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Leaching properties of some degradation products of alachlor and metolachlor

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Abstract

Once in soil, pesticides undergo degradation processes that give rise to a complex pattern of metabolites. Those presenting a significant percentage of formation, genotoxic and leaching properties may pose a threat to human health associated with the consumption of drinking water. The aim of this study is to assess the hazard potential of some metabolites that may occur in ground water. 2,6-diethylaniline, 2-chloro-2′,6′-diethylacetanilide, 2-hydroxy-2′,6′-diethylacetanilide, metabolites of alachlor and 2-ethyl-6-methylaniline, metabolite of metolachlor, were chosen for their genotoxic properties. Under laboratory conditions, these metabolites showed $DT_{50} = 1–5$ days and $K_{oc} = 45–357$. Their leaching potential, calculated according to Gustafson, is very low and, therefore, they should not be regarded as contaminants of ground waters. Aged residue leaching studies as well as preliminary studies on well waters seem to confirm these findings. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

After agricultural application, pesticides undergo biotic and abiotic processes in soil that give rise to a complex pattern of degradation products, mostly metabolites. Many of them have been determined in groundwaters (Lagas et al., 1989; Pereira et al., 1990), at concentrations that range from some units to tens of μg/l (Funari et al., 1995). The possible human health implications associated with the use of these waters for drinking are generally difficult to assess, as too few data are available on the biological properties of pesticide metabolites.

The World Health Organization has defined drinking water quality guidelines for many pesticides, but not for their metabolites (World Health Organization, 1993), and these products are not usually monitored in waters used for drinking.

In spite of this, some legislative measures have been established in order to control the possible impact of pesticide metabolites on human health and the environment. In Europe, in order to achieve authorisation to place new pesticides on the market, environmental studies on metabolites that form at significant percentages are mandatory (Council Directive 91/414/EEC).

The aim of our activity is to identify metabolites that pose a potential hazard to human health. Through an extensive survey of the scientific literature, we take note of those that show biological properties of concern (i.e. genotoxic activity). Then, we look at their chemiodynamic characteristics, the extension, rate and mode of application of their parent compounds, and their occurrence in soil, leachates and waterbodies. On the basis of this information, we select those of particular concern and define their leaching potential mostly under laboratory conditions (Donati et al., 1994; Bottoni et al., 1996).

This paper presents the results of laboratory studies on four metabolites of acetanilide herbicides. These studies included (i) the determination of mobility (expressed as K_{oc}), (ii) persistence in soil (expressed as

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DT_{50}) in order to define their leaching potential according to Gustafson (1989), and (iii) aged residue leaching experiments. K_{oc} and DT_{50} were determined for the metabolites, their parent compounds and atrazine, used as reference compound.

Moreover, preliminary studies have been conducted in order to analyse the metabolites in a limited number of well waters in an agricultural area where their parent compounds are extensively used.

The metabolites selected for these studies were 2,6-diethylaniline (DEAN), 2-hydroxy-2′,6′-diethylacetanilide (2OH), and 2-chloro-2′,6′-diethylacetanilide (2CL), which form in soil from alachlor (Tiedje and Hagedorn, 1975), and 2-ethyl-6-methylaniline (EMA), which forms in soil from metolachlor (Guzzella et al., 1996). DEAN and EMA are intermediates in the metabolism of alachlor and metolachlor in the rat liver where they are converted to nitroso metabolites, which are highly mutagenic in the Ames test (Kimmel et al., 1986). 2CL and 2OH are positive in the same test after metabolic activation with microsomes (Tessier and Clark, 1995).

As for their environmental fate and occurrence, only few data are available that show the presence of low levels of DEAN, 2OH, and 2CL in groundwater (Pereira et al., 1990; Potter and Carpenter, 1995; Kolpin et al., 1996). Their chemiodynamic characteristics are poorly known (Lyman, 1982; Liu et al., 1987; Heyer and Stan, 1995).

The parent compounds of these metabolites are widely applied herbicides. Alachlor has been classified by the WHO as a human carcinogen, metolachlor is carcinogenic in one mammal species (World Health Organization, 1993).

2. Materials and methods

2.1. Soils

DT_{50}s were determined using fresh soil samples collected from the surface layer (0–10 cm depth) of an agricultural field in the province of Modena (Italy). Their characteristics were: 29% clay, 49% silt, 22% sand, 1% organic carbon, pH 7.6 and 22% moisture (w/w).

For aged residue leaching experiments, a fresh soil sample coming from the province of Perugia (Italy) was used. Its characteristics were: 12% clay, 17% silt, 71% sand, 1% organic carbon, pH 8 and 12% moisture (w/w).

2.2. Products and reagents

Atrazine and metolachlor were purchased from Ciba-Geigy (99.2% and 99.5% of purity, respectively), alachlor from Monsanto (99.5%), 2,6-diethylaniline and 2-ethyl-6-methylaniline from Fluka (Fluka Chemie AG, Switzerland) (98 and 97%, respectively). 2-chloro-2′,6′-diethylacetanilide and 2-hydroxy-2′-6′-diethylacetanilide were prepared in our Institute as described below.

2-Chloro-2′,6′-diethylacetanilide: 0.1 moles of 2,6-diethylaniline were dissolved in a solution containing 50 ml of acetic acid and 50 ml of a saturated CH_3COONa liquid, 0.12 moles of chloroacetyl chloride were dripped in an ice bath under stirring. The mixture was stirred for 30 min. The precipitated solid was collected by filtration, washed with a solution of $NaHCO_3$ and water, then dried in a hot air furnace and crystallized.

2-Hydroxy-2′-6′-diethylacetanilide: 1 g of 2-chloro-2′,6′-diethylacetanilide was dissolved in a solution containing 10 ml of water with 1 g $NaCO_3$ and 10 ml of ethanol. The solution was bubbled for 6 h. The reaction was monitored by thin-layer chromatography (ethyl acetate/hexane 1:2) and stopped when the Rf was lower than that of the starting material. Then the bulk of ethanol was removed by rotary evaporation under vacuum and extracted with ethyl acetate. The precipitated solid was collected by filtration, washed with a solution of $NaHCO_3$ and water, then dried in a hot air furnace and crystallised.

The purity degree of these metabolites was $\geqslant 98\%$. Their identity was confirmed by Nuclear Magnetic Resonance Spectroscopy (NMR).

Distilled water for high performance liquid chromatography (HPLC) was further purified through a Norganic cartridge (Millipore, Bedford, MA). All the organic solvents used were of HPLC grade (acetone – Baker, Holland; methanol – Sigma Aldrich, Germany; acetonitrile – Merck, Germany).

2.3. Determination of K_{oc}

The K_{oc} of the examined compounds were determined by HPLC, according to published procedures (Donati et al., 1994; Kordel et al., 1995; Hong et al., 1997), using nine reference compounds: atrazine, diclofop-methyl, fenamiphos, isoproturon, linuron, methiocarb, monuron, quintozene and trifluralin (from Dr Ehrenstorfer, GmbH, purities $\geqslant 97\%$). These compounds were chosen because their K_{oc} cover a sufficient range of values and are available in the literature (Kordel et al., 1995).

2.4. Determination of soil half-life (DT_{50})

Degradation tests were conducted according to SETAC procedures (Lynch, 1995), in two replicates. The examined substances were applied at rates of 2.5 mg/kg and incubated at 21°C, keeping the soil moisture constant for all the experiments. All the residues were extracted from soil with methanol at a ratio of 2 g/8 ml, but for alachlor, for which 5 g/20 ml was used. The resulting suspensions were vigorously shaken for 10′ and then centrifuged for 10′ at 2420*g*. Supernatants were reduced to 1 ml under nitrogen flow, and then analysed by HPLC.

2.5. Determination of the leaching potential

The leaching potentials of the examined compounds were calculated as groundwater ubiquity score, using the following equation: $\mathrm{GUS} = \log \mathrm{DT}_{50} \times (4 - \log \mathrm{K}_{oc})$ of DT_{50} and K_{oc} (Gustafson, 1989).

2.6. Aged residue leaching

These experiments were carried out according to SETAC procedures (Lynch, 1995) with some modifications. Glass columns 45 cm high and with an inner diameter of 4 cm were filled with untreated soil up to a height of 30 cm.

Alachlor and metolachlor were separately incubated in soil samples for a period of time corresponding to their half-lives, under the above conditions.

Then, aliquots of incubated soils were transferred to the top of the columns in order to fill an additional height of 5 cm; 2000 ml of 0.01 M $CaCl_2$ solution were added on the top of each column for 4 days, by means of a peristaltic pump. This volume is equivalent to 300 mm rainfall per day and simulates a worst-case scenario.

The experiments were conducted in two replicates in the dark. Leachates were collected, filtered through cellulose acetate (0.45 μm), and concentrated using disposable solid phase extraction (SPE) cartridges (LiChrolut EN 200 mg, Merck).

2.7. HPLC apparatus

A liquid chromatograph Model 9010 (Varian, Walnut Creek, CA) equipped with a Rheodyne injector, having a 50 μl loop, a UV detector Model 9050 (Varian) and an autosampler Model 9100 (Varian) were used. The chromatographic raw data were elaborated with the software Star Integrator 4.1 (Varian).

For K_{oc} experiment, the capacity factors (k') of all the substances were derived from retention times measured with a 25 cm × 4.6 mm i.d. column filled with 5 μm of LC-CN (cyano) packing (Supelco Inc., Bellofonte, PA). The mobile phase was methanol–water (55:45 v/v), according to published procedures (Kordel et al., 1995).

For DT_{50} determination and aged residue leaching experiments, a 25 cm × 4.6 mm i.d. column filled with 5 μm, average particle size, of Supelcosil LC-18 packing (Supelco) was used.

Metabolites were separated from their parent compounds by applying a gradient program at the following conditions. The mobile phase was a mixture of acetonitrile (A) and water at the following percentages: from 0 to 5 min A keeps at 20%; from 5 to 20 min A linearly increases from 20% to 80%; from 20 to 25 min A remains at 80%, then linearly decreases to 20% from 25 to 30 min, and remains stable till 35 min.

The mobile phase flow was of 1 ml/min; the column was kept at 30°C and the detector set at the wavelength of 210 nm.

Each analytical data represents the mean value of three HPLC determinations.

3. Results

3.1. K_{oc}

Fig. 1 shows the relationship between the K_{oc} and k' values determined for the nine reference compounds. The correlation coefficient of the linear relationship between these values is not particularly high but it can be considered sufficient for the purpose of this study.

K_{oc} values for the metabolites and the parent compounds, obtained by applying the regression equation of Fig. 1, are shown in Table 1. K_{oc} values for atrazine, alachlor and metolachlor are within those published in the literature (Bottoni and Funari, 1992; Tomlin, 1995).

Only 2OH has a mobility higher than that of atrazine. The other metabolites as well as their parent compounds show a limited mobility.

3.2. DT_{50}

The degradation rates of the examined substances follow a first order kinetics (Figs. 2 and 3). All the examined substances have DT_{50} values lower than that of atrazine.

The metabolites show very low DT_{50} values that never exceed three days (Table 1). These values are much

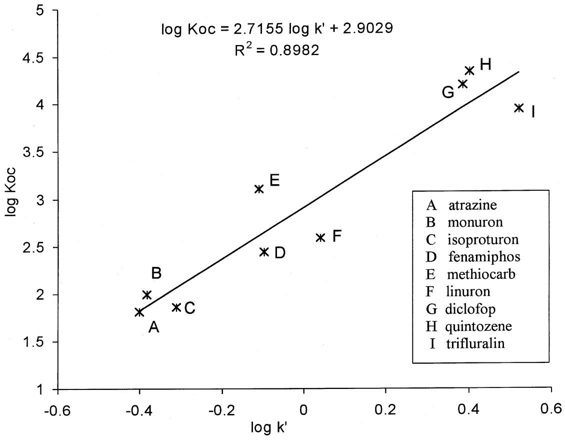

Fig. 1. Regression line derived from the correlation between K_{oc} and capacity factor (k') of the reference pesticides selected for this study.

Table 1
DT_{50}, K_{oc} values and GUS index for the examined parent compounds, their metabolites, and atrazine

Substances	DT_{50} (days)	K_{oc}	GUS[a]
Atrazine	59.2	66	3.87
Alachlor	5.2	312	1.07
2-Cl-2′,6′-diethylacetanilide (2CL)	2.4	148	0.7
2-OH-2′,6′-diethylacetanilide (2OH)	0.8	45	−0.18
2,6-Diethylaniline (DEAN)	1.3	357	0.19
Metolachlor	17.4	244	2.00
2-Ethyl-6-methylaniline (EMA)	1.7	197	0.38

[a] GUS index = $(\log DT_{50}) \times (4 - \log K_{oc})$; GUS > 2.8 leacher; 1.8 < GUS < 2.8 transient GUS < 1.8 nonleacher. These values have been calculated using mean DT_{50} values.

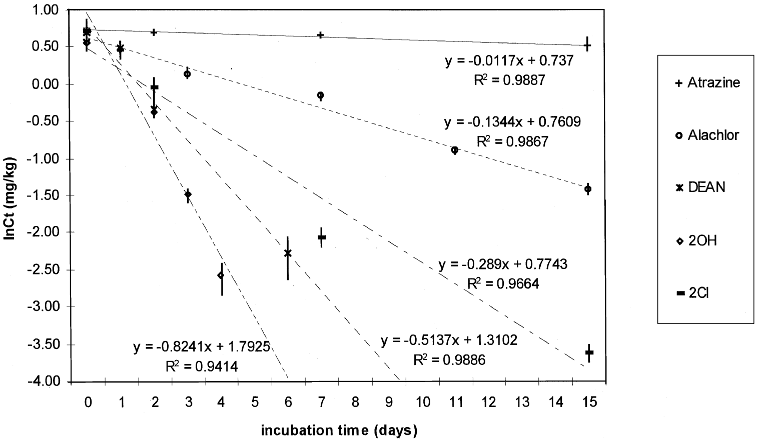

Fig. 2. Decrease in soil concentrations of alachlor, its metabolites and atrazine.

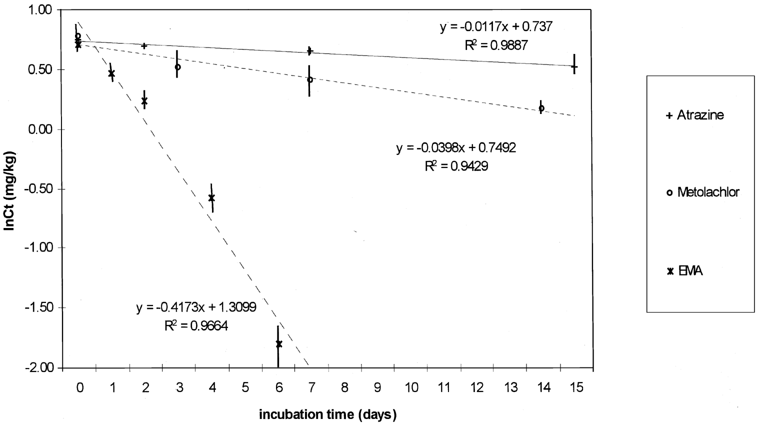

Fig. 3. Decrease in soil concentrations of metolachlor, its metabolite and atrazine.

lower than those for alachlor and metolachlor. From further experiments on thermally sterilized soil (Hsu and Bartha, 1974), 2OH and 2CL do not show important variations of concentration, meaning that the observed degradation in nonsterilized soil is due to biotic reactions. For DEAN and EMA, a significant percentage of the dissipation is due to non biotic factors, e.g. chemical degradation or bound residues formation. DT_{50} for alachlor and metolachlor are similar to those found in the literature (Bottoni and Funari, 1992).

3.3. Leaching potential (GUS)

The results show that only atrazine can be classified as a leacher, as it is shown in Table 1 and Fig. 4. Metolachlor has a GUS value which is characteristic of transition compounds. Alachlor and all the metabolites show leaching potentials belonging to non-leachers.

3.4. Aged residue leaching columns

Table 2 reports the residue concentrations and relative percentages of the examined compounds in the leachates at the end of the soil column experiment. The metabolites occur in the leachates at very low percentages with respect to their parent compounds. Only 2CL reached a percentage close to 10% of the applied dose. Taking into account that alachlor and metolachlor have been rarely found in ground water, and only at low concentrations, their metabolites represent a minor risk of groundwater contamination.

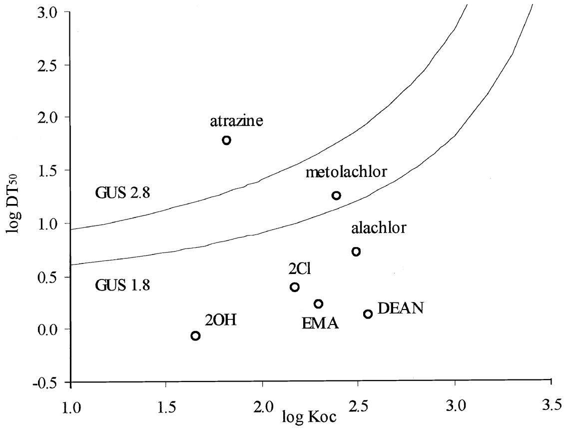

Fig. 4. GUS index of metabolites, their parent compounds and atrazine.

Table 2
Aged residues leaching experiments: concentrations (μg/l) and recovery (%) for all the compounds with respect to the initial dose of alachlor and metolachlor applied to soil[a]

Compounds	Concentrations (μg/l)	Recovery (%)
Column 1		
Alachlor	8.7–9.4	28–30
2CL	2.2–2.7	7–8.5
2OH	0.8	2.7
DEAN	1.0	3.2
Column 2		
Metolachlor	6.6	21
EMA	0.6	2

[a] Atrazine, used as tracer without incubation under the same experimental conditions, yielded an 80% recovery.

4. Discussion and conclusion

The experimental approach used in this study in order to define the leaching potential of the examined compounds seems correct as the results obtained for alachlor, metolachlor and atrazine are in the range of those published in the scientific literature.

The examined metabolites have very short half lives and consequently low leaching potentials; aged residue leaching studies show that they occur in the leachates at low concentrations and percentages, and are unlikely to contaminate ground water at concentrations significant to human health.

In conclusion, they may not be regarded as contaminants of groundwater. In spite of their biological properties, these metabolites do not represent a threat to human health when ground water resources are used for drinking.

These findings are confirmed by a preliminary study on a limited number of well waters from agricultural areas where alachlor and metolachlor have been widely used in the last years (data not shown).

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