Factors Influencing Transformation Rates and Formation of Products of Phenylurea Herbicides in Soil

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Transformation rates of phenylurea herbicides and their products were measured in native soil, sterile soil, soil suspensions, and soil inoculated with pure cultures of microorganisms. In native soil, transformation rates generally increased with decreasing adsorption of the herbicides, but correlations with adsorption coefficients were poor. In sterile soil, substitution patterns of the compounds influenced transformation rates. In soil suspensions, transformation rates increased with lipophilicity of the herbicides. In sterilized soil inoculated with specific microorganisms, transformation was mainly influenced by substrate specificities of the microorganisms to reactive sites of the phenylureas. In all cases, N-demethylation was an important, but not the only, transformation pathway. The data indicate that transformation rates of phenylureas in soils are affected by several parameters, related to the soil, the compounds, and the type of transformation. Although the results were gained in the laboratory under artificial conditions, they form a basis to establish quantitative structure−reactivity relationships and provide explanations for quality and quantity of the formed products.

Keywords: *Phenylureas; transformation; metabolism; adsorption; microorganisms; structure−reactivity relationships*

INTRODUCTION

Phenylurea herbicides are transformed in soil by dealkylation, followed by cleavage of the phenylurea bridge yielding aniline (Geissbühler, 1969). These products can form bound residues (Azam et al., 1988) or condense into the corresponding azobenzenes (Pieuchot et al., 1996). Hydroxylation of alkyl side chains and mineralization of the phenyl ring have also been observed (Mudd et al., 1983; Lehr et al., 1996a; Perrin-Garnier et al., 1996). Photolysis leads to demethylation and ring hydroxylation (Faure and Boule, 1997; Jirkovsky et al., 1997). Although the transformation mechanism is very similar for all phenylureas, their transformation rates are quite different.

Correlations of structure and transformation rates of pesticides in soils have only occasionally been reported. The availability of the pesticides is considered to have a high importance. It can be assessed from the adsorption coefficient (Briggs, 1990). Also, electronic properties and hydrophobicity were found to correlate with transformation of pesticides in soil, for example, for propyzamides, triazols, and triazines (Cantier et al., 1986; Patil et al., 1988; Kaune, 1997).

Phenylureas can be transformed in liquid cultures of soil microorganisms. Electronic properties have an important influence on the transformation of the herbicides by the bacterium *Bacillus sphaericus* (Engelhardt et al., 1973; Berger, 1993). Lipophilicity, however, was the main factor correlating with the transformation by soil fungi (Berger, 1998). No information is available on the transformation of phenylureas by defined microorganisms in a soil environment.

The purpose of our investigation was to explain the differences of transformation rates of different phenylurea herbicides in soil. Therefore, investigations in native and sterile soil, soil suspensions, and soil inoculated with microorganisms were performed to elucidate the effects of (i) adsorption to the soil, (ii) substrate specificities of microorganisms, and (iii) structural elements susceptible to chemical transformation. Transformation, meaning any changes in the molecular structure, of the parent other than mineralization was chosen as the endpoint because the phenyl ring is not completely mineralized, but bound residues are also formed.

MATERIALS AND METHODS

Soils and Chemicals. Samples were collected from the top 10 cm of three arable soils near Göttingen, Germany. The soil from Reinshof has 3.2% sand, 74.9% silt, 21.9% clay, a pH (0.01 M $CaCl_2$) of 7.3, 1.81% organic carbon, and a water holding capacity of 42.2%; the soil from Waake has 84.0% sand, 8.9% silt, 7.1% clay, a pH (0.01 M $CaCl_2$) of 5.8, 0.80% organic carbon, and a water holding capacity of 32.7%; and the soil from Weende has 6.3% sand, 78.9% silt, 14.8% clay, a pH (0.01 M $CaCl_2$) of 7.3, 1.0% organic carbon, and a water holding capacity of 47.3%. The total nitrogen content in these soils varies between 10 and 30 mg of N/kg for the soil layer 0−5 cm [if not sampled immediately after fertilization, refer to Berger and Heitefuss (1991)]. The soils were sieved through a 2 mm mesh size sieve and stored moist at 4 °C until use. In separate samples, the soil moisture content was measured on an oven-dry basis.

Herbicide standards and metabolites with their abbreviations are listed in Table 1. They were purchased from Riedel

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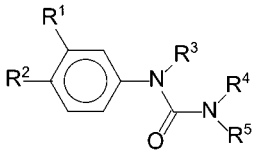
Table 1. Structures of the Phenylureas Investigated



compound (abbreviation)	R^1	R^2	R^3	R^4	R^5
fenuron (F)	−H	−H	−H	$-CH_3$	$-CH_3$
monuron (M)	−H	−Cl	−H	$-CH_3$	$-CH_3$
diuron (D)	−Cl	−Cl	−H	$-CH_3$	$-CH_3$
isoproturon (IPU)	−H	$-CH(CH_3)_2$	−H	$-CH_3$	$-CH_3$
chlorotoluron (CLT)	−Cl	$-CH_3$	−H	$-CH_3$	$-CH_3$
monolinuron (ML)	−H	−Cl	−H	$-OCH_3$	$-CH_3$
linuron (L)	−Cl	−Cl	−H	$-OCH_3$	$-CH_3$
metobromuron (MB)	−H	−Br	−H	$-OCH_3$	$-CH_3$
chlorbromuron (CB)	−Cl	−Br	−H	$-OCH_3$	$-CH_3$
methabenzthiazuron (MBT)		−benzthiazolyl	$-CH_3$	$-CH_3$	−H
3-(3,4-dichlorophenyl)-1-methoxyurea (DMXU)	−Cl	−Cl	−H	$-OCH_3$	−H
3-(3,4-dichlorophenyl)-1-methylurea (DMU)	−Cl	−Cl	−H	−H	$-CH_3$
3-(4-chlorophenyl)-1-methoxyurea (CMXU)	−H	−Cl	−H	$-OCH_3$	−H
3-(4-chlorophenyl)-1-methylurea (CMU)	−H	−Cl	−H	−H	$-CH_3$
3-[4-(1-methylethyl)phenyl]-1-methylurea (MMU)	−H	$-CH(CH_3)_2$	−H	$-CH_3$	−H
3-[4-(1-methylethyl)]phenylurea (MU)	−H	$-CH(CH_3)_2$	−H	−H	−H
3-(3-chloro-4-methylphenyl)-1-methylurea (CPMU)	−Cl	$-CH_3$	−H	$-CH_3$	−H
3-(3-chloro-4-methyl)phenylurea (CPU)	−Cl	$-CH_3$	−H	−H	−H

de Haen (Seelze, Germany) or provided by Hoechst AG (Frankfurt, Germany), Ciba Geigy Limited (Basel, Switzerland), and Bayer AG (Leverkusen, Germany). The labeled chemicals [*phenyl*-U-^{14}C]chlorotoluron (2.99 MBq/mg) and [*phenyl*-U-^{14}C]metobromuron (1.14 MBq/mg) were gifts from Ciba Geigy Limited, and [*phenyl*-U-^{14}C]isoproturon (2.01 MBq/mg), [*phenyl*-U-^{14}C]monolinuron (2.16 MBq/mg), and [*phenyl*-U-^{14}C]linuron (1.29 MBq/mg) were from Hoechst AG.

Microorganisms. Isolation, origin, and cultivation of the microorganisms *Phoma* sp., *Rhizoctonia solani* (Kühn), *Cunninghamella echinulata* (Thaxter), *Aspergillus niger* (van Tieghem), *Botrytis cinerea* (Pers. ex Nocca and Balb.), *Rhizopus japonicus* (Vuill.), and *Bacillus sphaericus* (Meyer and Neide) have been described earlier (Berger, 1998).

Analytical Procedures. High-performance liquid chromatography (HPLC) was done with a 300 C pump and a 250 B gradient former from Gynkotek (Munich, Germany), a 2157 autosampler and a 2155 oven from LKB Pharmacia (Uppsala, Sweden), and an SPD-6A variable wavelength detector from Shimadzu (Kyoto, Japan). Simultaneous determination of the 10 phenylureas was done as described earlier (Berger, 1997); experiments with only one herbicide used elution with isocratic acetonitrile + water (45 + 55 by volume). Liquid scintillation counting was performed with a Packard 2200 CA.

Transformation Studies. For the studies in native soil, moist soil was incubated at 20 °C for 7 days to accustom the soil microflora. Phenylureas were thoroughly mixed with soil (50 g of dry weight) using either a solution of all 10 herbicides (1.0 mL, 0.1 mg/mL each in methanol) or of single herbicides (1.0 mL, 0.1 mg/mL in methanol) by applying the herbicide solution with a 1 mL syringe to the soil, which was evenly spread out on a glass Petri dish (diameter = 15 cm). For some experiments, ground untreated straw (0.500 g) and/or an aqueous solution of NH_4NO_3 (1.0 mL, 30.1 mg/mL) were added. These amounts correspond to the addition of straw (75 dt/ha) and nitrogen (160 kg/ha) as fertilizers, presuming a distribution in the top 5 cm of the soil. The soil moisture was then adjusted to 20 or 40% (w/w) with water. The samples were placed in glass containers and incubated at 20 or 30 °C in the dark. Soil moisture was adjusted during the investigation period, and samples were analyzed in regular intervals.

Transformation in sterile soil was measured similarly after the soil had been autoclaved (121 °C, 15 min) on 3 consecutive days. The autoclaved soil was handled under a sterile hood and placed in sterile glass containers, and distilled sterile water was used to maintain soil moisture. As transformation in sterile soil at 20 °C is too slow to determine transformation rates, the sterile samples were incubated at 60 °C.

For studies on mineralization, mixtures of both unlabeled and labeled herbicides (linuron, monolinuron, metobromuron, isoproturon, and chlorotoluron) in methanol were added to 25 g of soil to yield concentrations of 2 μg/g soil and 200 Bq/g of soil. The flasks were sealed and incubated without shaking at 20 and 30 °C. CO_2 was trapped in vials with NaOH (1 mL, 0.1 M). At regular intervals, the NaOH solutions were replaced and the radioactivity was measured in the trapping solution. To compare transformation with mineralization, parallel experiments were performed with unlabeled herbicides only.

Transformation of phenylureas in soil suspensions was measured with soil from Reinshof and Waake. Soil (20 g) was incubated with half-strength nutrient broth according to the procedure of Joshi et al. (1985). The herbicide solution (1 mL, 0.1 mg/mL of each of the 10 phenylureas) was added immediately. Incubation was performed on a horizontal shaker with 100 rpm at 28 °C. Samples were taken in regular intervals by mixing an aliquot of 0.5 mL with 0.5 mL of an internal standard solution (5 μg/mL chloroxuron in acetonitrile). After centrifugation (15000*g*, Biofuge A, Heraeus, Germany), they were analyzed with HPLC.

For the transformation studies of the phenylureas in soil by defined microorganisms, the soil (50 g of dry weight) was mixed with supplements (0.500 g of cellulose, 0.500 g of starch, 0.500 g of ground, untreated straw, or 0.163 g of peptone) and then autoclaved (15 min, 121 °C) on 3 consecutive days. Five agar plates, on which the respective microorganism has been grown, were homogenized (with 300 mL of sterile water), and this solution (5 mL) was carefully mixed with the soil as described above. After incubation at 20 or 30 °C for 7−14 days, samples were treated with herbicide solutions as described above. All manipulations were done under a sterile hood.

Adsorption Coefficients. Adsorption coefficients in the soils were determined according to the method of Nicholls et al. (1982) at concentrations of 0.10, 0.05, 0.02, and 0.01 mg of each compound. Adsorbed amounts were calculated by finding the differences from initial concentrations. Adsorption coefficients [(micrograms of compound per gram of soil) divided by (micrograms of compound per gram of supernatant)] were calculated for the concentration range.

Data Analysis. Pseudo-first-order transformation rate constants were calculated according to the method of Timme et al. (1986). Correlation coefficients were >0.85 in all cases. If transformation did not follow first-order reaction kinetics or half-lives were not reached in the investigation period, the percentage transformation is given. Adsorption coefficients were calculated from the slopes after least-squares linear regression analysis. All values are means of three replicates.

Table 2. Half-Lives ($t_{1/2}$) ± Confidence Intervals (in Days) and Percent Transformation (within 28 Days) of Isoproturon and Linuron after Application of the Herbicide Alone or in Combination with Nine Other Phenylureas with and without the Addition of Straw and/or Nitrogen (NH_4NO_3), Soil Reinshof, 20 °C

supplement	herbicide application	isoproturon alone	isoproturon in combination	linuron alone	linuron in combination
no supplement	$t_{1/2}$	5 ± 0.3	9 ± 1	28 ± 4	>28[a]
	% transformation	97	86	52	24
straw	$t_{1/2}$	9 ± 1	8 ± 1	15 ± 2	>28[a]
	% transformation	86	90	77	31
nitrogen	$t_{1/2}$	10 ± 1	11 ± 2	36 ± 7[b]	>28[a]
	% transformation	84	76	48	23
straw and nitrogen	$t_{1/2}$	6 ± 1	8 ± 1	10 ± 1	24.8 ± 6.7
	% transformation	94	89	83	49

[a] The half-life was not reached within the investigation period of 28 days, and the reaction kinetic did not fit pseudo-first-order. [b] The half-life was not reached within the investigation period, but the reaction kinetic fitted pseudo-first-order.

Table 3. Half-Lives ± Confidence Intervals (in Days) and Adsorption Coefficients (± Standard Error) of Phenylurea Herbicides in Three Soils with Straw and Nitrogen (NH_4NO_3) (Ranked According to the Sum of the Half-Lives in All Three Soils; for Abbreviations, Refer to Table 1)

soil	MBT	D	MB	M	ML	CB	L	CLT	F	IPU
Reinshof										
30 °C, straw + NH_4NO_3	52 ± 22	27 ± 9	27 ± 6	30 ± 7	28 ± 6	26 ± 6	21 ± 5	10 ± 2	18 ± 5	5 ± 1
adsorption coefficient	3.8 ± 0.1	1.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.01	0.5 ± 0.1	4.6 ± 0.2	2.8 ± 0.1	0.7 ± 0.03	0.0 ± 0.0	0.3 ± 0.03
Waake										
30 °C, straw + NH_4NO_3	36 ± 14	23 ± 8	17 ± 4	11 ± 3	14 ± 4	19 ± 4	18 ± 4	10 ± 3	8 ± 2	4 ± 1
adsorption coefficient	2.6 ± 0.1	1.4 ± 0.1	0.8 ± 0.03	0.5 ± 0.04	0.6 ± 0.04	3.3 ± 0.2	2.2 ± 0.1	0.7 ± 0.02	0.2 ± 0.01	0.5 ± 0.01
Weende										
20 °C, no supplements	59 ± 11	63 ± 8	55 ± 7	56 ± 6	55 ± 9	51 ± 12	55 ± 3	42 ± 4	31 ± 3	14 ± 1
adsorption coefficient	2.2 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	2.0 ± 0.04	1.8 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.5 ± 0.1

Correlation coefficients were >0.96 in all cases. Correlations between reaction rates from different soils and between reaction rates and both adsorption coefficients and log K_{ow} were done with Molecular Analysis Pro (WindowChem Software, Fairfield, CA) and kinetic calculations with Kinetics (M. Cipollone, The 4S Co., Athens, GA). Cross-validation tests of the correlations were done by leaving out each observation once, calculating the regression equation, and checking if the missing observation can be predicted with the new model.

RESULTS AND DISCUSSION

Transformation in Native Soil. For comparison of transformation rates of pesticides, they should be determined simultaneously in the same soil sample to ensure equal conditions. In our studies, this resulted in an overall herbicide concentration calculated on the bases of the amount sprayed in agricultural practice assuming a distribution in the top 0.5 cm soil layer—in contrast to the experiments with single compounds, in which the concentrations are calculated on the basis of a soil distribution in the top 5 cm layer.

When 10 phenylureas were applied to the same soil, the transformation rates were significantly decreased, compared to the experiments in which a single herbicide was applied (refer to Table 2). This can be explained by the higher phenylurea concentration and has been observed earlier for other pesticides (Domsch, 1992; Berger et al., 1996). Our results indicate that herbicides with a long half-life, such as linuron, were more prone to this inhibiting effect than the relatively quickly transformed herbicide isoproturon. Therefore, enzymatic preferences could be an explanation, although in no case were transformation rates increased.

To compensate for this inhibiting effect, we stimulated the soil microflora by adding different supplements. The results are shown in Table 2 for linuron and isoproturon. The half-life of linuron—alone, not in combination—was significantly reduced after the addition of straw. Straw is known to stimulate pesticide transformation due to accelerated microbial activity (Hurle, 1982) or formation of bound residues (Azam et al., 1988). Addition of only nitrogen, however, resulted in slower transformation. This has also been described earlier for methabenzthiazuron and atrazine (Berger et al., 1996; Gan et al., 1996). It can be explained by changes in the soil microflora (Gan et al., 1996), for example, a reduced activity of those organisms able to degrade the pesticides, by fungitoxic effects due to increased salt concentrations, or because fertilizer nitrogen would serve as a more readily usable nitrogen source than the phenylurea nitrogen to soil microorganisms. In this study, the best stimulation of herbicide transformation in native soil could be obtained with a mixture of straw and nitrogen. This has been also observed by Yassir et al. (1998) for cellulose and ammonium, which stimulated the microbial N-demethylation of atrazine. Compared to the added amounts, the natural nitrogen contents in our soils were irrelevant. As an increase in temperature normally results in lower half-lives [refer to Jurado-Exposito and Walker (1998)], we further accelerated phenylurea transformation by incubation at 30 °C. Chemical hydrolysis in soil at this temperature was investigated and proved to be insignificant (data not shown). Prolonged investigation periods could not always result in measurable half-lives, as transformation rates can level out during the investigation period (data not shown) and suffer from decreasing microbial activities. Similar results as with linuron were obtained with the other eight phenylureas under investigation as well as in the soil from Waake (data not shown).

Table 3 shows the half-lives for all 10 phenylureas in three soils after simultaneous application. It has to be pointed out that the half-lives obtained in this study are relative and can be assessed only under these conditions, but they enable the comparison of half-lives under identical experimental conditions. In all three soils, isoproturon was transformed most quickly, followed by fenuron and chlorotoluron. Methabenzthiazuron, diuron, metobromuron, and monuron were most persistent. Although persistence generally increased

Table 4. Half-Lives ± Confidence Intervals (in Days) of Phenylurea Herbicides and Their Corresponding Products in Native Soil (Waake) at 20 °C and in Sterile Soil (Reinshof) at 60 °C (for Abbreviations, Refer to Table 1)

herbicide	soil	parent molecule	product	product
monolinuron	native (Waake)	ML	CMU[a]	CMXU[c]
		20 ± 2	17 ± 1	0.3 ± 0.01
linuron	native (Waake)	L	DMU[a]	DMXU[c]
		29 ± 5	24 ± 11	1 ± 0.04
isoproturon	native (Waake)	IPU	MMU[b]	MU[d]
		6 ± 0.4	10 ± 1	9 ± 1
chlorotoluron	native (Waake)	CLT	CPMU[b]	CPU[d]
		7 ± 1.0	12 ± 2	7 ± 1
monolinuron	sterile (Reinshof)	ML	CMU[a]	CMXU[c]
		4 ± 1	48 ± 28	2 ± 0.2
linuron	sterile (Reinshof)	L	DMU[a]	DMXU[c]
		5 ± 1	41 ± 11	3 ± 1
isoproturon	sterile (Reinshof)	IPU	MMU[b]	MU[d]
		4 ± 0.1	22 ± 6	11 ± 2
chlorotoluron	sterile (Reinshof)	CLT	CPMU[b]	CPU[d]
		4 ± 0.2	35 ± 24	22 ± 7

[a] Monodesmethoxylated product, with one methyl group. [b] Monodesmethylated product, with one methyl group. [c] Monodesmethylated product, with one methoxy group. [d] Didesmethylated product, neither methyl substituent nor methoxy substitution at the nitrogen of the urea moiety.

with higher adsorption, correlations of transformation rates with adsorption coefficients were poor. As all 10 phenylureas were present in the same soil sample, preferences of soil microorganisms for specific structural elements of some phenylureas seem to be the explanation for this effect, not differences in microbial activity, distribution of organic material, or local changes in pH values. A specificity of some microorganisms toward selected phenylureas has been observed by Cox et al. (1996).

Metabolism in Native Soil. Demethylated and demethoxylated products could be detected in significant amounts. No anilines were found. Studies with radiolabeled herbicides demonstrated that no measurable $^{14}CO_2$ was released within 56 days (data not shown), indicating that a significant mineralization of the phenyl ring did not occur under our experimental conditions. This is in accordance with the results from Perrin-Garnier et al. (1996), who reported only very little mineralization of the phenyl ring of isoproturon. Lehr et al. (1996b), Pieuchot et al. (1996), and Johnson et al. (1998) observed mineralization of the phenyl ring of that particular herbicide to a certain extent (14−23% in 67 days, 10−34% in 120 days, and 13−39% in 49 and 21 days, respectively). Divergent results regarding $^{14}CO_2$ evolving in different soils from ring-labeled phenylureas can be caused by different activities of the microorganisms due to specific experimental conditions such as soil moisture, temperature, and pH of the soil (Cox et al., 1996; Lehr et al., 1996b; Pieuchot et al., 1996) or by possible alternative substrates for bacteria mineralizing the phenyl ring.

To obtain data for kinetic calculations, transformation studies were undertaken with parent molecules and their respective products. Table 4 shows the results for the sandy soil (Waake). The half-lives of the products with one methyl group (CMU, DMU, MMU, CPMU) were similar to those of the parents, which has also been observed by Roberts et al. (1998) in pure culture studies with isoproturon and its demethylated metabolite.

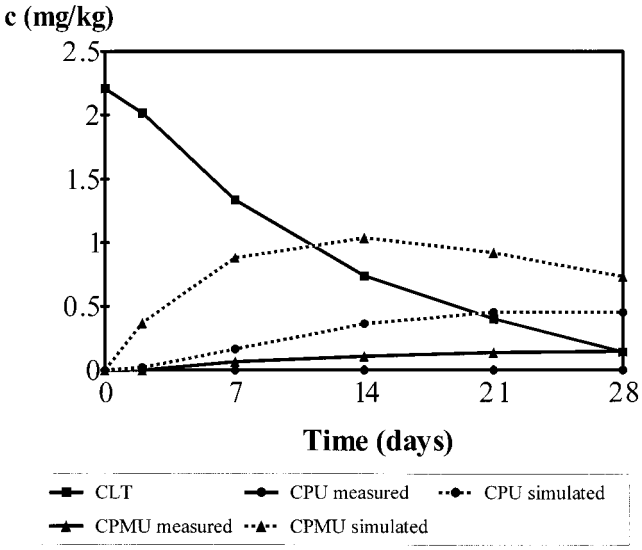


Figure 1. Transformation of chlorotoluron (CLT) and measured as well as simulated concentrations of the products after one (CPMU) and two (CMU) demethylation steps, in a soil (Waake) amended with straw and nitrogen and incubated at 30 °C.

These products can be observed during transformation studies with the parents. Didesmethylated products (MU and CPU) cannot be metabolized by oxidative N-demethylation any further. Nevertheless, they were transformed more quickly than the parents, which is in contradiction to the pure culture studies of Roberts et al. (1998), and it indicates the importance of transformation reactions such as hydrolysis of the urea moiety or hydroxylation reactions. Metabolites with a methoxy group (CMXU and DMXU) had significantly lower half-lives than the corresponding parents. The combination of the low negative atomic charge at the urea moiety (Berger, 1993) with the reduced steric hindrance due to demethylation may be the cause for this fast transformation. This observation is supported by the fact that these products were never found in transformation studies in native soil.

The measured transformation rates were used in the model Kinetics to simulate production and further transformation of the products. Therefore, transformation rates were calculated from measured half-lives. As the transformations followed pseudo-first-order reaction kinetics, concentrations of the parent and the transformation products could then be calculated for each day of the investigation period using the kinetic expressions for the consecutive transformation of the parents to the first and second transformation product. Measured concentrations of demethylated or demethoxylated products were significantly lower than those calculated. This result is demonstrated for chlorotoluron in Figure 1. It stresses that, although N-demethylation is the most important pathway, other reactions are also important. Hydroxylations of the aromatic moiety and alkyl side chains, in particular, have been observed by Tillmanns (1976), Mudd et al. (1983), and Lehr et al. (1996a) and could explain the overestimation of demethylated products in our kinetic studies, in which we assumed oxidative N-demethylation was the only pathway.

Transformation and Metabolism in Sterile Soil. No comparative studies have been undertaken until now to investigate the difference in chemical breakdown

Table 5. Percent Transformation of Phenylurea Herbicides in Sterilized Soil after Inoculation with Defined Microorganisms and the Addition of Supplements, Soil from Reinshof, $T = 30$ °C, and in Soil Suspensions, $T = 28$ °C (Ranked According to HPLC Elution Time from a Nucleosil RP-18 Column; for Abbreviations, Refer to Table 1)

	F	M	MBT	CLT	ML	IPU	D	MB	L	CB	supplement
microorganism											
R. japonicus[a]	30	2	73	20	28	49	11	11	12	7	starch
A. niger[b]	0	44	94	37	50	63	54	54	52	53	starch
Phoma sp.[b]	17	55	40	41	64	49	61	67	61	57	starch
C. echinulata[b]	51	51	52	55	71	75	53	68	57	55	cellulose
R. solani[a]	0	0	7	10	5	35	4	20	8	12	cellulose + peptone
B. cinerea[c]	14	21	14	36	59	54	10	41	46	47	starch
B. sphaericus[a]	5	5	19	12	76	6	6	67	66	64	straw
soil suspension											
Reinshof[b]	58	60	88	66	70	70	69	81	85	100	half nutrient broth
Waake[b]	10	13	60	35	38	10	43	59	72	100	half nutrient broth

[a] Investigation period of 28 days. [b] Investigation period of 56 days. [c] Investigation period of 84 days.

rates between parents and products. At 20 °C, the compounds are quite stable and hydrolysis was too slow to determine the rates of all compounds in a reasonable time. Therefore, our investigations were performed at 60 °C. The results are shown in Table 4.

Products with a methoxy substitution (CMXU, DMXU, ML, and L) were hydrolyzed very quickly. This can be explained with the destabilizing effect of the methoxy group (Berger, 1993). Products with one methyl group left, such as CMU, DMU, MMU, and CPMU, were hydrolyzed much more slowly than the parents or the other products. Steric effects are most likely of importance. Both observations are in accordance with the transformation studies in native soils with the respective parents, by which mainly products with one methyl group could be observed.

Transformation in Soil Inoculated with Microorganisms. Substrate specificities of a number of soil microorganisms for phenylureas are known from pure culture studies (Berger, 1998). The investigation of phenylurea transformation in sterilized soil inoculated with these microorganisms should provide additional information on the importance of these substrate specificities and of adsorption for transformation rates in soil.

The difficulties of such experiments have been outlined earlier (Goldstein et al., 1985). To be nevertheless able to achieve measurable transformation in soil by the microorganisms, supplements had to be added for their stimulation. This is in accordance with previous studies (Goldstein et al., 1985; Greer and Shelton, 1992). In preliminary experiments, starch and cellulose, both with and without peptone as an additional nitrogen source, gave better results than straw, as they are easily metabolized. As an example, for *A. niger*, 24% of isoproturon was transformed within 28 days without supplement; the addition of straw, cellulose, and starch resulted in transformations of 15, 45, and 86%, respectively; the addition of peptone had no influence (23% transformation); and the combination of the organic supplements with peptone also did not significantly increase the transformation compared with the organic supplements alone. Starch, an easily degradable supplement, was found to be suitable for most organisms. Inorganic nutrients did not accelerate, but even slowed, transformation. This was also observed by Gan et al. (1996) for atrazine and by Berger et al. (1996) for methabenzthiazuron. This stresses that the transformation of phenylureas by the investigated soil microorganisms is cometabolic. Increased investigation periods were used for some microorganisms (up to 84 days) but are not always suitable. Analyses of samples taken during the investigation period (data not shown) demonstrated that transformation of the compounds can considerably slow during long investigation periods.

For the simultaneous transformation study of all 10 phenylureas, the soil was amended with the supplement that gave the best performance in the preliminary experiment. For *B. sphaericus*, soil moisture had to be adjusted to 40% to achieve measurable transformation.

The results in Table 5 show the differences in the ability of the single microorganisms to degrade phenylureas in a soil environment. Some fungi had substrate specificities for certain herbicides: *R. japonicus* for methabenzthiazuron, isoproturon, and fenuron; *A. niger* for methabenzthiazuron and isoproturon; and *R. solani* for isoproturon. Other fungi, such as *C. echinulata*, *Phoma* sp., and *B. cinerea*, degraded most of the 10 herbicides to a similar extent. Adsorption did not influence transformation. *B. sphaericus* behaved differently compared with the soil fungi. Transformation in soil was related to the substitution pattern of the urea moiety, as only herbicides with a methoxy substituent were transformed in significant amounts.

Metabolism in Soil Inoculated with Microorganisms. The metabolism of phenylureas in the sterilized soil inoculated with specific microorganisms was different from their metabolism in native soil. Products resulting from N-demethylation accumulated in significant amounts. Even DMXU and CMXU reached up to 10% of the initial herbicide concentration. These products result from N-demethylation of methyl-methoxy-substituted phenylureas and could never be observed in native soils, where their rapid transformation is probably due to fast microbial attack by other microorganisms. This underscores the importance of microbial communities for the mineralization of phenylureas, which has been pointed out earlier by Roberts et al. (1993) and Lehr et al. (1996a). Possible modified sorption capacities due to autoclaving cannot explain the differences in the metabolisms between inoculated soil and native soil. In addition, no visible alteration of the soil surfaces was observed.

Figure 2 shows the transformation of IPU and the formation of its monodesmethylated product (MMU) in soil after inoculation with *R. japonicus* as a representative transformation curve. IPU transformation started after an initial lag period of 7 days, and at the end of the investigation period 66% of the herbicide was transformed. The concentration of the product reached only 38% of the initial herbicide concentration. In studies with *R. solani*, *B. cinerea*, and *C. echinulata*

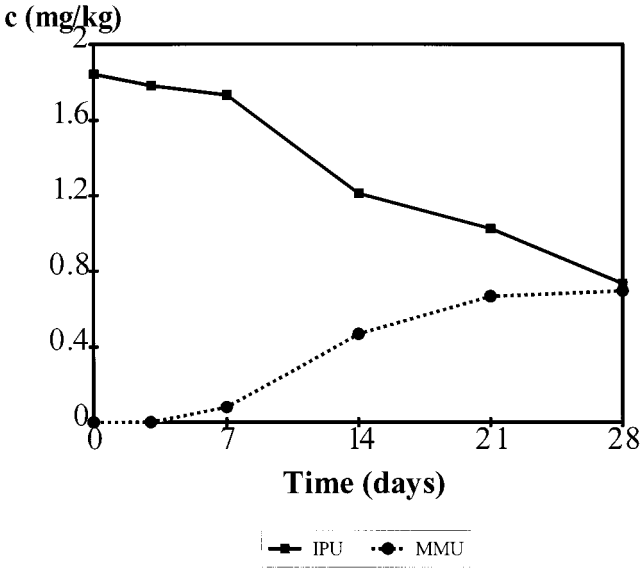


Figure 2. Transformation of isoproturon (IPU) and formation of the product after demethylation (MMU) in a sterilized soil (Reinshof) amended with starch and inoculated with *R. japonicus* at 30 °C.

concentrations of N-demethylated products such as MMU and CMU were lower than 15% of the initial concentration of the parents. This again indicates that transformation processes other than N-demethylation must have been significant, for example, the formation of hydroxylated metabolites, which have been observed in pure cultures for some of these microorganisms (Tillmanns et al., 1978; Engelhardt et al., 1979; Ozaki and Hayase, 1983; Berger, 1998). As *B. sphaericus* cleaves the urea bridge of phenylurea herbicides in the process yielding aniline (Engelhardt et al., 1973), no demethylated or demethoxylated products were observed in our investigation. Anilines were also never detected in soil because, according to Azam et al. (1988), they can form bound residues and thus lose their extractability.

The results indicate that the metabolism of phenylureas in sterilized soil inoculated with soil microorganisms is similar to the metabolism observed in pure cultures (Berger, 1998) but not to the metabolism observed in native soil.

Transformation in Soil Suspension. Transformation studies in soil suspension give information about the substrate specificities of native soil microbial communities, as adsorption is not important under these conditions. The use of the protocol from Roberts et al. (1993) for isolation of an enrichment culture able to transform phenylureas was not successful, and the protocol suggested by Joshi et al. (1985) for sulfonylureas led to measurable transformation in the soil suspension only after the amount of soil added as inoculum was significantly increased. The results are shown in Table 5.

Transformation increased with increasing log K_{ow} of the compounds as a parameter for lipophilicity, and the correlations could be cross-validated if fenuron was excluded from the calculations ($r = 0.84$ and 0.89 for Reinshof and Waake soils, respectively). Lipophilic compounds can more easily pass through the membranes of microorganisms than hydrophilic compounds. This can become the time-limiting step under conditions when sorption to soil is not or less important—as in soil suspension. The effect has also been observed in pure culture studies of some soil fungi (Berger, 1998). As in native soils, the transformation rates were different in the two soil suspensions. This can be explained by different microbial communities. The correlation of the amounts transformed in the two soil suspension ($r = 0.93$) indicates, however, that the substrate specificities of the native microbial populations toward phenylureas are similar in both soils.

Explanations for the Different Transformation Rates (Structure−Reactivity Relationships). The correlation between reaction rates in soil from Weende and Waake ($r = 0.88$; a calculation with the soil from Weende is not useful due to different incubation conditions) indicates also that the spectra of organisms able to biotransform the phenylureas in these two soils are similar. Specific capabilities of certain microorganisms to biotransform some herbicides are obviously compensated in microbial communities of native soil.

For the biotic transformation of complex molecules, such as pesticides, relationships between transformation and molecular descriptors are obviously very complex. Different attempts to establish such relationships have failed (Pussemier et al., 1980; Bastide et al., 1981; Patil et al., 1988). Kaune (1997), however, found a relationship between mineralization of triazines and parameters describing molecular size and solubility. According to Briggs (1990), there is evidence that the persistence of a given compound in soil is related to both adsorption and degradability of its functional groups. This was confirmed for phenylureas during this study, as, although transformation in native soil increased with decreasing adsorption of the compounds, cross-validation of the correlation was negative. This is an indication that other factors besides adsorption, such as the reactivity of certain structural elements, are important for the estimation of the reaction rates.

The poor correlations of reaction rates measured in soil inoculated with defined microorganisms with adsorption coefficients support these results. In some cases, specific reactive sites of the molecules significantly affected transformation rates. An example that can be assessed with molecular charges is the transformation of phenylureas by *B. sphaericus* in soil. This has already been demonstrated for the reaction of the isolated enzyme from the same bacterium (Berger, 1993).

In soil suspensions, where the availability of the compounds is not limited by adsorption, transformation increased with higher lipophilicity. This confirms earlier results with pure cultures of soil microorganisms (Berger, 1998).

The results of the experiments under the different conditions demonstrate that transformation of phenylurea herbicides is influenced by the following:

- the lipophilicity of the compounds—as demonstrated in soil suspensions. This could be assessed with descriptors such as log K_{ow}.
- the availability of the compounds to the soil microorganisms (this was, however, never the only factor)—as demonstrated in experiments with native soil. The availability could be assessed with adsorption coefficients.
- the reactivity of specific structural elements—as demonstrated in inoculated and sterile soil. This could be assessed with quantum chemical parameters.

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