.

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The phenylpyrazole-based insecticide Fipronil [**3**] is a relatively new generation of insecticides as its mode of action differs from the biochemical pathways of the traditional insecticides based on phosphates and carbamates to which some insects have developed some resistance. Accordingly, it is used to control various insects; and is also used in granular turf products, seed treatments, topical pet care products, gel baits, and liquid termiticides, among others. In Japan, Fipronil is popularly used in rice nursery-box application for the control of sucking insects, nephotettix cincticeps, planthoppers, and *cnaphalocrocis medinalis* in rice production [**2**]. Fipronil and its metabolites have been detected in several water bodies in urban and agricultural areas, albeit at low concentrations throughout the U.S., with the highest recorded concentration recorded in Louisiana, while the Fipronil-sulfone (**I**), Fipronil-sulfide (**V**), Fipronil-desulfinyl (**III**), and Fipronil- amide (**II**) metabolites have been detected in Colorado, California and in Louisiana's surface waters mostly originating from agriculture [**1**]; however, domestic urban use of Fipronil is substantial, accounting for about half of all detected Fipronil and its degraded metabolites. Honey bees are particularly affected by this toxic insecticide so that it was recommended not to be applied to vegetation when bees are foraging [**7**]. Fipronil is also toxic to humans when ingested orally (self-poisoning) causing vomiting, agitation, and seizures [**8**].

Studies on different soils (loam, silt loam, clay loam, and sand) under normal environmental conditions and on irradiation with natural sunlight at wavelengths ≥ 300 nm both in the United States and Europe [**9, 10**] by the original manufacturer of Fipronil (Rhone-Poulenc) identified five principal metabolites on photolysis of the insecticide Fipronil: 5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethyl)sulfonylpyrazole (**I**), 5-amino-3-carbamoyl-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]pyrazole (**II**), 5- amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethyl)pyrazole (**III**), 5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]pyrazole-4-sulfonic acid (**IV**), and 5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)thio]pyrazole (**V**); different degradation pathways were proposed: hydrolysis, photolysis, oxidation, and reduction [**11**].These studies were later confirmed by Hainzl and Casida [**12**], who discovered that under environmental conditions Fipronil degraded photochemically (natural sunlight) to the Fipronil-desulfinyl derivative (**III**) as the major photoproduct in addition to the Fipronil-detrifluoro-methylsulfinyl, Fipronil-sulfone (**I**), and the Fipronil-sulfide (**V**) as minor products; the metabolism of Fipronil in mice yielded only the Fipronil-sulfone (**I**). Apparently, desulfinylation and detrifluoro- methylsulfinylation occurs via diradical intermediates. Bobé and co-workers [**11**] also confirmed the formation of photoproducts (**II–IV**) using a Suntest solar simulator to irradiate a methanolic aqueous dispersion containing dry soil. By contrast, the photodegradation of Fipronil on three different soil surfaces revealed only the formation of photoproduct (**III**). A later study by Ngim et al. [**13**] showed that photolysis of Fipronil in aqueous media at wavelengths ≥ 300 nm produced all five Fipronil derivatives (**I–V**) – **Figure 1**.

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In addition to the Fipronil-desulfinyl derivative (**III**), Raveton and co-workers [**14**] reported that on illumination (wavelengths, 290–350 nm) of an ethanolic aqueous media of Fipronil a large number of minor photoproducts were observed that included various substituted phenylpyrazoles and anilines subsequent to cleavage of the pyrazole ring from the Fipronil structure; the photoproduct (**III**) was relatively stable under both UV light and sunlight, with only limited changes occurring in the substitution of the aromatic ring; it accumulated to about 30–55% levels with respect to the quantity of degraded Fipronil. The authors deduced that two distinct pathways were operating in the photodegradation of Fipronil: (i) desulfuration at the 4-position of the pyrazole ring yielding the Fipronil-desulfinyl derivative (**III**) and (ii) a different modification of the 4-substituent that led to cleavage of the pyrazole ring and the formation of anilines. Germane to the present study, it is important to recognize that all the above photodegradation studies on the Fipronil insecticide and its metabolites were done either under sunlight irradiation under environmental conditions in the field, or otherwise under laboratory conditions with irradiation either by a solar simulator or a xenon or a halogen lamp, but in all cases at wavelengths ≥ 290-300 nm.

Many different types of fluorine-bearing organic products, such as pharmaceuticals, medicinals and agrochemicals have been developed extensively and utilized widely in various fields; these products display such characteristic properties as photoresistance, heat stability and, not least, chemical properties owing to the high C–F bond energy. In fact, the C–F bond is the strongest single bond and is relatively short due to its partial ionic character. As well, the bond also strengthens and shortens as more fluorine atoms are added to the same carbon atom. Together with antibiotics, many halogenated drugs are causing considerable harm to the environment as bacteria become increasingly resistant to such compounds. In this regard, pollution of aqueous ecosystems by discharged recalcitrant pharmaceuticals and medicinals, such as Fluoxetine (FLX), diclofenac, carbamazepine, 17-α-ethynylestradiol and their metabolites, are particularly significant [**15**] owing to the difficulty in cleaving the relatively strong C–F bond. Consequently, defluorination by a photoreductive process in aqueous media may be rendered somewhat difficult. Not surprisingly then to find that the C–F bond cannot be easily cleaved by activated sludge in a water purification plant. Hence such fluorinated systems tend to accumulate in rivers, lakes, and swamps. Additionally, some of these fluorinated products also tend to have endocrine disrupting capability.

Germane to the present study, the photochemical decomposition of the fluorinated substrate perfluorooctanoic acid (PFOA; often used as a model substrate to test both photocatalysts and processes) and its defluorination in homogeneous aqueous media by irradiation of the water-soluble heteropolyacid $H_3PW_{12}O_{40}$ was remarkably low, and recovery of the polyacid by separation or filtration was impracticable [**16**].The use of an insoluble metal oxide in a heterogeneous medium might facilitate the separation process and metal-oxide recovery. Our earlier attempt to defluorinate photoreductively various fluorinated substrates by UV-irradiated TiO_2 suspensions demonstrated that the process was rather inefficient (ca. 22% yield) as evidenced by the defluorination of heptafluorobutanoic acid (HFBA); the efficiency of defluorinating this acid in the

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presence of the $TiO_2/H_3PW_{12}O_{40}$ mixed system was somewhat greater (ca. 64%), albeit after 60 hrs of UV illumination [**17**]. Other attempts have been reported on the defluorination and degradation of the highly fluorinated PFOA in heterogeneous media using titanate nanotubes [**18**], TiO_2-MWCNT composites [**19**], and indium oxide [**20, 21**], as well as in homogeneous media using hydrated electrons in a sulfite-mediated UV photochemical system [**22**]; the latter showed degradation of PFOA within 1 hr of irradiation at 254 nm, although it required 24 hrs to achieve 88.5 % defluorination.

Our laboratory has been concerned for some time on the presence of recalcitrant pollutants in the aquatic environment, and thus we began a search for a process that would eliminate these pollutants. As part of our systematic studies in the latter, we recently examined the photo-degradation and defluorination of such persistent fluorinated substances as the fluorine-bearing pharmaceutical drugs Fluoxetine (FLX; Prozac) and Fluvoxamine maleate (FOM), together with several other substrates (fluorobenzoic acid and various fluoroaliphatic compounds including PFOA) by UVC illumination and assisted by the presence of the wide band gap metal oxide β-Ga_2O_3 in heterogeneous aqueous media [**23**]. Near-quantitative defluorination occurred within 3 hrs of UVC illumination for some substrates, while others required about 24 hrs to achieve same; the extent of defluorination at both times correlated with the molecular mass of the substrates – i.e., the heavier ones required more time to be defluorinated.

The present study summarizes our efforts in degrading and defluorinating the agrochemical insecticide Fipronil by a procedure we used for the fluorinated pharmaceuticals; that is, using UVC irradiation at 254 nm and the metal oxide β-Ga_2O_3(band gap, 4.8–5.0 eV) [**24–27**], and for comparison in the presence of TiO_2 under otherwise identical conditions. The choice of gallium sesquioxide rested from observations by Zhao and Zhang [**28**] who showed that it was more effective in decomposing PFOA than TiO_2 (band gap, 3.0–3.2 eV) under UV illumination, no doubt due to the position of the conduction band energy of the β-Ga_2O_3 (E_{CB} = −2.95 eV) being more positive than that of TiO_2 (E_{CB} = −4.21 eV) relative to vacuum [**24**], as well as by the observations long ago by Basov and co-workers [**29**], and reported recently by Emeline et al. [**30**], that the greater the band-gap of pristine metal oxides (up to ca. 10–12 eV) the more photoactive they were in the photoreduction of molecular oxygen and the photooxidation of methane. The photodegradation of Fipronil was followed by absorption spectroscopy and by time-of-flight mass spectrometry, while defluorination (formation of F^- ions), dechlorination (Cl^- ions), desulfonation (SO_4^{2-} ions), and transformation of the nitrogens to NO_3^- and NH_4^+ ions were followed by HPLC techniques. Samples of the Fipronil were subjected to UVC illumination at 254 nm under N_2 and air (O_2) atmospheric conditions, and with and without the presence of the metal oxide β-Ga_2O_3.

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2. Experimental

2.1. Materials

The insecticide Fipronil was 99.4% pure according to the supplier (Wako Pure Chemical Ind. Co. Ltd.), and thus was used as received. The β–form of gallium sesquioxide was also supplied by Wako Pure Chemical Ind. Co. Ltd.; it was heat-treated at 600 ^{o}C in an electric furnace prior to use. Titanium dioxide was Degssa P25. Other chemicals were reagent grade.

2.2 Photoassisted reaction setup and analytical procedures

A solution of Fipronil was made that contained 0.0437 g L^{-1} of the insecticide to give a concentration of 0.10 mM; however, to the extent that the solubility of Fipronil is 0.0019 g L^{-1} at pH 5 and 0.0024 at pH 9 [**4**], that is about 20 times more than could dissolve in water, much of the insecticide remained undissolved in the aqueous media. A tightly closed 115-mL quartz cylindrical photoreactor that contained 100 mL of the Fipronil suspension was added, when necessary, 0.050 g of β-Ga_2O_3; the suspension was irradiated externally with two low-pressure mercury lamps (Toshiba Lighting & Technology Corp, GL 20-A sterilization lamp; *ca.* 60 cm length; light irradiance 10.4 mW cm^{-2} measured at maximal emission of 254 nm; Topcon Corp. UVR-2 and UD-25 radiometers). The magnetically stirred suspension was purged with nitrogen gas at atmospheric pressure for 30 min prior to irradiation for the reductive-type experiments; otherwise the suspension was air-equilibrated. The temperature was maintained at *ca.* 35 ^{o}C by air cooling. The loading of Ga_2O_3 in the suspension was such (0.50 g L^{-1}) that insured all the photons from the UV light source (254 nm) were absorbed by the metal-oxide photomediator. Similar experiments were carried out by replacing Ga_2O_3 by TiO_2 for comparison purposes. All spectra and analyses were performed after separation of the metal oxide by centrifugation and ultimate filtration through an Advantec 0.2 μm filter. Quantitative analyses of fluoride, chloride, nitrate and sulfate anions were carried out by ion chromatography using a Tosoh Ion Chromatograph Model IC-2001, together with a TSK Gel Super IC-AZ column (i.d. 4.6 nm x 15 cm); the eluent consisted of a mixed aqueous solution of 1.1 mM Na_2CO_3 and 7.5 mM $NaHCO_3$ and the flow rate was 0.8 mL min^{-1}. The detector was a suppressed conductivity detector operating with a column maintained at 40 ^{o}C. The quantity of fluoride ions was assayed using a calibration curve determined using standard solutions of sodium fluoride supplied by Merck. By contrast, the quantity of ammonium cations was analyzed with a JASCO HPLC chromatograph. The standard column was IC Y-521 for cationic analysis and the eluent was an aqueous 4 mM HNO_3 solution. The flow rate was 1.0 mL min^{-1} and the detector was also a suppressed conductivity detector operating with a column maintained at about 40 ^{o}C. Reported yields of the ions produced are based on the theoretical expected yields for complete degradation.

The time-dependent profiles of the aromatic components in Fipronil were observed by absorption spectroscopy using a JASCO V-570 spectrophotometer, and by ESI^+ time-of-flight mass spectrometry (ESI^+/TOF-MS); the applied needle voltage for electrospray ionization (ESI^+) was 2000 V; detection voltage was 2500 V; temperature of vaporization of the degraded sample was 100 ℃ and that of orifice 1 was 80 ℃; ring lens voltage was 15 V, while that of orifice 1 was 80 V and that of orifice 2 was 1 V.

2-3 Ames test measurement

The Ames mutagenicity test was performed on the initial substrate (0.50 mM in mixed solution of 55% acetone and 45%water) and on the intermediate products subsequent to dehalogenation/decomposition using both the *Salmonella typhimurium* TA-98 strain (of the flame-shift type) and the TA-100 strain (of the base-pair substituted type) [**31–33**]. The Ames test is an experimental method to detect a chemical substance that causes possible mutations. The two strains were independently cultured by vigorous shaking the samples at 37 °C for 10 to 11 hrs to prepare the suspended solution containing a fixed concentration of each strain. After pre-incubation of the dispersion (0.10 mL) at 37 °C for 20 min, an aliquot (2 mL) of a soft agar solution was added onto a minimum glucose plate, which was subsequently incubated at 37 °C for more than 48 hrs. After incubation, the colony number of revertants was evaluated with a colony counter. The term S9 denotes a supernatant that consists of homogenized rat liver subsequent to centrifugation at 9000 rpm. Various drug-metabolizing enzymes were collected in this fraction. Since the enzyme groups needed a co-enzyme, the rat liver S9 was added to the tested aliquot (denoted S9mix); when added the term +S9mix is indicated, while the non-added one is referred to as −S9mix. Generally, a compound having a nitro group or a compound (e.g., an epoxide) attached directly to DNA yields a positive response without the S9mix, whereas a compound having an amino or an aromatic group is usually activated by addition of the S9mix.

2-4 Gaussian calculations

Frontier electron densities and charges on the atoms of Fipronil were calculated and optimized using the hf/6-31g* configuration of the Gaussian 09 software. Electron densities on each of the atoms infers the ease with which an •OH radical can attack atomic positions bearing the highest electron density in the molecule. The calculated charges on each atom are helpful in inferring which atom would most likely adsorb on the surface of the metal oxide and be a candidate for reactions with water (hydrolysis).

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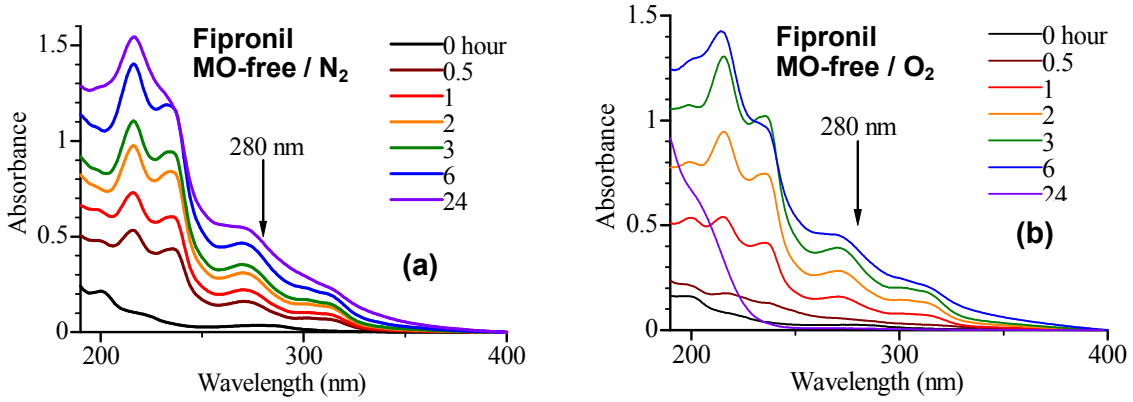
3. Results and discussion

3.1 Photoassisted and Ga_2O_3-photoassisted transformation of Fipronil

The time profiles of the spectra of solutions and suspensions of Fipronil in aqueous media in reductive (N_2) and oxidative (air O_2) atmospheric conditions and with the presence and absence of the metal oxide β-Ga_2O_3 are illustrated in **Figures 2a–2d**, whereas the time profiles (0 to 24 hrs of irradiation) of the band intensities at 280 nm, expressed as $(Abs)_t/(Abs)_o$, are displayed in **Figures 2e.** Unlike the usual decrease in the absorption band, as observed in several earlier studies, the band intensity increased with irradiation time up to 6 hrs of irradiation following which the band intensity began to decrease; after 24 hrs of irradiation the bands decreased to near-zero intensity for systems under air-equilibrated oxidative conditions in the absence (**Figure 2b**) and presence (**Figure 2d**) of the metal oxide mediator, as attested by the behavior of the 280-nm band (**Figure 2e**). Under oxidative conditions (air) but in the presence of Ga_2O_3 the band increased to its maximal value after 1 hr of UVC illumination, and within 2 hrs the band decreased to its minimal value. These observations demonstrate that oxidative conditions led to the total breakup of the phenylpyrazole skeletal structure. By contrast, under a N_2 reductive atmosphere, the decrease of the 280 nm band after 24 hrs of illumination was far less pronounced when reactions were performed in a quartz reactor with the 254 nm irradiation from the Hg light source. This rather curious spectral behavior is rationalized in terms of a photoassisted hydrolysis [**34–43**] at certain positions of the phenylpyrazole architecture that renders the initially hydrophobic Fipronil (only ca. 5% dissolved in water initially) into a hydrophilic substrate such that more of it solubilized concomitantly with its degradation – this will be treated later.



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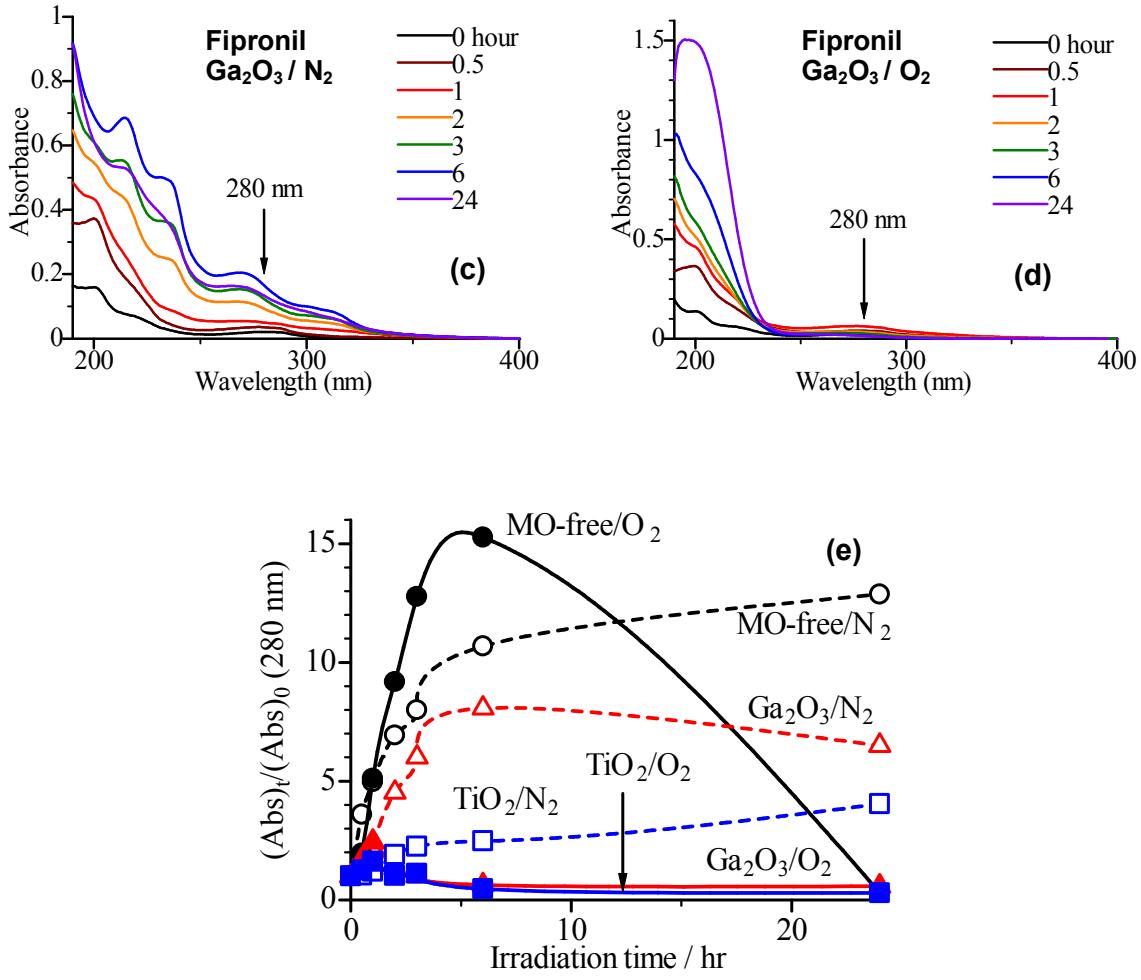

Figure 2. – (a-d) Time changes of the spectra of Fipronil in aqueous media in a metal-oxide free N_2 (a) and air O_2 (b) atmosphere; (c) and (d) show same but with added Ga_2O_3. (e) Ratio of absorbance at 280 nm at various irradiation times against the initial absorbance of 0.10 mM of Fipronil with irradiation carried out in a quartz reactor under various conditions of the photocatalysts Ga_2O_3 and TiO_2. Conditions: two low-pressure mercury lamps; light irradiance, 10.4 mW cm^{-2}; 0.050 g of β-Ga_2O_3; volume, 100 mL. Irradiation was carried out at 254 nm under nitrogen and air oxygen atmospheric conditions with and without the presence of the metal oxide.

Figure 3 summarizes the time profiles of the yields of desulfonation, defuorination, dechlorination, and deamination at different experimental conditions **(a)** Ga_2O_3/N_2, (**b**) MO-free/N_2, **(c)** Ga_2O_3/O_2 and **(d)** MO-free/O_2 for the photoassisted degradation of Fipronil carried out with the quartz reactor. Under reductive N_2 conditions the extent of desulfonation was the most significant process (**Figure 3a**) followed by defluorination, dechlorination and deamination**:** SO_4^{2-} (45 %) > F^- (32 %) > Cl^- (19 %) > NH_4^+ (7 %) ≥ NO_3^- (6 %) for the Ga_2O_3/N_2 system, whereas in the absence of the metal oxide the yields were lower (**Figure 3b**): SO_4^{2-} (33 %) > F^- (23 %) > Cl^- (15 %) > NH_4^+ (1.5 %) > NO_3^- (0 %) – no nitrates formed. Clearly, it does not appear that the Fipronil

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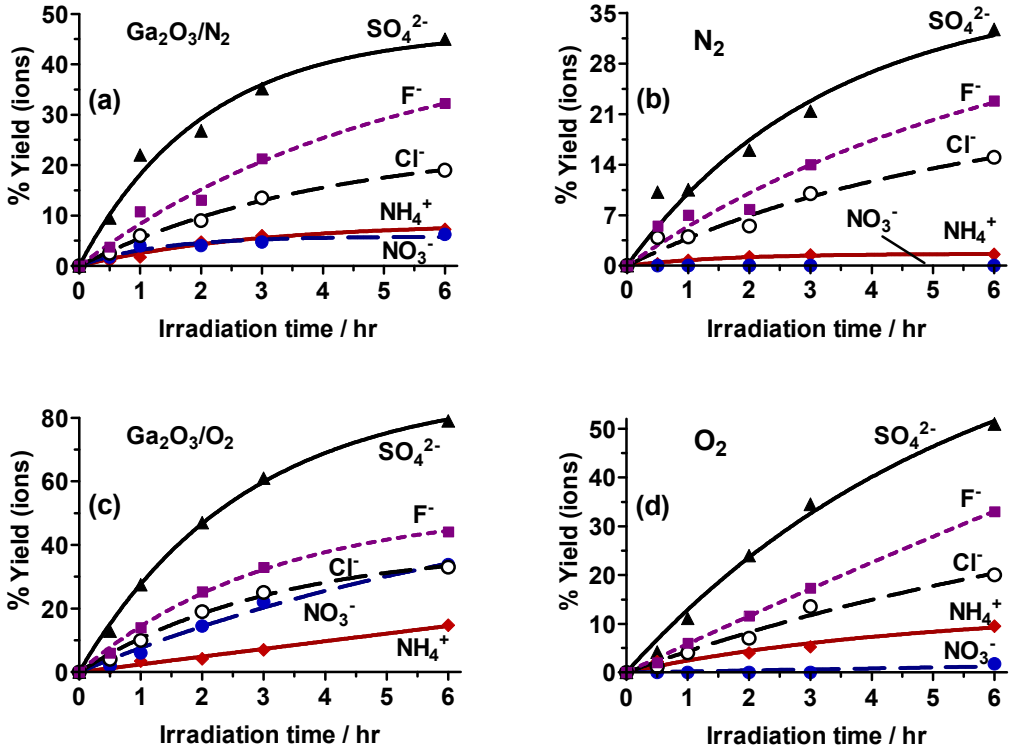
insecticide underwent very significant degradation in the absence of oxygen.

On the other hand, desulfonation, defluorination, dechlorination and deamination events were significantly greater under air-equilibrated oxidative conditions, especially in the presence of the Ga_2O_3 photomediator, as illustrated in **Figures 3c** and **3d**. The corresponding yields for the air-equilibrated system (O_2) were: SO_4^{2-} (51 %) > F^- (33 %) > Cl^- (20 %) > NH_4^+ (10 %) > NO_3^- (~2 %), whereas for the Ga_2O_3/O_2 conditions the yields were: SO_4^{2-} (79 %) > F^- (44 %) > Cl^- (33 %) ~ NO_3^- (34 %) > NH_4^+ (15%). For comparison, the ionic yields for the TiO_2/O_2 system were, after 6 hrs of UVC irradiation (**Figure 3f**): SO_4^{2-} (74 %) > F^- (38 %) ~ Cl^- (36 %) > NO_3^- (20 %) > NH_4^+ (15%) under oxidative conditions, whereas under reductive conditions (TiO_2/N_2) the corresponding yields were significantly lower (**Figure 3e**): SO_4^{2-} (32 %) > F^- (18 %) ~ Cl^- (16 %) > NH_4^+ (8.5 %) > NO_3^- (2.8 %).

The yields of sulfate, fluoride, chloride, nitrate and ammonium ions are also displayed in **Figures 4a-4e** to compare the events under oxidative (Ga_2O_3/O_2, TiO_2/O_2 and air O_2) and reductive conditions (Ga_2O_3/N_2, TiO_2/N_2 and N_2). Evidently, the greatest yields for all the ions were obtained for the oxidative conditions in which the Ga_2O_3 and TiO_2 photomediators no doubt had a significant role, while the lowest yields were obtained for the reductive N_2 atmospheric conditions (no metal oxides).



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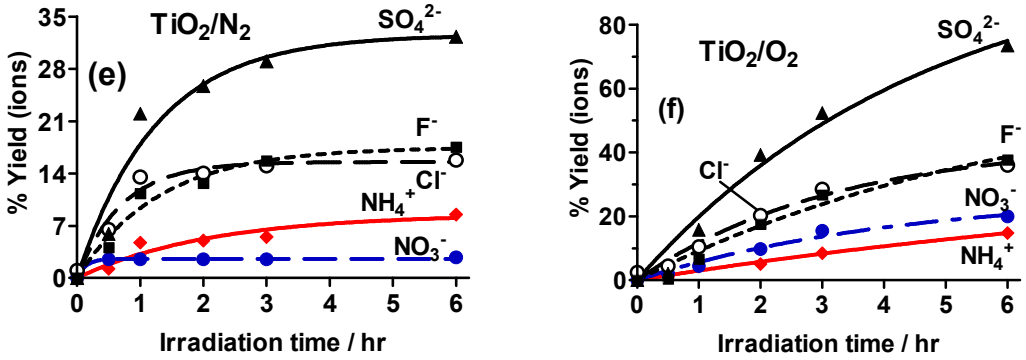
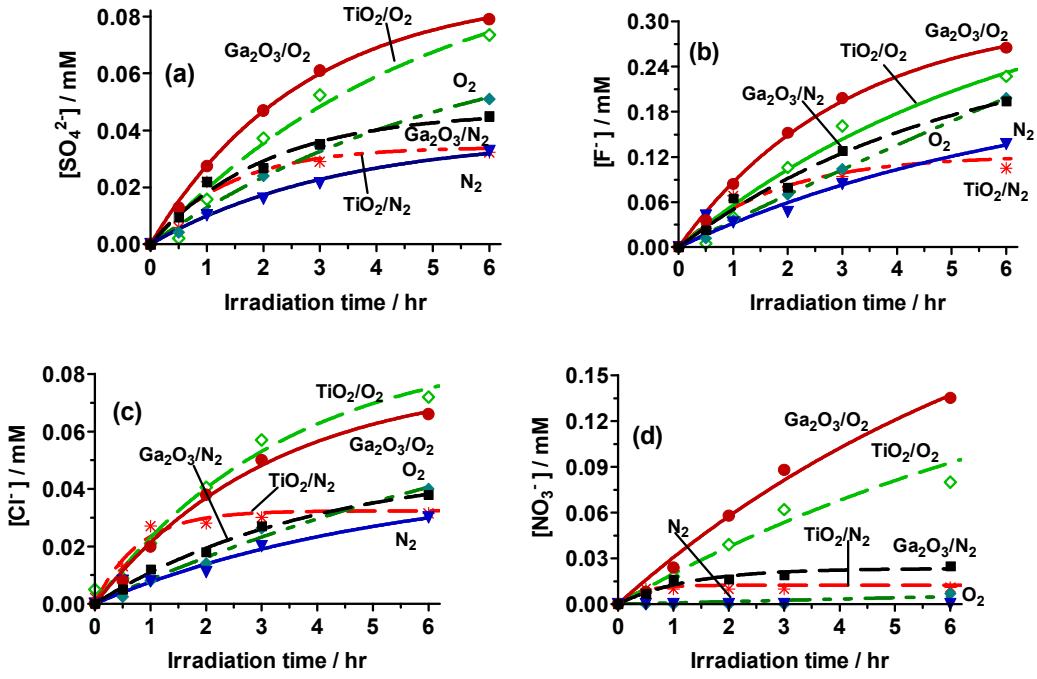


Figure 3. – Plots of mineralization yields as attested by the formation of sulfate, fluoride, chloride, nitrate and ammonium ions in aqueous media from the photochemical (**b** and **d**; no gallium oxide), Ga_2O_3-photoassisted (**a** and **c**) and TiO_2-photoassisted (**e** and **f**) transformation of the insecticide Fipronil (0.10 mM) under a nitrogen atmosphere or in air oxygen. Conditions: UVC illumination with low-pressure Hg light source; metal-oxide loading, 50 mg; volume of medium, 100 mL. Reported yields are based on the theoretically expected yields if complete degradation were achieved.

That the metal-oxide photomediators had a significant role in the photoassisted degradation of Fipronil is witnessed by the observation that the yields from the air-equilibrated conditions (no metal oxide present) and from the reductive N_2 conditions but with (at least) Ga_2O_3 present are not significantly different. Also noteworthy are the results for the nitrate ion formation displayed in **Figure 4d**, which shows that nitrate ions are formed only, or mostly only, in the presence of the metal oxides regardless of whether or not oxygen was present. The yields of sulfate, fluoride, chloride, nitrate and ammonium ions are reported in **Table 1**.



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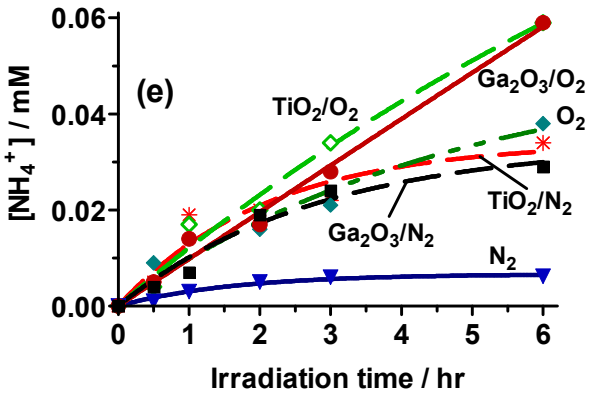


Figure 4. – Plots illustrating the formation of (a) sulfate, (b) fluoride, (c) chloride, (d) nitrate, and (e) ammonium ions in the transformation of the insecticide Fipronil (0.10 mM, 100 mL) in aqueous media under nitrogen and air oxygen conditions as well as in the presence of the metal oxides Ga_2O_3 and TiO_2.

Table 1. – Percent yields of various ions after 6 hrs of irradiation of an aqueous solution of Fipronil (0.10 mM; 100 mL) with UVC irradiation under various conditions.

Ions formed	**% Yields of various ions**					
	Ga_2O_3/N_2	**TiO_2/N_2**	**N_2**	**Ga_2O_3/O_2**	**TiO_2/O_2**	**O_2**
SO_4^{2-}	45	32	33	79	74	51
F^-	32	18	23	44	38	33
Cl^-	19	16	15	33	36	20
NO_3^-	6	2.8	--	34	20	~ 2
NH_4^+	7	8.5	1.5	15	15	10

3.2 Mass spectral analysis

We have already alluded to earlier to the active toxicity of the Fipronil insecticide and its five photoproducts under natural and simulated sunlight UVA/UVB irradiation (compounds **I** to **V**). It was therefore imperative that the intermediates that form under UVC irradiation (254 nm) also be identified, and ultimately examine their toxicity (see below). To this end, **Figure 5** displays two representative electrospray time-of-flight mass spectra at time 0 (m/z from 0 to 1000) and after 6 hrs of UVC illumination in aqueous media under O_2 oxidative conditions for mass numbers; other mass spectra are available in **Figure S1** of Supporting Information. Spectra show some significant peaks above m/z 500 for the non-illuminated suspension even though the supplier's fact sheet indicated purity to 99.4%. Nonetheless, whatever the nature of the species with m/z > 500 their presence was no longer detectable after UVC illumination for 0.5 hr (**Figure S1**).

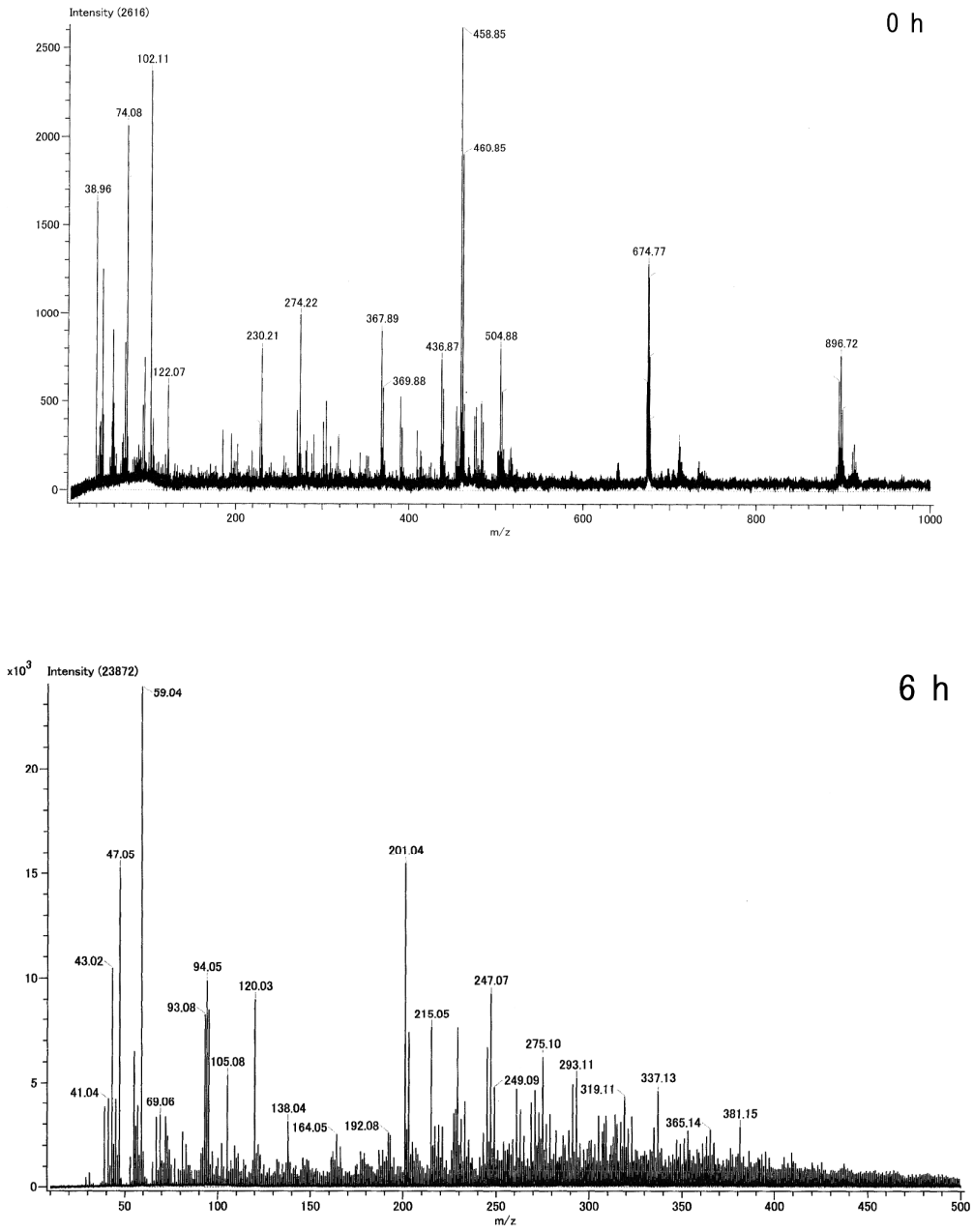
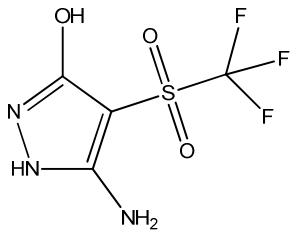


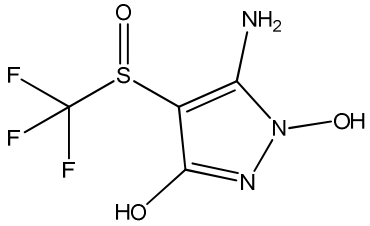
Figure 5. – Representative electrospray time-of-flight mass spectra (ESI^+/TOF-MS) of the photodegraded intermediates from the degradation of Fipronil in aqueous ethanol media under irradiation at 254 nm and under air-equilibrated oxidative conditions without the Ga_2O_3 or TiO_2 photomediators. The data for 6 hrs were assayed for the removed solution of Ga_2O_3 through filtration. Other mass spectra are reported in **Figure S1** of the Supporting Information.

The mass spectrum at time = 0 shows significant mass peaks at 459 and 461 (not identified) as well as some important peaks at m/z < 110; nevertheless, Fipronil is identified at m/z

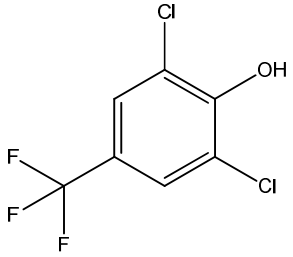
= 437 [M]) and more significant the initial mass spectrum also shows a mass peak at m/z = 455 that we identify with Fipronil-amide (compound **II**); the mass peak at m/z = 389 is attributed to Fipronil-desulfinyl (compound **III**) – see **Figure 6**. After 0.5 hr of UVC illumination, the latter mass peak (and at m/z = 391) becomes predominant indicating loss of the S=O function from Fipronil to yield (**III**) and a significantly decreased mass signal for Fipronil, together with an important mass peak at m/z = 231 which is consistent with such structures as 5-amino-4-[(trifluoromethyl)sulfonyl]-1*H*- pyrazol-3-ol (**VI;** m/z = 231 [M]), 5-amino-4-[(trifluoromethyl)-sulfinyl]-1*H*-pyrazole-1,3-diol (**VII;** m/z = 231 [M]), 2,6-dichloro-4-(trifluoromethyl)phenol (**VIII**; m/z = 231 [M]), 4-[(trifluoro- methyl)sulfonyl]-1*H*-pyrazole-1,3-diol (**IX;** m/z = 231 [M–H]), and 5-amino-4-[(trifluoromethyl)- sulfonyl]pyrazolidin-3-ol (**X;** m/z = 231 [M-H]). Moreover, the small mass peak at m/z = 215, seen after illumination for 0.5 to 6 hrs, is attributed to 1,3-dichloro-5-(trifluoromethyl)benzene (**XI**; m/z = 215 [M]) or to 5-amino-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazol-3-ol (**XII**; m/z = 215 [M]); the mass signal at m/z = 201, which becomes significant with further irradiation time to 6 hrs, is likely due to 4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazol-1-ol (**XIII**; m/z = 201 [M+H]). Not seen initially, there is a significant mass signal at m/z = 59 that becomes the most intense peak upon further irradiation to 6 hrs and was even seen after 24 hrs of illumination; we assign this peak to isothiocyanic acid (**XIV**; m/z = 59 [M]).



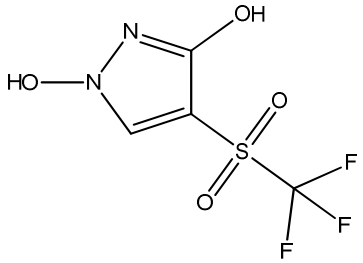
(**VI**) m/z = 231 [M]



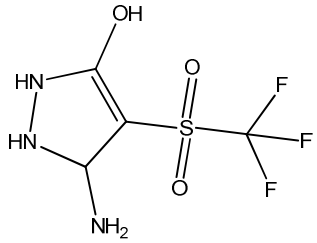
(**VII**) m/z = 231 [M]



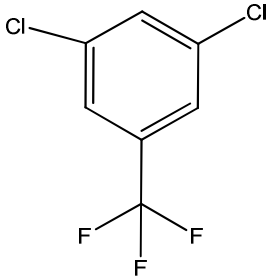
(**VIII**) m/z = 231 [M]



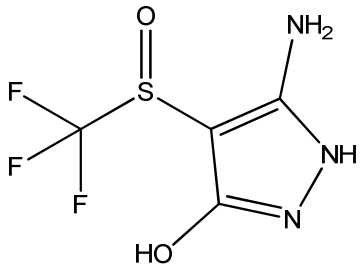
(**IX**) m/z = 231 [M-H]



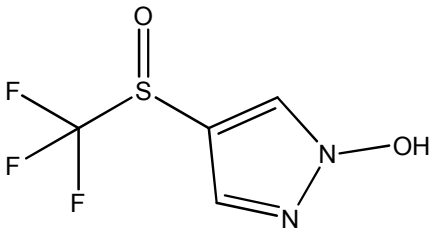
(**X**) m/z = 231 [M-H]



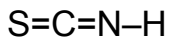
(**XI**) m/z = 215 [M]



(**XII**) m/z = 215 [M]



(**XIII**) m/z = 201 [M+H]



(**XIV**) m/z = 59 [M]

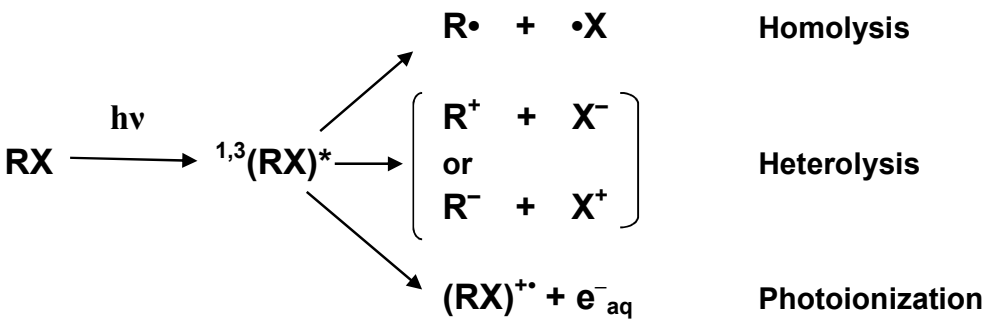
Figure 6. – Intermediates identified from electrospray TOF-MS data in the photodegradation of Fipronil in aqueous media under UVC irradiation of air-O_2 oxidative conditions.

Finally, it is worth noting that after 24 hrs of UVC illumination, the relevant mass spectrum shows but only significant peaks at m/z < 100 confirming the complete breakup of the phenyl-pyrazole skeletal structure.

3.3 Mechanistic considerations

3.3.1 Photochemically-assisted degradation – Although our scope was not to do a detailed mechanistic analysis of the photodegradation of Fipronil, it is nonetheless of interest to consider the various possibilities, albeit somewhat speculative at the stage of presently available knowledge.

Direct irradiation of the insecticide would promote the ground state to excited singlet states, which then intersystem cross to produce ultimately the lowest triplet state. Either the triplet state and/or the lowest singlet state could then undergo various processes among which are (i) homolysis, (ii) heterolysis, and (iii) photoionization [**44**] – **Scheme 1**.



Scheme 1. - Summary of consequences of the excited states of an organic compound.

Consequently, various paths open up for Fipronil to degrade by direct photolysis in aqueous media. Homolysis would yield two radicals that can react with water and oxygen and lead

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to consequent intermediates, while heterolysis would generate cationic and anionic species that could also react with water. Formation of aqueous electrons and a cation radical by a photoionization process would also lead to further consequences with their reaction with water and oxygen. Moreover, it is not unreasonable to suppose that on UVC (254-nm) irradiation in the presence of oxygen, singlet oxygen $^1O_2$ could also form [**45**] as a result of interactions between triplet oxygen, $^3O_2$, and the triplet excited state of a photosensitizer, which in the present instance would be Fipronil itself (FP) – reactions 1–4:

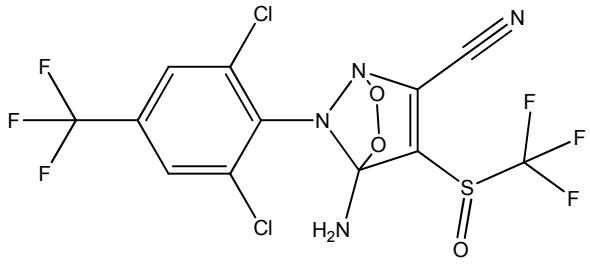
$$(FP)_o + h\nu \rightarrow {}^1(FP) \quad (1)$$

$$^1(FP) \rightarrow {}^3(FP) \quad (2)$$

$$^3(FP) + {}^3O_2 \rightarrow {}^1O_2 + (FP)_o \quad (3)$$

$$(FP)_o + {}^1O_2 \rightarrow {}^1(FP{:}O_2) \quad (4)$$

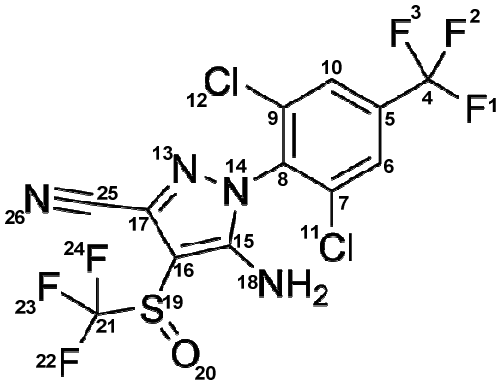
This would then mean that Fipronil would sensitize its own demise through formation of the [4+2] adduct 4-amino-7-[2,6-dichloro-4-(trifluoromethyl)phenyl]-5-[(trifluoromethyl)sulfinyl]-2,3-dioxa-1,7- diazabicyclo[2.2.1]hept-5-ene-6-carbonitrile (**XV**). Even though we have no evidence for formation of (**XV**), our data do not preclude the involvement of singlet oxygen.



(XV)

A more realistic pathway for the demise of Fipronil on irradiation at 254 nm would be the photoassisted hydrolytic process, especially in the absence of the metal oxide Ga_2O_3 and TiO_2, for which there are plenty of examples in the literature [**34–44**]. For instance, trifluoromethyl-substituted compounds are known to undergo hydrolysis, with the final product of $–CF_3$ hydrolysis being a carboxylic acid function following a release of three molar equivalents of fluoride ions into solution; presumably, nucleophilic attack by water followed by tautomerization to the acyl fluoride and ultimate hydrolysis of the acyl fluoride would yield the carboxylic acid [**34**].

Table 2. – Structure of Fipronil with numbered atoms used in the calculations of frontier electron densities and partial charges on the atoms of this insecticide (hydrogens have been omitted from the Table but were not in the calculations).



No.	Atom	Electron density	Charge	No.	Atom	Electron density	Charge
1	**F**	9.393	-0.3931	**14**	**N**	6.834	0.1662
2	**F**	9.344	-0.3438	**15**	**C**	5.523	0.4773
3	**F**	9.392	-0.3922	**16**	**C**	6.718	-0.7178
4	**C**	3.979	**2.0209**	**17**	**C**	5.252	**0.7585**
5	**C**	***7.403***	-1.4032	**18**	**N**	***7.904***	-0.9037
6	**C**	5.164	-0.1245	**19**	**S**	***14.783***	**1.2169**
7	**C**	6.080	-0.1218	**20**	**O**	8.858	-0.8577
8	**C**	6.087	-0.0949	**21**	**C**	5.087	**0.9839**
9	**C**	6.149	-0.1492	**22**	**F**	9.381	-0.3809
10	**C**	6.139	-0.1386	**23**	**F**	9.354	-0.3566
11	**Cl**	16.729	0.2707	**24**	**F**	9.352	-0.3623
12	**Cl**	16.309	0.2730	**25**	**C**	6.548	-0.5483
13	**N**	***7.234***	-0.0971	**26**	**N**	***7.369***	-0.3687

Some indications as to the positions of the hydrolytic event(s), which would take place between the atoms with the greatest positive charge and negative dipole end of the water molecule, can be obtained from the theoretical calculations of frontier electron densities and partial charges of all the atoms in Fipronil (**Table 2**). Three carbon atoms have a relatively large partial charge, not unexpectedly for the C-4 (2.02) and C-21 (0.98) carbons of the trifluoromethyl groups and the C-17 carbon (0.76) attached to the nitrile group –C≡N, together with the sulfonyl sulfur atom (S-19; 1.22); they all bear the largest positive charges. Accordingly, the negative end of the water dipole

interacts at the C-4, C-21 and S-19 positions and hydroxylates the Fipronil rendering it more hydrophilic; concomitantly, fluoride ions are released with the carbons ultimately turning into carboxylic acid functions [**34**]. The next possible hydrolytic attack could occur at carbon C-17 of the pyrazole ring bonded to the nitrile group; this could be a possible pathway for the formation of the amide group in Fipronil-amide (**II**).

3.3.2 Gallium oxide/titanium dioxide photoassisted degradation – The presence of the gallium oxide opens up other new pathways reminiscent of the reductive and oxidative events occurring on another well examined metal oxide: namely TiO_2. **Figures 7a** and **7b** display the proposed, albeit simplified occurrences taking place upon 254-nm excitation of the metal oxide, Ga_2O_3, whose band gap is 4.8–5.0 eV. Taking a clue from the behavior of titanium dioxide, irradiation of gallium oxide with 254-nm radiation would also generate •OH radicals through oxidation of water by valence band holes, and concomitantly generate photoelectrons in the conduction band. Following electron attachment to the Fipronil structure, dehalogenation would then ensue and generate fluoride and chloride ions while the •OH radicals would likely attack the Fipronil architecture at positions bearing the largest electron density, namely at S-19 leading to desulfonation (sulfate ions) and formation of the photoproduct Fipronil-desulfinyl (III) almost immediately upon irradiation (mass spectral results, **Figure 5**; m/z = 389), and at the N-18 (7.90) of the amino function yielding hydroxylamine ($HO\text{-}NH_2$) that could be oxidized to NO_3^- or reduced to ammonia (seen as NH_4^+ ions); the N-13 (7.23) nitrogen of the pyrazole ring and the N-26 (7.37) nitrogen of the nitrile function would also be potential targets for the •OH radicals.

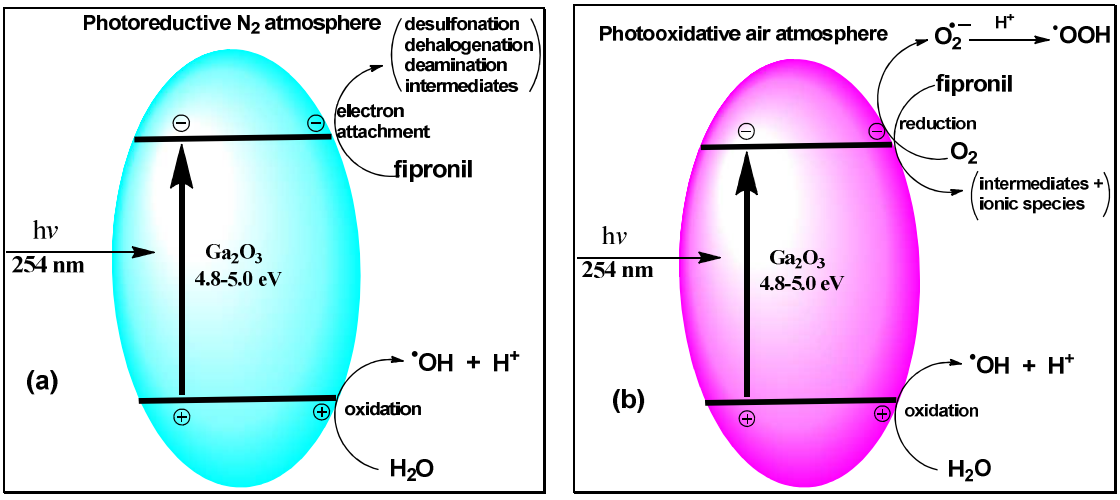


Figure 7. – Illustrations showing the simplified events occurring in the photoreductive (a) and photooxidative (b) degradatiion of Fipronil in the presence of gallium oxide illuminated with UVC at 254 nm.

The excited states of the insecticide can also undergo electron transfer from the ^{1,3}(FP) excited states to the conduction band of the gallium oxide or to some defect level within the band gap of this metal oxide, thereby forming a Fipronil radical cation (FP)$\bullet^+$ that subsequently can be attacked by water in a manner reminiscent of the self-decomposition of dyes in the presence of TiO_2 reported some time ago by Zhao and coworkers [**46**] – reactions 5 and 6.

$$^{1,3}(FP) + (Ga_2O_3) \rightarrow (FP)\bullet^+ + (Ga_2O_3)^- \quad (5)$$

$$(FP)\bullet^+ + \text{Water} \rightarrow \text{hydroxylated intermediates} \quad (6)$$

A comparison of the results reported in **Figure 3a** and **Figure 3b** that illustrate differences between the presence and absence, respectively, of Ga_2O_3 under nitrogen atmospheric conditions is revealing. Sulfate ions are formed to a greater extent in the presence of the metal oxide as are the dehalogenation and deamination processes. Nitrate ions are formed only in the presence of Ga_2O_3 indicating the possible role of •OH radicals subsequent to the activation of this metal oxide with UVC radiation; with no gallium oxide present, formation of NO_3^- ions is basically non-existent under oxygen-free conditions and even under air-equilibrated conditions (presence of O_2) – **Figure 3b** versus **Figure 3d**. Moreover, results of the desulfonation, dehalogenation and ammonium ion formation displayed in the latter two figures also reflect an involvement of oxygen and water following homolysis of the singlet/triplet excited states of Fipronil. The possible involvement of •OH radicals in the presence of the metal oxide is also suggested by the greater extent of nitrate ion formation in the Ga_2O_3/O_2 system than under Ga_2O_3/N_2 conditions (compare **Figure 3c** with **Figure 3a**) and by the greater yields of sulfate, fluoride chloride and ammonium ions.

Clearly, there is not one single pathway but several mechanistic pathways to rationalize the degradation of the Fipronil insecticide under the four different conditions used: homolysis, heterolysis and photoionization of the singlet/triplet excited states, photoassisted hydrolysis, and (although not demonstrated) the photocatalytic process when the metal oxide was present.

3.4 Mutagenicity (Ames) tests

In any photoassisted process involving environmental pollutants, it is imperative that the resulting intermediates and/or final products be no more toxic or mutagenic than the initial pollutant substrate. Studies on the mutagenic activity of products from photoassisted processes have tended to be rather scarce. Accordingly, it was imperative to carry out the Ames test to verify that the intermediates were neither toxic nor mutagenic. In the present study, the Ames test was used to assess the mutagenic activities of the agrochemical insecticide Fipronil using the two strains TA-98 and TA-100 without (−S9mix) and with the S9mix (+S9mix). In particular, the assays for Fipronil

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(0.50 mM) were performed in a mixed solution of acetone (55 %) and water (45 %).

The mutagenic activity of intermediates and products that resulted from the photodegradation of the Fipronil insecticide was also assayed with both TA-98 and TA-100 strains under the conditions of −S9mix and +S9mix; results are displayed in **Figure 8**. Although the background level of each assay varied between 17 and 27 revertants, we confirmed that the number of revertant colonies increased linearly with increasing concentration of the positive controls 4-nitroquinoline-N-oxide and benzo[a]pyrene under identical experimental conditions. These findings indicate the validity of the assay system.

The validity of the assay system was also confirmed by the dose dependency of the number of revertant colonies obtained with the positive control employed, 4-nitroquinoline-N-oxide. In the other assays, repeated colony selection resulted in reducing the background level with enhanced sensitivity. Since the background of –S9mix for the TA-100 strain commonly showed a background level of around 151 (**Figure 8c**), no problems were encountered under these conditions, although the mutagenicity test data were slightly lower.

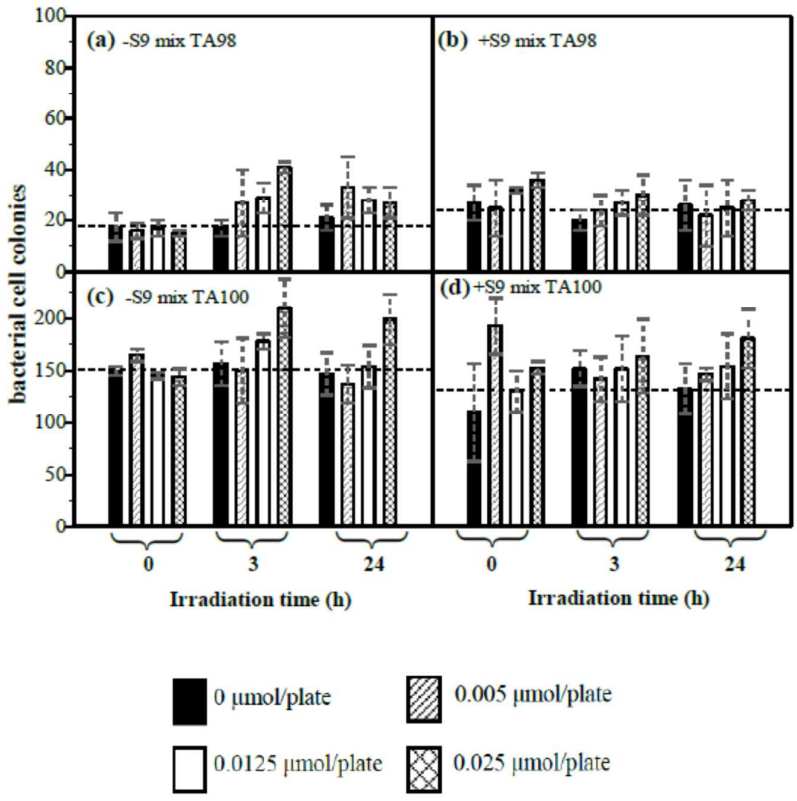

Figure 8. - Mutagenic activities of TA-98 and TA-100 strains in the photodegraded solution of Fipronil (0.50 mM) in a mixed solution of acetone (55%) and water (45%) by β-Ga_2O_3 particles. For each irradiation time of 0, 3 and 24 hrs, three or four bars are shown to indicate the loading of 0, 0.005, 0.0125 and 0.025 µmol/plate. The dotted line means the average level of each background.

While the data of the –S9mix with the TA-98 strain after 3 hrs of illumination exhibited nearly twice the number of bacterial cell colonies than the initial ca. 18 (**Figure 8a**), a fluctuation in the number of bacterial cell colonies around 20 would generally be taken as being experimental error. When the TA-98 strain was employed, the background level (+S9mix) was 24 (**Figure 8b**). The number of revertant colonies of 40 would then be taken as being relatively small in this test. However, if the number were 100 to 200, then mutagenicity would undoubtedly be recognized as there being no mutagenic activity.

Mutagenic activities for the TA-98 and TA-100 strains were also assessed for the photodegraded solution of Fipronil (0.50 mM) in the presence of β-Ga_2O_3 particles (**Figures 8**). For each irradiation time of 0, 3 and 24 hrs, three or four bars are shown to indicate the loading of 0, 0.005, 0.0125, and 0.025 μmol per plate. Experiments were carried out as follows: after incubating the strain solution (100 μL) overnight, it was mixed with a phosphate buffer (500 μL) and various solution loadings (10, 25, and 50 μL). An increase of the sample concentration showed no enhancement of mutagenic activity compared to the background level.

We also discovered that the cell toxicity for the starting reagent for the TA-100 strain was stronger at the higher concentration. The bacterial cell colonies of the TA-98 strain (background level, 18; **Figure 8a**) increased slightly with increasing irradiation time on the standard base of the initial sample prior to irradiation. No mutagenic activity of the photodegraded Fipronil solutions was evidenced, regardless of the 10, 25 and 50 μL loadings. When the TA-100 strain was employed, the background level (+S9mix) was 132 (**Figure 8d**). The number of bacterial cell colonies for the initial solution hovered around this value in the case of the 25 μL loading. After various UV illumination times, the number of colonies was relatively low in comparison with the number in the initial solution. The number of colonies tended to increase gradually with increasing irradiation time. However, the illuminated solution still showed a relatively low value. Consequently, we deduce that there was no mutagenic activity of the intermediates and products from the photodegradation of Fipronil, even after relatively long UVC illumination times.

4. Concluding remarks

Earlier studies on the fate of the insecticide Fipronil in a natural or simulated natural environment had shown [**1–6, 9–13**] that it decomposed into no less than five photoproducts or metabolites (**I–V; Figure 1**), perhaps even more toxic than the original insecticide**.** By contrast, the present study has demonstrated that the photodegradation of Fipronil can be achieved under nitrogen-purged and air-equilibrated conditions in aqueous media both photochemically in the

absence of a metal oxide, and photocatalytically in the presence of wide band gap metal oxides such as β-Ga_2O_3 and TiO_2, also in N_2-purged and air-equilibrated conditions, albeit using UVC irradiation (254 nm) from a low-pressure mercury light source. Desulfonation, defluorination, dechlorination and deamination processes occur in relatively short time, as evidenced by the formation of SO_4^{2-}, F^-, Cl^-, NO_3^- and NH_4^+ ions, although in some cases no nitrate ions formed. As expected, the extent of formation of these ions from the photochemical (direct photolysis) degradation of Fipronil was significantly greater under air-equilibrated (oxidative) conditions relative to the extent under reductive conditions (nitrogen-purged solutions). Under reductive conditions but with the TiO_2 photocatalyst present (TiO_2/N_2 case – see **Table 1**), the extent of formation of the ions was not different from the direct photolysis of Fipronil, in contrast to the case where the Ga_2O_3 was the photocatalyst present in the suspension (Ga_2O_3/N_2 case) in that the extent of formation relative to TiO_2/N_2 was some 41% and 78% greater for desulfonation and defluorination, respectively. By comparison, under oxidative conditions the extent of desulfonation, defluorination and dechlorination with both Ga_2O_3 and TiO_2 photocatalysts occurred to nearly the same extent, except for denitrification of the insecticide (formation of NO_3^- and NH_4^+ ions) wherein the wider band gap Ga_2O_3 photocatalyst proved far superior by nearly a factor of 2.5. Evidently, the gallium sesquioxide is to be preferred albeit it necessitates UVC radiation to activate it, unlike TiO_2 that can also be photoactivated by UVB/UVA radiation. We hasten to note, however, that the above comparisons should be taken *cum grano salis* as a more quantitatyive comparison would require a determination of the number of photons absorbed by the two photocatalyst – this was outside the scope of the present investigation.

Under the presently available data and current knowledge, it is proposed that the degradation likely occurred by several complex pathways that could involve homolysis, heterolysis and photoionization from the singlet/triplet excited states of Fipronil, as well as by photoassisted hydrolysis, and photocatalytically in the presence of either gallium sesquioxide or titanium dioxide nanoparticulates. A possible role of singlet oxygen originating from excited Fipronil could not be precluded by our data. Mass spectral data revealed the formation of Fipronil-desulfinyl and Fipronil-amide photoproducts in fairly short time (ca. 30 min) together with an intermediate with m/z = 59 that we have identified with isothiocyanic acid. The Ames test of the initial insecticide and the intermediates revealed no mutagenic activity of the substrates.

Further studies should focus on detailed examinations of the five metabolites, particularly those that are most toxic, identify possible intermediates and carry out more detailed toxicological tests other than the Ames test.

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