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Degradational Behavior of the Pesticides Glyphosate and Diflubenzuron in Water

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Glyphosate (N-(phosphonomethyl)glycine) is a nonselective postemergence herbicide used in weed control (Worthing 1987) which is applied against weeds in a wide variety of aquatic ecosystems. It is effective against emergence and bankside weeds (Barret 1985) and this fact can result in the direct contamination of watercourses. It can enter in the aquatic environments in several ways and so clearly one of the most important aspects of the evaluation of its potential risk to the environment will be to study its degradation rate in water.

Diflubenzuron (N-(4-chlorophenyl)-N'-2,6-difluoro benzoyl)-urea, belongs to a group of insecticides acting by inhibition of the chitin synthesis and so interfering with the formation of cuticle (Mulder and Gijswijt 1973). It is used in crop protection against leaf-feeding larvae, lepidopterous insects in cotton, soybeans, fruits and horticultural crops (Grosscurt 1978). It is also widely used in forest pest control (Worthing 1987). Although it has a low water solubitity in water this way may represent a significant route through which nontarget organisms can be exposed to this herbicide (Sainz de los Terreros and García de Jalón 1991), as when is used as mosquito larvicide Mulla et al (1974). However, although diflubenzuron has a considerable potential as insect control agent, little has been reported about its effects in water. Shaefer and Dupras (1976 and 1977) have reported that dilute solutions of diflubenzuron are not stable in natural waters, particularly when temperature and pH are relatively high. Ivie et al (1980) have also investigated the breakdown pathways of diflubenzuron in water, using high concentrations and with high values of temperature and pH. Although Metcalf et al (1975) and Ivie et al (1980) have studied the environmental degradation of diflubenzuron, no attempt has been made

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to determine the degradation rates of this insecticide at low concentrations and under ambient conditions.

In the present work, it was studied the degradational behavior of glyphosate and diflubenzuron residues in aqueous media in conditions as closely related as possible with those occurring in the natural waters.

MATERIALS AND METHODS

Dechlorinated tapwater, according to the showed characteristics in tables 1a and 1b, was used in all experiments. Samples were kept at room temperature (20±2°C) and under natural sunlight illumination in air opened 12 L containers under no sterile conditions. Two parallel experiments, with and without water aeration, were carried out. 100 mL samples of each container were taken at different times and then were analyzed to

Table 1. Running Water Parameters on Diflubenzuron and Glyphosate Degradation Experiments.

1a. Diflubenzuron

PARAMETER	With aeration (*)	Without aeration (*)
pH	7.3 (5.8-6.9)	7.2 (5.7-6.9)
Conductivity (mg/L)	64.0 (76.8)	70.4 (83.2)
Temperature (°C)	19.6 (18.8)	18.9 (20.4)
Dissolved Oxygen(%)	85.0 (83.2)	86.5 (79.4)

1b. Glyphosate

PARAMETER	With aeration (*)	Without aeration (*)
pH	7.0 (5.9-6.8)*	7.3 (5.6-6.9)
Conductivity (mg/L)	76.8 (76.8)	70.4 (76.8)
Temperature (°C)	19.3 (18.8)	19.9 (19.4)
Dissolved Oxygen (%)	83.0 (82.0)	86.5 (79.0)

* Without parenthesis, data at the begin of experiments, and between parenthesis, data at the end of the experiment.

determine the concentration of these pesticides.

For both pesticides, two water containers were contaminated with the appropiate quantities of a 48% herbicide formulated (Roundup, Monsanto), and with a 99.2% analytical standard Diflubenzuron (U.S. Environmental Protection Agency, Washington DC) solutions in acetone, to give a final glyphosate concentration of 10 mg/L as free acid, and a final diflubenzuron concentration of 0.4 mg/L.

To determine the diflubenzuron, water samples (100 mL) were filtered through a Wathmann n° 1 filter paper and extracted with two 50 mL portions of methylene chloride analytical grade (Montplet & Esteban S.A., 08110 Barcelona, Spain). The organic extracts were mixed and dried over 10 g anhidrous sodium sulphate (Merck, Darmstadt, FRG). Methylene chloride extract volume was reduced to near dryness with vacuum rotatory evaporator (<40°C) and redissolved in 2-3 mL methanol. Methanol extract volume was reduced to less than 1 mL and final volume was adjusted to 1 mL; then was filtered through 0.5 μm pore size membrane filter (Nihon Millipore Kogyo K.K., Yonezawa, Japan) before the analysis by liquid chromatography.

Samples were analyzed on a Perkin-Elmer series 4 liquid chromatograph, which was equipped with a Rheodyne Model 7125 S injector (6 μL charge loop), LC-95 variable wavelength U.V. detector operated at 245 nm and a 3,700 chromatographic data station. A 15 cm × 4,6 mm i.d. stainless column, packed with 5 μm particle size Spherisorb ODS-2 (Tracer Analítica S.A., 08024, Barcelona, Spain) was used throughout. Mobile phase, 75% methanol in water, was pumped at a flow rate of 0.9 mL/min. Diflubenzuron analytical standard (U.S. Environmental Protection Agency, Washington DC: 99.2% stated purity.) solutions in methanol at appropiate concentrations were used for quantification.

To determine glyphosate, water samples (100 mL) were filtered through a Wathmann n° 1 filter paper and 1 mL of K_2HPO_4 was added. Samples were evaporated to near dryness on a rotatory evaporator at 50°C and diluted to 5 mL with deionized water (Milli-Q water purification system, Milipore Corp., Bedford, MA 01730). 1 mL aliquot was filtered through 0.2 μm membrane; then 0.1 mL of the filtrate was derivatized (FMOC) with N-fluorenylmethyl chloroformate (Merck, Darmstad, FRG) according the procedure described by Miles et al (1986). Derivatized samples were analyzed on the same Perkin-Elmer series 4 liquid chromatograph, equipped with a Rheodyne Model 7125 S injector (6 μL charge

Table 2. Glyphosate residue levels in water over 20 days.

TIME (days)	RESIDUE LEVELS (mg/L) with aeration	RESIDUE LEVELS (mg/L) without aeration
0	10.00	10.00
0	7.23	7.80
2	7.66	9.12
4	8.33	7.85
6	8.85	7.90
8	8.10	8.89
10	8.94	8.34
13	7.64	8.21
20	----	9.61

loop), LC-95 variable wavelength U.V. detector operated at 263 nm and a 3,700 chromatographic data station. A 20 cm x 4 mm column packed with 5 µm particle size Spherisorb SAX was used throughout. The mobile phase, phosphate buffer 0.05 M, pH 6.0/ phosphoric acid 0.05 M/acetonitrile (40/10/50) was pumped at flow rate of 1.5 mL/min. Glyphosate analytical standard (U.S. Environmental protection Agency, Washington DC: 99.9% stated purity.) solutions at appropiate concentrations were derivatized as has been describe above.

The degradational behavior in water of two pesticides were studied using the statistical methods described by Timme et al (1980, 1986). Six decay models according to Timme et al (1986), (1st order, 1.5th order, 2nd order, RF 1st order, RF 1.5th order and RF 2nd order) were checked for adjustment, and the model which offered the best correlation coefficient was finally selected for half life calculations.

RESULTS AND DISCUSSION

The chromatographic analytical system for glyphosate, described by Miles et al (1986) was sligthly modified in order to achieve shorter retention times. No interference peaks for glyphosate has appeared on the chromatogram for these samples. Although FMOC derivatization is mainly intended for use with fluorescence detection, these derivatives are chromophores enough suitable to allow UV absorption detection at 263 nm if high sensitivities are not needed. The lowest detectable amount of glyphosate-FMOC

Table 3. Glyphosate residue levels in water over 78 days.

TIME (days)	RESIDUE LEVELS (mg/L) with aeration	RESIDUE LEVELS (mg/L) without aeration
0	10.00	10.00
1	8.23	8.47
15	8.76	7.89
22	8.43	8.62
36	9.12	8.26
50	8.60	8.82
64	7.81	8.43
71	8.31	8.22
78	8.37	7.93

derivative was 0.6 ng (2 x noise peak heigth) under the proposed conditions.

Measurements of glyphosate residues in water in the first experiment, carried out over 2 weeks, are presented in table 2, showing the data for all taken and analyzed individual samples. Although they were not taken several samples by day, these data shows that herbicide residues levels are quite erratic, probably due to glyphosate linkage to glassware surface on the process of samples (Miles et al 1986); don't seems have breakdown tendence in both experiments: with or with out aeration.

Results of a further experiment carried out over a longer time period are shown in table 3. There was not an appreciable degradation, chemical or microbiological, over a 78 days period. The degradation data from 0 to 78 days had not shown statistical significance. In this experiment it was observed a severe microbiologic contamination of water in both containers.

The statistical treatment of data in the second experiment, offered an RF 1st order fitting ($R = a/b^t$) for residue data in the oxigenated container, and an 2nd order fitting ($R = 1/a+b.t$) for the container with out aeration. The correlation coefficients for both regression curves shows very low values, indicating that residue levels don't have dependence on time. The significance levels of statistical analysis were higher than 95%. Calculations from regression curves offer extrapolated half lifes of 162 and 176.5 years in

Table 4. Diflubenzuron residue levels in water over 10 days.

TIME (days)	RESIDUE LEVELS (mg/L) with aeration	RESIDUE LEVELS (mg/L) without aeration
0	0.4	0.4
1	0.15	0.19
2	0.10	0.13
3	0.07	0.06
8	0.02	0.02
10	N.D.[a]	N.D.[a]

[a] below 0.011 mg/L

presence or absence, respectively, of aeration. Of course, it must been taken into account that extrapolated half life results from regression lines only have a relative value, mainly due to herbicide decay behavior, and in minor extent, to the glyphosate erratic analytical behavior, and so they must be considered as an approach to the actual half life values. Further it was made an Anova statistical analysis to study the glyphosate residue in water in the two former experiments. The results of this Anova showed that there were not significance values in both experiments.

According to Bronstad and Friestad (1984), glyphosate is chemically stable in aqueous media with very low tendency to hydrolytic decomposition. Despite this fact, this herbicide is considered to be fastly dissipated from aquatic ecosystems. Microbial degradation to yield aminomethylphosphonic acid and CO_2, has been noted by Rueppel et al (1977) as the major pathway for its degradation, but it was not observed in these experiments. According this authors, glyphosate also showed to be stable to sunlight, as in these experiments.

To study diflubenzuron degradation in water, the reversed phase liquid chromatographic system described by Di Prima et al (1978) was sligthly modified in order to achieve a higher sensitivity. The actual retention time of diflubenzuron was 5.77 min. and no interference peaks had appeared for these samples. The lowest detectable concentration of diflubenzuron was 1.1 µg/mL (2 x noise peak heigth) in the proposed conditions.

Table 5. Parameters of the regression curve for diflubenzuron decay data in presence and in absence of an aeration.

5a. Presence

Parameter	Value	Lower limit[a]	Upper limit[a]
Intercept:	-0.93713	-0.95318	-0.92166
Slope:	-0.46505	-0.41228	-0.51782
r^2:	0.09961		
D:	0.11978		
Half life (days):	0.41899	0.32391	0.51407

5b. Absence.

Parameter	Value	Lower limit[a]	Upper limit[a]
Intercept:	1.70915	1.68272	1.73686
Slope:	0.68943	0.62200	0.75686
r^2:	0.99717		
D:	0.12027		
Half life (days):	0.96685	0.77749	1.15621

[a] 95% confidence limits

Measurements of diflubenzuron residues in water over 10 days both in presence or absence of aeration, are presented in table 4. These results shows a rapid decay of Diflubenzuron residue levels.

The statistical treatment of experimental data, offers an RF 1st order fitting (R = 1/a+b.t) for residue data in the oxigenated container (table 5a), and an 1.5th order fitting (R =1/a+b. t)^2) for the container with out aeration (table 5b), with high correlation coefficients for both regression curves. Calculations from these regression curves, offer half lifes of 0.42 and 0.97 days in presence and absence of an aeration.

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