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Photochemistry and Photoinduced Toxicity of Acifluorfen, a Diphenyl-Ether Herbicide

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ABSTRACT

Photochemistry studies can be helpful in assessing the environmental fate of chemicals. Photochemical reactions lead to the formation of by-products that can exhibit different toxicological properties from the original compound. For this reason the photochemical behavior of the herbicide acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) in the presence of different solvents was studied. Photochemical reactions were carried out using a high-pressure mercury arc and a solar simulator. Kinetic parameters and quantum yields were determined. The identification of photoproducts was performed by mass spectrometry and [¹H] nuclear magnetic resonance (NMR). Nitrofluorfen, hydroxy-nitrofluorfen, 2-chloro-4-(trifluoromethyl)-phenol, 5-trifluoromethyl-5'-nitrodibenzofuran, and other derivatives were identified. The photochemical reactions were also carried out in the presence of either a singlet or a triplet quencher, and in the presence of either a radical initiator or a radical inhibitor. Substances used as inhibitors of the excited levels T₁ and S₁ showed that photodegradation of acifluorfen begins from a singlet state S₁ through a π,π^* transition. The role of free radicals in the photodegradation of acifluorfen was determined and a radical mechanism was proposed. Toxicity tests against *Daphnia magna* Strauss showed that acifluorfen was not toxic at a concentration of 0.1 mM; however, photoproducts formed after 36 h of UV exposure of the herbicide induced a remarkable toxicity to the test organism.

DEGRADATION and transformation reactions of herbicides lead to the formation of by-products that exhibit, in some cases, toxicological properties that are very different from the original compound (Kirkwood, 1986; Scheunert, 1992). With increasing concern and awareness of the fate and effects of these chemicals in the environment, it is important to develop sensitive methods that can characterize the presence and toxicity of residues or reaction by-products at trace levels. This challenge is most evident in the detection of water-soluble and polar compounds, and their degradation products.

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Diphenyl-ethers are highly active herbicides used for the selective control of broadleaf weeds in large-seeded legume crops. Acifluorfen 1, as illustrated in Scheme 1 (Fig. 1), is a nitro-diphenyl ether herbicide (Johnson et al., 1978), acting as protoporphyrinogen oxidase inhibitor, and is used to control monocotyledonous and broad-leaved weeds at rates in the range of 0.38 to 0.6 kg of active ingredient (a.i.) ha⁻¹. Solubility in water is 120 mg L⁻¹. It has acute oral toxicological effects on mammals with oral LD₅₀ values between 1370 and 2050 mg kg⁻¹ in rats, an acute percutaneous LD₅₀ value of 3680 mg kg⁻¹ for rabbit, and moderate fish toxicity between 17 and 61 mg L⁻¹ (Worthing and Hance, 1994).

Herbicides in this class require light to exhibit phytotoxic activity (Fadayomi and Warren, 1976; Gillham and Dodge, 1987; Vanstone and Stobbe, 1979) and under irradiation they can give rise to several photodegradation products (Scrano et al., 1999). However, the nature of the light-activated mechanism is still unknown.

Photochemical reactions of substituted diphenyl ethers in liquid phases have received attention (Nakagawa and Crosby, 1974; Ruzo et al., 1980), but no information is available on the photochemical behavior of acifluorfen and its photoinduced toxicity. It is only known that in aqueous solutions acifluorfen degraded following first-order kinetics, with loss of the carboxylic acid group (Pusino and Gessa, 1991). Diphenyl ether, 1,4-diphenoxybenzene, and some substituted diaryl ethers (methyl and/or methoxy derivatives) have been irradiated using unfiltered UV light from a high-pressure mercury lamp at 25°C. The reactions observed were cleavage of the ether bond(s), followed by H-abstraction from the solvent yielding phenols and benzene derivatives, and a photo-Claisen type rearrangement yielding 2- and/or 4-hydroxybiphenyl derivatives (Hageman et al., 1970). Another experiment showed that diphenyl ether was converted into *o*-phenylphenol, *p*-phenylphenol, and a small amount of phenol, by UV light in various solvents. *p,p'*-Ditolyl ether was similarly converted into 2-(*p*-tolyl)-4-methylphenol and *p*-cresol, indicating that the photochemical rearrangement proceeds via C–O bond cleavage and recombination of the radical (quinoid) fragments. These reactions were reported as intramolecular and occurred via an excited singlet state or via a short-lived triplet (Ogata et al., 1970).

Sunlight photolysis of the herbicide nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) in aqueous methanol was represented as a photonucleophilic displacement of nitrophenate by the hydroxide ion of water, giving rise to 2,4-dichlorophenol and *p*-nitrophenol. Oxygen did not influence the formation of derivative photoproducts (Nakagawa and Crosby, 1974).

Other substituted diphenyl ethers were studied under 300 nm irradiation in water, cyclohexane, and methanol solutions. The major reaction pathways involved reductive dehalogenation, decarboxymethylation, reduction of nitro substituents, and cleavage of the ether linkage to yield phenols. Nucleophilic substitution by the solvent was also observed (Ruzo et al., 1980).

Photodegradation of 3-phenoxybenzoic acid was also investigated under xenon lamp irradiation ($\lambda > 290$ nm) in aqueous phosphate buffer at pH 7. In these conditions hydroxylation of both phenyl rings and ether cleavage were the main degradation pathways, and ether cleavage proceeded via reaction with a hydroxyl radical excluding the photonucleophilic substitution by water molecule (Katagi, 1992).

The photochemistry of diphenyl ethers is relevant to the dependence of their activity on light absorption. Our objectives were to determine the photochemical behavior of acifluorfen in various solvents and the photoinduced toxicity arising under UV irradiation. Data obtained in this study will be useful in understanding the environmental behavior of this herbicide.

MATERIALS AND METHODS

Photophysical and Photochemical Experiments

All solvents (pesticide grade), reagents (analytical grade), and filters (disposable sterilized packet) were purchased from Fluka and Sigma-Aldrich (Milan, Italy). Ultrapure water was obtained with a Milli-Q system (Millipore, Bedford, MA). Acifluorfen (CAS RN [50594-66-6]), pure standard (98%, 361.7 MW), was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Ultraviolet spectra were recorded using a Uvikon 930 spectrophotometer (Kontron Instruments, Milan, Italy). Fluorescence and phosphorescence spectra were obtained with a Jobin Yvon (Opera-Milan, Italy) 3D spectrofluorimeter. The fluorescence spectrum was recorded in anhydrous acetonitrile using diphenyl-anthracene (DPA) as the actinometer. The phosphorescence spectrum was recorded, under liquid nitrogen, in the mixture of ethylether, 2-methylbutane, and ethanol (5:5:2) (EPA), using acetophenone as the actinometer.

The photodegradation of acifluorfen was studied for 140 h in a solar simulator (Suntest CPS+; Heraeus, Hanau, Germany) furnished with a 1.1 kW xenon arc (protected with a quartz plate; total passing wavelength: 300 nm $< \lambda < 800$ nm). The irradiation chamber was maintained at 25°C using both the circulating water from a thermostatic bath and a conditioned airflow. Another experiment was carried out for 50 h by use of a Pyrex reactor (250 mL) (Steroglass, Perugia, Italy) equipped with a high pressure mercury arc (Philips HPK 125 W; total passing wavelength: 240 nm $< \lambda < 580$ nm; Philips Lighting, Monza-MI, Italy) protected with a quartz thimble and surrounded by a water jacket to maintain temperature at 25°C.

Solutions (0.1 mM) of the herbicide in water, acetonitrile,

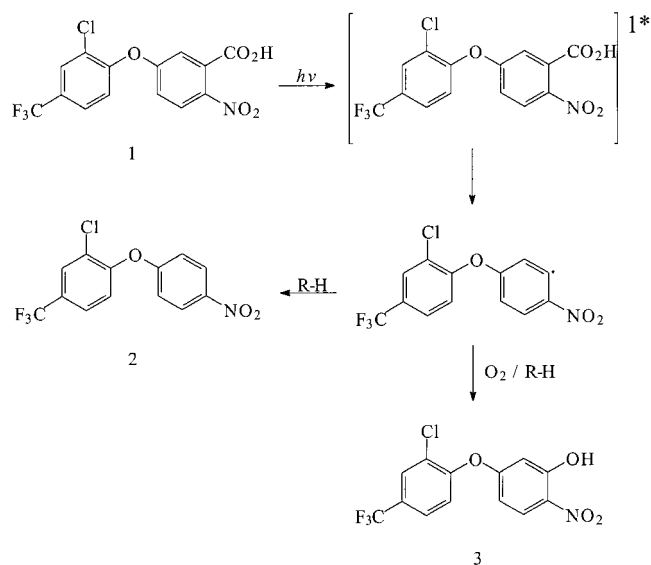


Fig. 1. Illustration of Scheme 1, showing the mechanism of acifluorfen photodegradation (20% transformed).

methanol, and *n*-hexane were used in the degradation tests. Aqueous solutions were prepared using pH 7 phosphate buffers with an ionic strength of 0.05 M. All glassware was sterilized by autoclaving for 60 min at 121°C before use. All solvents and solutions were filtered using sterilized ultrafilters (0.2 μ m). Aseptic handling materials and laboratory facilities were used throughout the study to maintain sterility. All working solutions were divided into two parts. One of these portions was used for the irradiation experiment, the other as a control, which was kept in the dark. All reactions were performed in triplicate in the atmospheric environment. In the experiments carried out under xenon lamp irradiation, quantum yields (Guittonneau et al., 1995) were also determined using uranyl oxalate as the actinometer (Murov et al., 1993).

In order to understand the mechanism of the photochemical degradation of acifluorfen the following experiments were performed with the solar simulator system (Suntest):

(i) Photodegradation of Compound 1 (0.10 mM in acetonitrile) either in the presence of naphthalene (1 mM), as a singlet quencher, or in the presence of biphenyl (0.25, 0.5, and 1 mM), as a triplet quencher;

(ii) Photodegradation of Compound 1 (0.10 mM in acetonitrile) either in the presence of 2,2'-azobisisobutyronitrile (AIBN, 1 mM), as a radical initiator, or in the presence of benzoquinone (1 mM), as a radical inhibitor;

(iii) Photodegradation of Compound 1 (0.10 mM in acetonitrile) without the presence of oxygen, by using argon to remove air from the reaction vessel.

A separate experiment was carried out under mercury arc irradiation for the identification of photoproducts. To avoid the formation of low quantities of many by-products, which could be a hindrance for understanding the degradation pathway, the reaction was stopped after 2 h of irradiation, when 20% of starting compound was converted, and after 36 h, when the reaction could be considered at completion (98% acifluorfen degradation).

Analytical Procedures

The depletion with time of Compound 1 was monitored via high performance liquid chromatography (HPLC) using a HP 1090 instrument (Hewlett-Packard, Palo Alto, CA) equipped

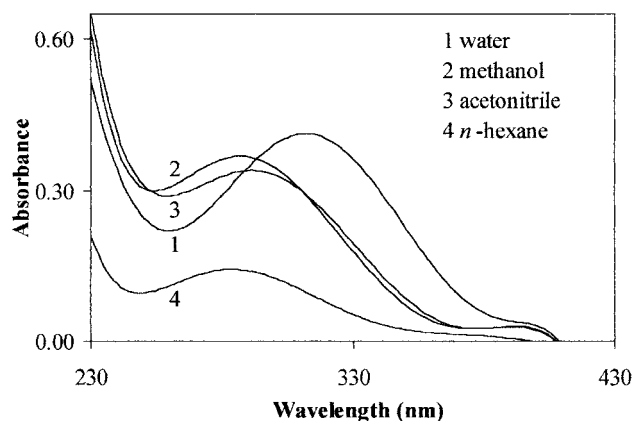


Fig. 2. Ultraviolet spectra of acifluorfen (0.1 mM) in different solvents.

with a diode array detector. A C18 PerkinElmer (Wellesley, MA) HS-5HCODS column (250 mm long, 4.6 mm i.d.), 5- μ m particle size, was used at a 0.6 mL min^{-1} flow rate. An acetonitrile and water (7:3 [v/v]; H_3PO_4 , pH = 3) mixture was used as the mobile phase. The retention time of acifluorfen was 5.84 min.

For the identification of photoproducts, a HP 5971 mass selective detector on a HP 5890 gas chromatograph was used (OV-1 capillary column between 70 and 250°C [$12^\circ\text{C min}^{-1}$]). Further analyses were achieved on a Finnigan SSQ 7000 mass spectrometer (ThermoFinnigan, Bremen, Germany), coupled with another HP 5890 chromatograph (DB 5-MS capillary column between 90 and 300°C [$10^\circ\text{C min}^{-1}$]).

The injection of pure Compound 1 in the gas chromatography-mass spectrometer (GC-MS) gave Compound 2, as illustrated in Scheme 1 (Fig. 1), as the unique detectable product because of the thermal degradation of acifluorfen in both the gas chromatographic systems. For this reason, flow injection technique (FIA) coupled with electrospray ionization (ESI) on quadrupole ion trap (QIT) mass spectrometer (Finnigan LCQ) was also used to characterize the reaction mixture obtained either at 2 or 36 h of irradiation. Standard or sample solutions (1 μL) were injected at a flow rate of 200 $\mu\text{L min}^{-1}$ using a solvent system of acetonitrile and water (1:1 [v/v]; 5 mM NH_4OAc , 1% formic acid). The heated inlet capillary was maintained at 130°C and nitrogen was employed as both the sheath and auxiliary gas (10 L min^{-1}). Electrospray ionization was performed in the negative and positive ion modes and a typical ESI voltage was 1.3 kV. Under these conditions no thermal decomposition of the pure standard acifluorfen was observed.

The reaction mixture was extracted with ethyl acetate, concentrated under a mild vacuum in a rotary evaporator, and nitrogen fluxed. Separation of photoproducts was achieved by thin layer chromatography (TLC) on 60F₂₅₄ plates (Merck Eurolab, Milan, Italy) using a solvent system of acetonitrile, chloroform, and methanol (30:5:65 [v/v/v]). Recovered products were analyzed by [^1H] NMR spectroscopy. A Bruker (Rheinstetten, Germany) 300 AM instrument was used and the solvent was CDCl_3 .

Daphnia magna Test

Toxicity tests on *D. magna* were performed in triplicate following OECD Guideline 202 for testing of chemicals (Organisation for Economic Co-operation and Development, 1993). A *D. magna* Strauss monoclonal culture was used. The vitality of the test population used in each test was determined by a reference toxicant test with $\text{K}_2\text{Cr}_2\text{O}_7$. The endpoint for effect

Table 1. UV absorption maxima and extinction coefficients of acifluorfen in different solvents.

Solvent	λ nm	ϵ $\text{L mol}^{-1} \text{cm}^{-1}$
<i>n</i> -Hexane	274	4990
Methanol	287	8078
Acetonitrile	288	7988
Water	310	4771

determination was immobility after 24 h, which was used for calculation of the 24-h EC_{50} (effective concentration 50%, i.e., the concentration at which 50% of examined *Daphnia* were immobilized). An organism was defined to be immobile if it was not able to swim within 15 sec after gentle stirring. Probit analysis was used for the calculation of EC_{50} and 95% confidence limits.

The pure active ingredient of the herbicide (50 mg L^{-1}) was dissolved in a buffer solution (pH 7). The test was performed in 20 mL of water saturated with oxygen. In each of the 20-mL flasks, 20 individuals not older than 24 h were used. The same experiment was carried out on the herbicide solutions after 2 h (20% acifluorfen degraded) and 36 h (98% of acifluorfen degraded) of irradiation, respectively. The test was carried out in the dark at 20°C. The water fleas were not fed during the test. The test compounds were by visual examination completely dissolved at the different test concentrations. A pre-test was necessary to choose the dilution range in the main test.

RESULTS AND DISCUSSION

Photophysical Properties

The UV spectra of Compound 1 in different solvents (methanol, acetonitrile, water, and *n*-hexane) are illustrated in Fig. 2. Each spectrum exhibits an absorption in the range of 274 to 310 nm, which could be assigned to $\pi \rightarrow \pi^*$ transitions. Table 1 summarizes the absorption maxima and the corresponding extinction coefficients evaluated by the Lambert-Beer law. Acifluorfen shows a low emission band in acetonitrile at 300 nm ($\lambda_{\text{exc}} = 288$ nm) in the fluorescence spectrum (Fig. 3), and an emission band at 410 nm in the phosphorescence spectrum with $\Phi_{\text{ph}} = 1.5 \times 10^{-3}$ (Fig. 4). These data allow the calculation of the energy of the first excited singlet state at 402.9 kJ mol^{-1} ($\tau = 8.61 \times 10^{-9}$ s) and the energy of the lowest excited triplet state at 291.8 kJ mol^{-1} . These values are compatible with the excited

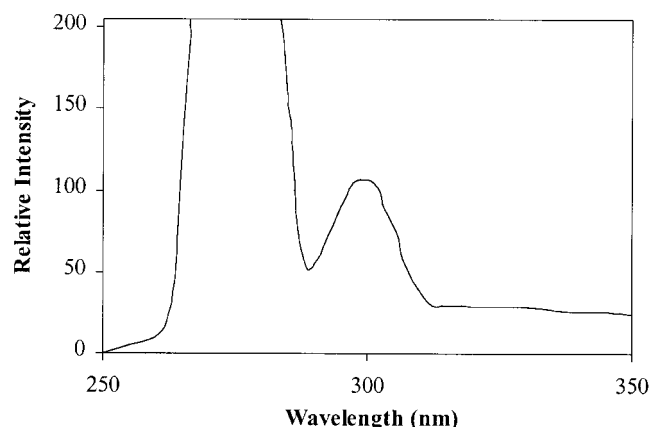


Fig. 3. Fluorescence spectrum of acifluorfen (0.1 mM in acetonitrile vs. diphenyl-anthracene [DPA] as the actinometer); $\lambda_{\text{exc}} = 288$ nm, $\lambda_{\text{em}} = 300$ nm.

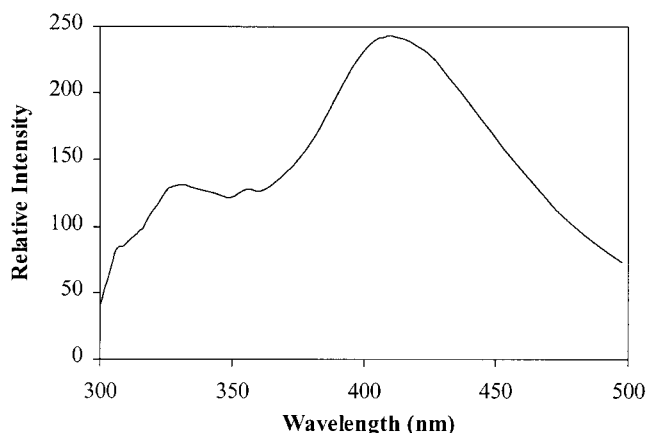


Fig. 4. Phosphorescence emission of acifluorfen (0.1 mM in the mixture of ethylether, 2-methylbutane, and ethanol [5:5:2] [EPA] vs. acetophenone as the actinometer); $\lambda_{exc} = 290$ nm, $\lambda_{em} = 410$ nm.

electron transfer from the higher level to the lower one (the direct transfer from the ground state to the triplet state is forbidden).

Photodegradation Kinetics

In the dark, no significant reaction of acifluorfen was observed in the solvents used for the experiments. Figures 5 and 6 show the degradation curves of Compound 1 obtained in the above solvents under xenon and UV mercury arc irradiation, respectively. The kinetic parameters of the degradation reactions of acifluorfen are shown in Table 2. All the reactions fit a first-order kinetic relationship. The quantum yields under Suntest irradiation (xenon arc) ranged from 1.23×10^{-4} to 3.60×10^{-4} for polar solvents and increased to 1.70×10^{-2} in the nonpolar solvent. The observed behavior can be explained considering the different solubility of oxygen in the solvent we used (see the Mechanism of Photodegradation section, below, for the effect of oxygen). In fact, solubility at 1 atm O_2 in acetonitrile, methanol, and *n*-hexane is reported to be 9.1, 10.3, and 15×10^{-3} mol L^{-1} , respectively, while in water it is 1.39×10^{-3} mol L^{-1} (Murov et al., 1993). The observed increments of reaction rate are in agreement with O_2 solubility data with the exception of water. However, in this case, we have to consider another effect. Table 1 shows a bathochromic shift of maximum absorption wavelength with solvent, but the differences among *n*-hexane, meth-

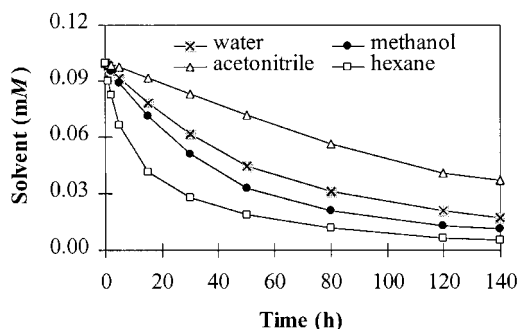


Fig. 5. Degradation curves of acifluorfen (0.1 mM) in different solvents (xenon arc [Suntest irradiation]).

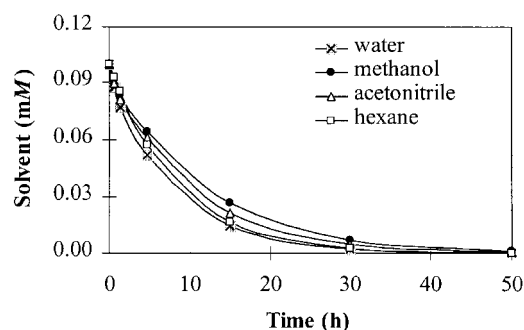


Fig. 6. Degradation curves of acifluorfen (0.1 mM) in different solvents (UV mercury arc).

anol, and acetonitrile are not as relevant as in water. Acifluorfen in water can more efficiently absorb light at wavelengths higher than 300 nm, used in the Suntest under xenon arc irradiation. The differences in the absorption spectra can explain the anomalous behavior of acifluorfen in water.

During the postemergence (of crops) use of this herbicide, a large amount of the technical product remains on the exposed side of leaves (Bentson, 1990), and may interact with the nonpolar waxy and fatty substances that cover the surface of the latter (Cabras et al., 1997). The high value of quantum yield observed in *n*-hexane suggests that the photolysis of acifluorfen could be an important process in organic environments. This behavior implies that the photolytic degradation of acifluorfen on the leaf surface is possible, particularly in sunny areas and seasons.

Identification of Photoproducts

When the reaction was stopped after 2 h of irradiation (Sample A) the gas chromatography–mass spectrometry (GC–MS) spectra allowed the identification of two derivatives (Scheme 1 [Fig. 1]). The first, 2-chloro-1-(4-nitrophenoxy)-4-trifluoromethylbenzene (nitrofluorfen), is referred to as Compound 2 [MS, m/z (relative abundance): 319 (33%), 317 (M^+ , 100), 298 (13), 289 (15), 287 (40), 236 (67), 217 (14), 196 (13), 195 (12), 179 (11), 139 (13), 132 (10), 92 (14), 75 (19), 63 (18)]. The second, 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrophenol (hydroxy-nitrofluorfen), is referred to as Compound 3 [335 (18%), 333 (M^+ , 52), 280 (17), 252 (100), 223 (11), 179 (8), 132 (8), 69 (16), 63 (19)].

When the photochemical reaction was allowed to run

Table 2. Kinetic parameters of acifluorfen photodegradation: τ , half-life; k , kinetic constant; Φ , quantum yield (degraded molecules per absorbed photon).

Solvent	Irradiation source	τ	k	Φ
		$s \times 10^3$	$s^{-1} \times 10^{-5}$	$\times 10^{-4}$
Acetonitrile	xenon arc	386.3	0.18	1.23
	Hg-UV arc	23.90	2.89	
Methanol	xenon arc	112.0	0.62	3.60
	Hg-UV arc	28.08	2.47	
<i>n</i> -Hexane	xenon arc	52.56	1.32	170
	Hg-UV arc	20.88	3.32	
Water	xenon arc	155.2	0.45	1.85
	Hg-UV arc	18.68	3.71	

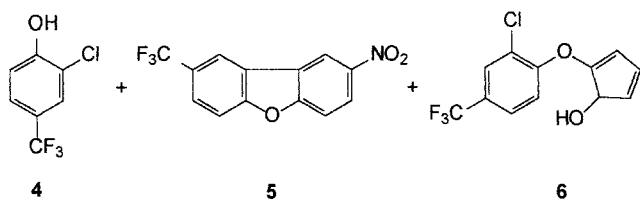


Fig. 7. Further by-products found after 36 h of irradiation (98% acifluorfen transformed).

its course, after 36 h irradiation (Sample B), in addition to Compounds 2 and 3, other substances were detected in the reaction mixture (Fig. 7). These included 2-chloro-4-(trifluoromethyl)phenol, referred to as Compound 4 [198 (33%), 196 (M^+ , 100), 179 (15), 177 (35), 161 (15), 146 (22), 132 (25), 113 (13), 63 (19)]; 5-trifluoromethyl-5'-nitrodibenzofuran, referred to as Compound 5 [281 (M^+ , 100%), 262 (10), 251 (25), 235 (23), 222 (20), 207 (68), 157 (11)]; and 2-[2-chloro-4-(trifluoromethyl)phenoxy]-cyclopentadien-1-ol, referred to as Compound 6 [278 (12%), 276 (M^+ , 28), 242 (14), 241 (100), 207 (9), 69 (16)]. In the last case (Sample B), many signals due to a host of small molecules ranging from 53 to 99 m/z were also recorded.

The ions (amu) detected in the Sample A with electrospray ionization (ESI) mode in flow injection technique-mass spectrometry (FIA-MS) analysis were: Compound 1 [positive, 362 ($M + H$) $^+$ + 384 ($M + Na$) $^+$; negative, 360 ($M - H$) $^-$ + 316 + 195]; Compound 2 [positive, 318 ($M + H$) $^+$ + 340 ($M + Na$) $^+$ + 300; no negative ions were detected]; Compound 3 [positive, 334 ($M + H$) $^+$; negative, 332 ($M - H$) $^-$ + 252]. Otherwise, in Sample B, Compounds 2 and 3 were confirmed together with Compound 4 [positive, 197 ($M + H$) $^+$ + 219 ($M + Na$) $^+$; negative, 195 ($M - H$) $^-$ + 176].

The [1H] NMR spectra showed the formation of two photodegradation products in Samples A and B (chemical shifts are reported in δ): Compound 2, 7.70 (s, 1 H) and 7.30 to 7.00 ppm (m, 6 H); and Compound 3, 7.72 (s, 1 H), 7.51 (s, 1 H), 7.2 to 7.0 (m, 4 H), and 3.5 ppm (bs, 1 H). Compound 5 was also recognized in Sample B: 7.81 (s, 1 H), 7.75 (s, 1 H), and 7.2 to 6.9 ppm (m, 4 H).

Mechanism of Photodegradation

In order to elucidate the mechanism of the photochemical degradation of acifluorfen we wished to clarify whether the photochemical reaction occurred through the first excited singlet state or through the first excited triplet state.

Kinetic data of the reactions performed in the presence of either a singlet (naphthalene [$E_S = 384$ kJ mol $^{-1}$]) or a triplet quencher (biphenyl [$E_T = 274$ kJ mol $^{-1}$; $E_S = 391$ kJ mol $^{-1}$]) are shown in Table 3. The presence of a singlet quencher seems to inhibit the reaction while the presence of triplet quencher does not; on the contrary, we observed an increase of the degradation rate. This behavior is probably due to the capability of biphenyl to sensitize the reaction by energy transfer; in fact, the energy of the first excited singlet state of biphenyl is 418 kJ mol $^{-1}$ (Murov et al., 1993), a value very close to acifluorfen (E_S , 402.9 kJ mol $^{-1}$). These results

Table 3. Halfives (τ) and kinetic constants (k) of acifluorfen degradation (0.1 mM in acetonitrile) in the presence of triplet quencher (biphenyl, at different concentrations), singlet quencher (naphthalene), radical initiator (AIBN), radical inhibitor (benzoquinone), and argon as oxygen remover.

Added substance	Concentration	Irradiation source	$s \times 10^3 \text{ s}^{-1} \times 10^{-5}$	
			τ	k
–		xenon arc	386.3	0.18
Triplet quencher	1 mM	xenon arc	110.7	0.63
Singlet quencher	1 mM	xenon arc	1112	0.06
Radical initiator	1 mM	xenon arc	118.4	0.58
Radical inhibitor	1 mM	xenon arc	6701	0.01
–		Hg-UV arc	23.82	2.91
Argon (oxygen remover)		Hg-UV arc	77.19	0.90

are in agreement with a mechanism involving the first excited singlet state. Furthermore, the degradation of Compound 1 was accelerated in the presence of a radical initiator (2,2'-azobisisobutyronitrile, AIBN). The presence of a radical inhibitor (benzoquinone) decreased the reaction rate (Table 3). These data also support the presence of radicals in the reaction.

Finally, using argon to saturate the solution and to remove oxygen, a decrease in the degradation rate was observed (Table 3), showing that oxygen could play an important role in the degradation of acifluorfen. In these conditions only 6.3% of the initial content of Compound 1 was transformed after 2 h of irradiation and Compound 3 was not detectable in the reaction mixture with adopted analytical methods.

On the basis of all previous results we can formulate our opinion as to the initial mechanism of acifluorfen photodegradation (Scheme 1 [Fig. 1]): the first excited singlet state may undergo a homolytic photolysis of the carboxyl bond to give Compound 2 after abstraction of a hydrogen atom from the solvent. On the other hand, the radical intermediate can react with oxygen, giving Compound 3. The derivative Compound 2 is due to the decarboxylation of Compound 1, according to Pusino and Gessa (1991). Defoin et al. (1986) found the same behavior for phenylglyoxylic acid and ascribed a $\pi \rightarrow \pi^*$ transition to the CO_2 loss under UV irradiation. In Compound 3, a hydroxyl group replaces the carboxylic function, as also found by Katagi (1992) on 3-phenoxybenzoic acid.

The products detected after 36 h of irradiation are depicted in Fig. 7. Compound 4 can be a residue due to the cleavage of the ether linkage as reported in the literature (Hageman et al., 1970). Product 5 could be derived from the cleavage of the ether linkage in Compound 1 followed by the rearrangement of the molecule (Scheme 2 [Fig. 8]) in agreement with Ogata et al. (1970). In our case the dibenzofurane intermediate was not successively converted to the diphenyl derivative.

In our opinion, Compound 6, deriving from a ring contraction, is not a real degradation product, but an artifact due to the thermal transformation of Product 2 in the gas chromatographic system.

Toxicity Evaluation

Daphnia magna has been used for many years as a standard aquatic test species. In fact, chronic and acute

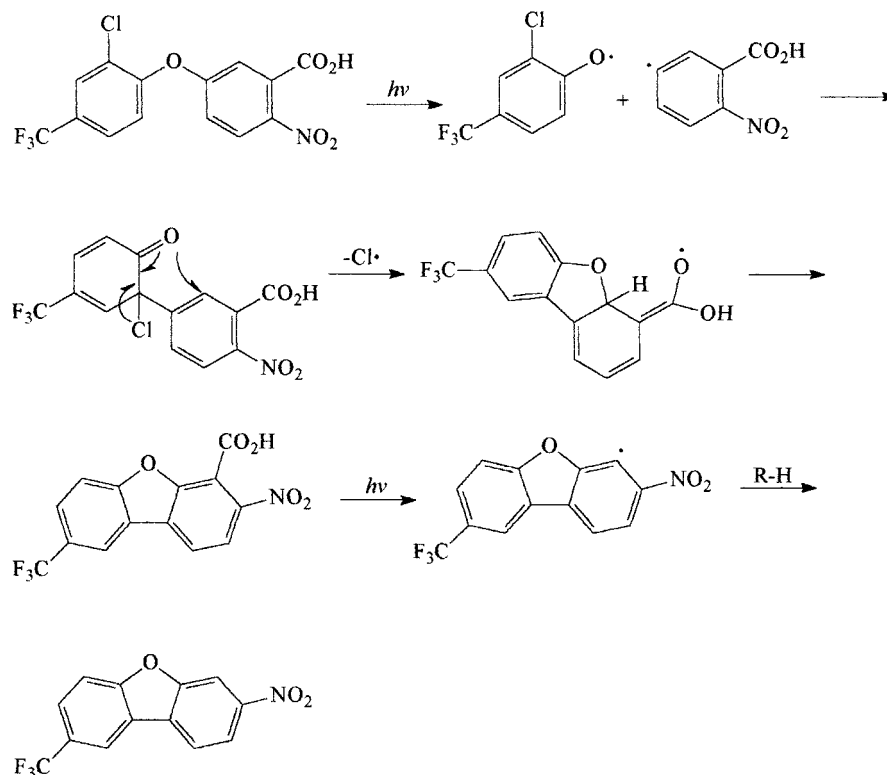


Fig. 8. Illustration of Scheme 2, showing the mechanism of cleavage of the ether linkage of acifluorfen followed by the rearrangement of the molecule to give the product 5-trifluoromethyl-5'-nitrodibenzofuran.

tests with this microcrustacean are among the most frequently performed in aquatic toxicology (Behechti et al., 1998; Mark and Solbé, 1998). The choice of *D. magna* for use as a standard test species was strongly influenced by the following factors:

- (i) It can be cultured in the laboratory, although culture on defined synthetic media is rather expensive;
- (ii) It represents the zooplankton community, a major element of the freshwater food chain;
- (iii) As a species of worldwide occurrence, the ecological relevance of the test results is recognized.

Acifluorfen in the concentration range used for the test (5–50 mg L⁻¹) did not show acute toxicity to the model organism. The 24-h EC values for the *D. magna* immobilization in the reaction mixtures were calculated referring to the initial content of the pure active ingredient. Organism swimming was not significantly reduced during the test performed in the dilution range of the mixture after a 2-h irradiation (20% acifluorfen transformed, giving 13% Compound 2 and 7% Compound 3). Otherwise, the products obtained after 36 h of irradiation were toxic since the 24-h EC₅₀ was 35.1 mg L⁻¹ (95% confidence limit, range 29.7–41.4 mg L⁻¹) and 24-h EC₁₀ was 6.7 mg L⁻¹ (95% confidence limit, range 5.9–9.7 mg L⁻¹). In the 36-h mixture, acifluorfen was 98% transformed, giving 24% Compound 2, 38% Compound 3, and many other by-products that were not identified with exception of substances shown in Fig. 7 (not quantified). Unfortunately, owing to poor recovery of each by-product, it was not possible to perform *Daph-*

nia tests for individual photoproducts to establish if one substance in particular is responsible for the toxicity found in the 36-h mixture.

To better understand such a result, octanol–water partition coefficients (K_{ow}) were calculated from structures of acifluorfen and discovered metabolites by using the Clog P 4.0 program from BioByte (Claremont, CA) (Leo, 1993). The log K_{ow} of acifluorfen was -4.73. All values for metabolites were positive, ranging from 3.35 to 5.35. The K_{ow} coefficient has been assumed as a valid index for the evaluation of the capability of chemicals to interact with the organic and/or biological environment (Scheunert, 1992). We could not find a correlation between log K_{ow} and the toxic effects of the metabolite mixture (after a 36-h irradiation) on zooplankton organisms. Our opinion is that the immobilization of *D. magna* may be due to a possible synergistic effect of several acifluorfen by-products in the aqueous environment.

CONCLUSIONS

Acifluorfen photodegradation occurs through the first excited singlet state, giving a radical mechanism that confirms the decarboxylation reaction cited in the literature. The common name of the decarboxylation product is nitrofluorfen, a potent herbicide no longer used commercially. The degradation is favored by the presence of oxygen in the reaction environment since it induces the parallel hydroxylation of the radical intermediate. On the other hand, more reactions can transform the initial compound, giving rise to a reaction mixture that

contains a multitude of small molecules. Both solar (simulated) and UV irradiation are effective for the degradation of parent molecules; however, more time is necessary under solar irradiation. Photolysis of acifluorfen is an important process in organic environments and for this reason a rapid degradation could occur on plant leaves (not verified here). Acifluorfen was not toxic to *D. magna* under the experimental concentrations used at the beginning of the degradation reaction, although toxicity was induced upon photodegradation.

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