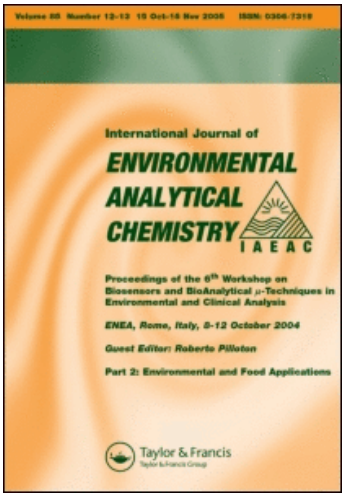

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Microbial and Photolytic Degradation of the Herbicide Acetochlor

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MICROBIAL AND PHOTOLYTIC DEGRADATION OF THE HERBICIDE ACETOCHLOR

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Time-course of the microbial degradation of the herbicide acetochlor was studied in commercial black mold and products of degradation were followed using gas chromatography-mass spectrometry (GC-MS). After 1-year-exposure in soil only 9% of the parent molecule was found and two metabolites were identified. The estimated half-life ($t_{1/2}$) of the acetochlor was approximately 90 days. In photolytic degradation studies irradiation of acetochlor resulted in 50% conversion of the parent molecule yielding at least six products, of which five photoproducts accounted for 43 % were tentatively identified using GC-MS and their structures were confirmed by chemical synthesis. Products formed by microbial degradation were less phytotoxic to maize, oat and ryegrass seedlings than parent acetochlor.

Keywords: Acetochlor; soil; photolysis; metabolism

INTRODUCTION

The herbicide acetochlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(ethoxymethyl) acetamide) has been used in Hungary for many years for preemergence weed control of maize. In plants, its initial conjugation with glutathione followed by further transformations of the sulfur-linked acetochlor has been reported as the major metabolic pathway[1,2]. As to environmental fate of this herbicide there is relatively little information available[3] despite degradation pathways of its structural analogs such as alachlor and metolachlor have been extensively studied[4,5].

The objectives of this study were a) to monitor the time-course of acetochlor disappearance and of the formation of its non-conjugate degradation products in

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soil and b) to determine products of photodegradation by UV irradiation as well as c) to investigate the phytotoxicities of the metabolites.

EXPERIMENTAL

Materials

Acetochlor (purity >99%) was provided by Nitrokemia (Fuzfogyartelep, Hungary). Reference materials of degradation products were synthesized as was described previously[6]. Soil was a commercial black mold (pH=7.0; organic matter 10.9%; moisture content 10.3%).

Soil degradation studies

Ten grams of soil placed in glass vials was treated by applying 100 μg of acetochlor dissolved in 0.5 ml of 20% acetone-water solution on the surface from a syringe. Vials containing soil samples were kept in plant growth room at controlled conditions (26/21°C day/night temperature; 65% relative humidity; 16 h photoperiod) and were watered every other day up to the original weight of the vial plus the soil sample in order to maintain the original moisture content. Samples of soils were taken in different time intervals and a suspension of soil samples (10g) with 20 ml of water was extracted with distilled toluene (3 × 50 ml). Recoveries of extractions for acetochlor were over 90%. Extracts were purified on Florisil column by dichloromethane eluent. After concentration of extracts in nitrogen stream the samples were analyzed by gas chromatography (GC). Perkin-Elmer F22 gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) with nitogen selective detector was equipped with a 1 m × 3 mm i.d. glass column packed with 3% OV-17 on 80–100 mesh Gaschrom Q. GC conditions were as follows: nitrogen carrier gas: 45 mL/min; column inlet temperature: 220°C; column temperature: programmed from 120 to 180°C by 6°C/min; detector temperature: 250°C. Retention times were as follows: acetochlor, 17.27 min; deethoxymethyl acetochlor, 10.95 min; chloroacetyl-indoline, 19.45 min. Samples of purified extracts were also subjected to gas chromatography – mass spectrometry (GC-MS) analyses. MS analysis (MM-12F1A, V.G. Micromass Ltd., Winsford, U.K.) was done in electron impact (EI) mode with an ionization potential 70 eV at a source temperature of 160°C. GC (Pye Series 104 Chromatograph, Pye Unicam, Cambridge, U.K.) was equipped with a 2m × 3 mm i.d. glass column packed with 3% OV-17 Q. GC conditions were as follows: helium carrier

gas: 20 mL/min; column temperature: programmed from 150 to 250°C by 6°C/min.

Photodegradation studies

Acetochlor (1% w/v) in 20% ethanol-water mixture was irradiated for 24 hours with high-pressure mercury arch in water-jacketed Pyrex quartz reactor equipped with magnetic stirrer. The light spectrum of the arch was between 311 and 437 nm with the most intensive emission at 360 nm. The irradiated samples were analyzed by GC-MS under the above-mentioned conditions. Relative amounts of photodegradation products were estimated by GC. Authentic reference materials were synthesized and used for confirmation of structures of photodegradation products.

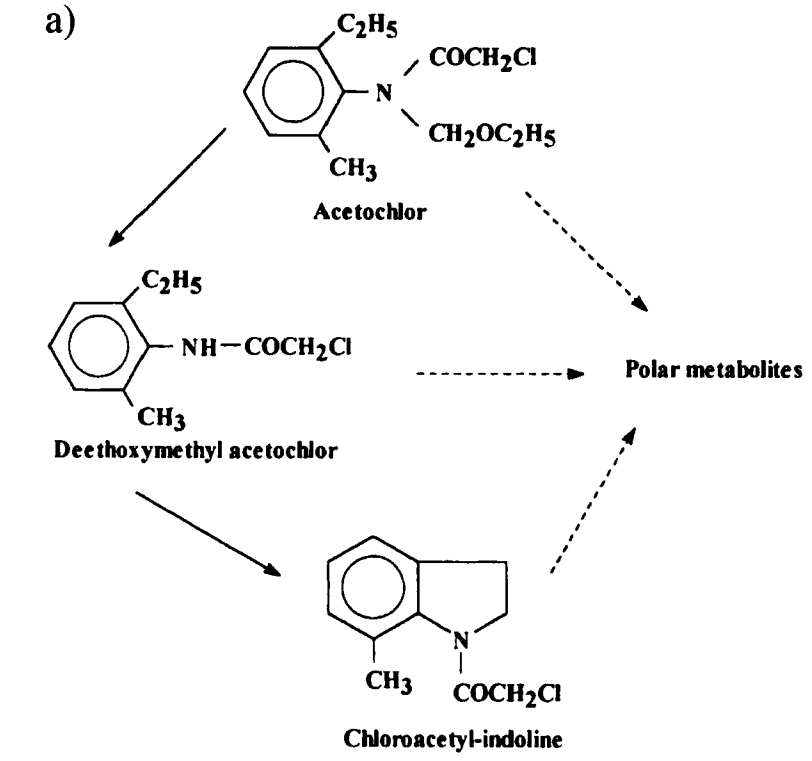
Phytotoxicity studies

Growth-response experiments were carried out by applying various rates (1.25 – 10 kg/ha) of reference materials formed in degradation experiments pre-emergence[7]. Maize, oat and ryegrass seedlings grown in sand were used as test plants and harvested 2 weeks after planting.

RESULTS AND DISCUSSION

Soil degradation

Since degradation in soil mainly occurs by microorganisms a commercial black mold was used in the soil degradation experiments due to its high organic matter content which gives suitable environment for microorganisms to live. In addition, high organic matter soils exhibit high degradability to the herbicide applied. Time-course of the acetochlor herbicide breakdown was followed through a 1-year-period using GC and GC-MS analyses (Figure 1a. and b.). In the soil treated with 10 mg acetochlor/kg soil, two major degradation products were detected over the time. Upon 1 month after treatment, 66% of the parent herbicide remained without degradation. The major degradation product was the deethoxymethyl acetochlor (2-chloro-N-(2-ethyl-6-methylphenyl)acetamide, M=211) formed probably by a hydrolytic cleavage of the ethoxymethyl side chain. In the MS spectrum of this compound, in addition to the molecular ion at *m/z* 211, a very intensive peak at *m/z* 162 which means the loss of a CH_2Cl was



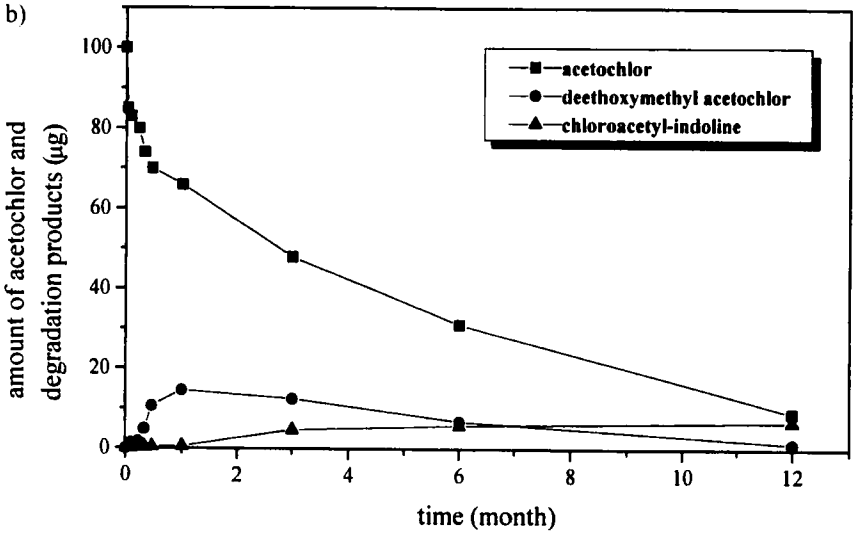


FIGURE 1 Degradation pathway (a) and time-course of the degradation of acetochlor (b) in soil

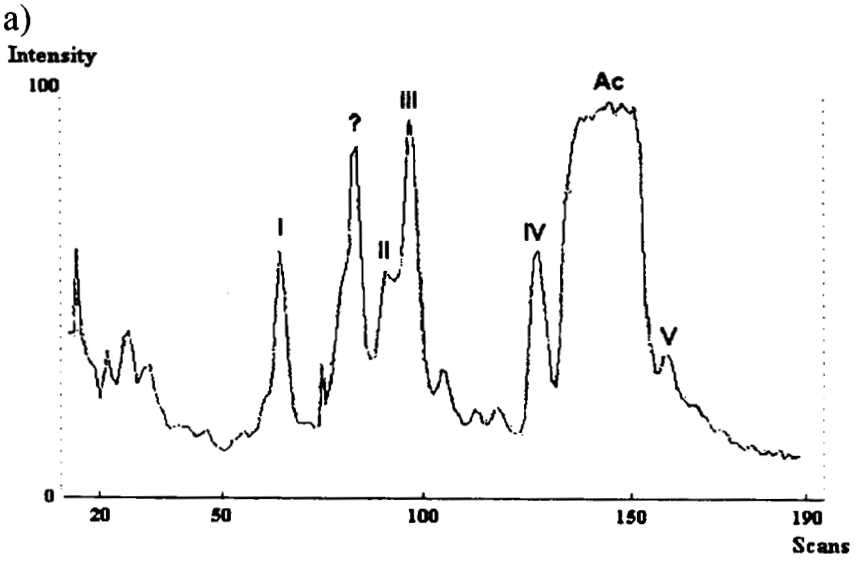
detected. Chemical synthesis of this compound confirmed the postulated structure. Additional metabolism resulted in decrease in the amount of both the parent molecule and the initial deethoxymethylated derivative. Up to 1 year, 9% of the applied acetochlor and only traces of the initially formed deethoxymethyl acetochlor were detected. At this time, chloroacetyl-indoline (1-(chloroacetyl)-7-methyl-2,3-dihydro-1H- indole, M=209) and two unidentified metabolites were detected as major toluene-extractable degradation products. The chloroacetyl-indoline product had a parent peak at *m/z* 209 and three major ions at *m/z* 194, 160 and 132 corresponding to the loss of CH_3, CH_2Cl, and $COCH_2Cl$. This spectrum matched with that for standard compound[6]. Indoline formation from structurally similar chloroacetanilide herbicides alachlor[8] and Antor[9] by the soil fungus *Chaetomium globosum* has been reported. The major products of this metabolic degradation were polar compounds.

The half-life of the acetochlor in our studies was determined from the plot obtained for acetochlor disappearance (Figure 1b). The estimated half-life ($t_{1/2}$) was approximately 90 days in our experiments. In comparision, the half-life of acetochlor in soil at 30°C in a small-scale incubation was estimated 7 days[3]. Depending on soil type, temperature, and moisture content, $t_{1/2}$ values for alachlor and metolachlor showed a great variability between 7 and 203 days[4]. Nevertheless, polar compounds such as oxanilic, sulfonic, and sulfinyl-acetic acids has been identified as major metabolites in soil from alachlor[10], metolachlor[11], propachlor[12] and AC 206784[13].

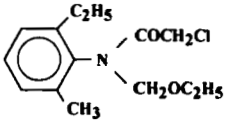
Photolytic degradation

In photolytic degradation studies, acetochlor was irradiated in a photoreactor for 24 hours. The photodegradation resulted in 50% conversion of the acetochlor yielding at least six products (Figure 2a) of which five photoproducts accounted for 43 % (Figure 2b.) were tentatively identified using GC-MS. The structures of photodegradation products were confirmed by using synthesized reference materials.

The EI spectrum of photoproduct **I** (N-(2-ethyl-6-methylphenyl)formamide, MW=163) showed intensive peaks at *m/z* 163, 134, and 120. The ion at *m/z*134 is due to the loss of a CHO from the molecular ion, while the ion at *m/z* 120 probably results from the cleavage of the NHCHO substituent with concurrent hydrogen migration. Photodegradation product **II** was identified as 2-chloro-N-(2-ethyl-6-methylphenyl)acetamide (MW=211) which was also formed in soil degradation studies. Photodegradation product **III** identified as N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide (MW=235) had characteristic peaks at *m/z* 206, 164, and 148 corresponding to the loss of C_2H_5, C_2H_5

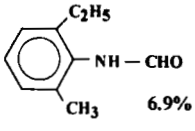


b)



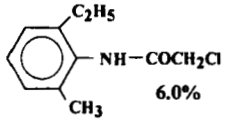
50.5%

Acetochlor, M=269



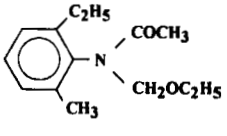
6.9%

I, M=163

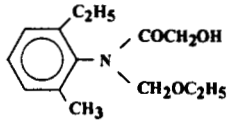


6.0%

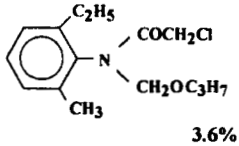
II, M=211



III, M=235 12.2%



IV, M=251 7.3%



3.6%

V, M=283

FIGURE 2 GS-MS chromatogram (a) and photodegradation products obtained for acetochlor photolysis (b)

plus CH_3CO, and C_2H_5O plus CH_3CO with concurrent hydrogen migrations, respectively. This molecule appeared as the major photodegradation product accounted for 12% in the mixture. Product **IV** was tentatively identified as 2-hydroxy-N-(2-ethyl-6-methylphenyl)acetamide (MW=251). The identification was confirmed by matching the spectrum with that of the synthesized reference material. The fragmentation of this molecule resulted in ions at *m/z* 222, 205, and 192 which may be due to the loss of C_2H_5, C_2H_5O, and $COCH_2OH$. Photodegradation product **V** was identified as 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(propyloxymethyl) acetamide (MW=283) and its structure was confirmed by comparision with synthesized reference material. The major ions in its EI spectrum were at *m/z* 223 ($M-C_3H_7O$), 174 ($M-C_3H_7O-CH_2Cl$), 146 ($M-C_3H_7O-COCH_2Cl$).

Sunlight photodegradation of metolachlor resulted in structurally analogous molecules[5] to componds **III** and **IV** indicating that dechlorination and hydroxylation reactions are common pathways in the photolysis of chloroacetanilides. N-Dealkylation also seems to be an important transformation following irradiation of the acetochlor. Dehydrochlorination with subsequent morpholine ring formation was also reported as metolachlor photoproduct[5]. Indoline ring formation took place in sunlight degradation of alachlor[14]. However, we could not observe chloroacetyl-indoline formation under our photolytic conditions.

TABLE I Shoot growth inhibition by soil and photolytic degradation products of acetochlor

Compound	*Shoot fresh weight (% of control)*[a]		
	Maize	*Oat*	*Ryegrass*
Acetochlor	53[b]	0[b]	0[b]
Deethoxymethyl acetochlor	100	100	77
Chloroacetyl-indoline	100	64	36
Photoproduct IV	92	7	0
Photoproduct V[b]	90[b]	0[b]	0[b]

a. At 10 kg/ha rates.
b. At 1.25 kg/ha rates.

Phytotoxicities of degradation products

In growth-response experiments using maize, oat and ryegrass, seedlings phytotoxicity for degradation products was characterized by the magnitude of their shoot growth inhibition. Microbial degradation products formed in soil incuba-

tion studies were less phytotoxic to the test plants than parent acetochlor (Table I). However, photodegradation product **V** exhibited comparable herbicidal efficacy with that of acetochlor. Compounds **I** and **III** had very low residual toxicity (data not shown). Since only marginal phytotoxicities were observed for most of the degradation products we can conclude that both soil and photodegradation of acetochlor is a part of its detoxication after application.

References

[1] I. Jablonkai and F. Dutka, *J. Radioanal. Nucl. Chem., Letters*, **94**, 271–280 (1985).
[2] E.J. Breaux, *J. Agric. Food Chem.*, **34**, 884–888 (1986).
[3] P.C.C. Feng, *Pestic. Biochem. Physiol.*, **40**, 136–142 (1991).
[4] G. Chesters, G.V. Simsiman, J. Levy, B.J. Alhajjar, R.N. Fathulla and J.M. Harkin, *Rev. Environ. Contam. Toxicol.*, **110**, 1–74 (1989).
[5] J. Kochany and R.J. Maguire, *J. Agric. Food Chem.*, **42**, 406–412 (1994).
[6] I. Jablonkai and F. Dutka, *J. Agric. Food Chem.*, **40**, 506–508 (1992).
[7] I. Jablonkai and K.K. Hatzios, *J. Agric. Food Chem.*, **41**, 1736–1742 (1993).
[8] J.M. Tiedje and M.L. Hagedorn, *J. Agric. Food Chem.*, **23**, 77–81 (1975).
[9] L.L. McGahen and J.M. Tiedje, *J. Agric. Food Chem.*, **26**, 414–419 (1978).
[10] D.B. Sharp, in: *Herbicides: Chemistry, Degradation and Mode of Action* (P.C. Kearney and D.D. Kaufman, eds. Dekker, New York, 1988) pp. 301–333.
[11] H.M. LeBaron, J.E. McFarland, B.J. Simoneaux, and E. Ebert, in: *Herbicides: Chemistry, Degradation and Mode of Action* (P.C. Kearney and D.D. Kaufman, eds. Dekker, New York, 1988) pp. 335–382.
[12] M. Hussain, I.P. Kapoor, C.C. Ku, and S. Stout, *J. Agric. Food Chem.*, **31**, 232–236 (1983).
[13] G.L. Lamoreux and D.G. Rusness, *Pestic. Biochem. Physiol.*, **34**, 187–204 (1989).
[14] C.H. Fang, *J. Chinese Agric. Chem. Soc.*, **15**, 53–59 (1977).