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Registry No. Glyphosate, 1071-83-6; (aminomethyl)-phosphonic acid, 1066-51-9.

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Degradation of Dinocap in Three German Soils

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The degradation of [*ring-U*-^{14}C]dinocap in three German soils was investigated under laboratory conditions using 100 g of soil and uniform distribution of 9 mg of dinocap/kg of soil. The mineralization of the ring carbon was monitored at 15 and 25 °C with 50% of the maximum moisture capacity. Organic amendments in the form of alfalfa meal (0.5 g of dry mass/100 g of soil) stimulated the degradation only during the first 3 weeks of incubation. The degradation rate in a parabraunerde soil was nearly 1 order of magnitude higher than that in two standard soils recommended for degradation studies where only 3.3 or 5.1%, respectively, was mineralized to $^{14}CO_2$ at 25 °C during 100 days. Desorption and solvent extraction studies showed that the dinocap-bound residue fraction increased with incubation time, the highest amount being in soil amended with alfalfa meal. Dinocap and the major metabolite DNOP were identified by HPLC and TLC. In addition, four unknown metabolites were separated.

Fungicidally active compounds reach the soil initially in part during spraying application or are later washed off the plants by precipitations. Once in the soil, they are subject to degradation, rearrangement, and incorporation processes. These processes are dependent upon the physical and chemical behavior of the compound as well as on the multiple reactions in the soil such as sorption, translocation, distribution, and chemical and biochemical degradation. For new fungicides these processes are studied as a prerequisite for registration. However, for some of the organic pioneer compounds that replaced mercury- and sulfur-containing fungicides, comparatively little information is available. Therefore, the degradation behavior was examined for the chemical dinocap (^{14}C labeled) in three soils of the Federal Republic of Germany. In addition to the measurement of the rates of mineralization for up to 100 days at two different temperatures (15 and 25 °C), the influence of an organic amendment (alfalfa meal) on biomass development and hence mineralization

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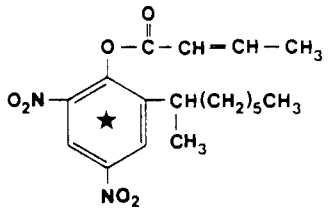

Figure 1. Structural Formula and ^{14}C labeling (★) of dinocap [2-butenoic acid, 2-(1-methylheptyl)-4,6-dinitrophenyl ester]

rates was examined. After 30 and 100 days, the soils were exhaustively extracted.

EXPERIMENTAL SECTION

To a large extent, the degradation experiments were based on the guidelines for determining the fate of pesticides in the soil, as part of the registration process of the Federal Institute of Biological Research (Weinmann and Schninkel, 1976).

Materials. The technical Karathane consists of six different dinocap isomers. In this study isomer no. 1, probably the major isomer, uniformly ^{14}C labeled in the phenyl ring (Figure 1),was supplied by the Rohm and Haas Co., Springhouse, PA. The specific activity was 10.13 μCi/mg. Shortly before the start of the experiment the purity of the dinocap was checked by using high-performance liquid chromatography. It was determined that the sample contained 91.2% dinocap, 3.6% 4,6-dinitro-2-(2-octyl)phenol (DNOP), and 5.8% impurities.

Soils. Two standard soils 2.2 (Neuhofen Neu) and 2.3 (Hatzenbuhl) recommended by the Federal Institute of Biological Research were used. These soils do not represent any particular type of soil. Therefore, in addition the Ap horizon of a representative farm soil from Eschweiler/Rhineland was later included in the experiment. This soil is a degraded loess of the parabraunerde type (alfisol), found throughout the Federal Republic of Germany (Mückenhausen, 1977), and is predominantly under agricultural use. The properties of the soils are summarized in Table I.

Four weeks prior to application, 9 kg of each of the two standard soils was taken from the soil stock and sieved (<2 mm). Five grams of a ground complete fertilizer (12% N, 12% P_2O_5, 17% K_2O, 2% MgO) was well mixed into each soil sample. The soil was then kept for 2 weeks at 50% of its maximum moisture capacity and at approximately 20 °C in order to attain equilibrium and allow regeneration of microorganisms (Greaves et al., 1980). The soil was then air-dried before the application of dinocap.

Application of Dinocap to the Soil. In the whole experiment, a dinocap concentration of 8.9 mg/kg of dry soil (standard soils 2.2 and 2.3) and 9.0 mg/kg for the Eschweiler soil was attained, calculated from the ^{14}C activity. The radioactivity was approximately 9 μCi/100 g of soil. The entire amount of [^{14}C]dinocap was dissolved in 25 mL of acetone, and aliquots of 4 and 8 mL were applied to the soil batches of 0.9 and 1.8 kg, respectively, and thoroughly mixed for 25 min in an automatic stirring vessel after removal of the solvent. The soil was then subdivided for the degradation experiment, six replicates for each treatment.

Degradation Experimental Procedure. Two separate degradation experiments were conducted. In the first experiment only the standard soils were tested, whereas in the second experiment the parabraunerde soil was included. For degradation according to guideline No. 36 of the Federal Institute of Biological Research (Weinmann and Schinkel, 1976), an amount corresponding to 100 g of dry soil was weighed into an Erlenmeyer flask and adjusted to 50% of its maximum moisture capacity. The Erlenmeyer flasks were kept at 25 or 15 °C in a darkened water bath, moist CO_2-free air, flowing at a rate of about 0.2 L/min, was passed over the soil and then through a trap containing 100 mL of ethylene glycol monomethyl ether, and finally the CO_2 was absorbed into 75 mL 1 N of NaOH.

Table I. Chemical and Physical Properties of the Soils

origin	Neuhofen Neu[a] (standard soil 2.2) humic sand	Hatzenbühl[a] (standard soil 2.3) sand	Eschweiler/ Rhineland parabraunerde (alfisol)
pH ($CaCl_2$)	6.8	4.7	5.9
org C, %	2.1	1.7	1.4
total N, %	0.26	0.12	0.12
clay, %	8.3	6.9	12.0
silt, %	6.3	13.6	28.4
fine sand, %	73.8	38.6	58.3
coarse sand, %	11.6	40.9	1.4
T value[b]	15.3	6.3	11.2
S value[c]	14.1	3.6	12.8
$CaCO_3$, %	0.3	0.0	0.0
maximum moisture capacity, mL/100 g	44	32	42

[a] Provided by the Agricultural Experiment Station (LUFA) Speyer, FRG. [b] Total sorption capacity (mequiv/100 g of soil). [c] Actual exchangeable cations (mequiv/100 g of soil).

Extraction and Metabolite Spectra. After 30 and 100 days, three replicates of each of the six variants were used for analysis. The soil moisture content was determined, and aliquots corresponding to 10 g of dry soil were weighed into stainless steel centrifuge tubes for determination of the adsorption/desorption with 50 mL of 0.01 M $CaCl_2$ solution (Kerpen, 1975; Kerpen and Schleser, 1977). Additional aliquots were taken and (a) freeze-dried for total ^{14}C determination and (b) extracted with organic solvents. The remaining soil was stored at −20 °C.

Several extraction procedures applying benzene and methanol as extractants were tested before choosing a method using dichloromethane as the major extractant followed by methanol/water (Figure 2). The organic extracts were evaporated to dryness on the rotary evaporator and then taken up in 5 mL of either acetonitrile or methanol.

Thin-layer chromatography (TLC) was used for separating dinocap and the following metabolites: 4,6-dinitro-2-(2-octyl)phenol (DNOP), 6-(acetylamino)-4-nitro-2-(2-octyl)phenol, and 6-amino-4-nitro-2-(2-octyl)phenol. Acceptable or good separations were obtained by developing with methanol/chloroform (10/90) in two dimensions on Merck silica gel plates (60 F_{254}). The separated compounds on the TLC plates were visualized (a) by UV light (254 nm) and (b) by radioautography.

For the purification and separation of the extract fractions, a high-performance liquid chromatograph (HPLC: Waters Associates, Inc.) with the following components and separation conditions was used: two Model 6000 A pumps, a Model 660 solvent programmer, a Model 440 detector, and a Model U6K injector; column, RP18 Lichrosorb (E. Merck), 10 μm, 25-cm length, 4-mm diameter; temperature; ambient (23 °C); mobile phase, 70 acetonitrile/30 H_2O/0.2 acetic acid/1 2-propanol (Rothman, 1980); flow rate, 2.2 mL/min; UV detection, 254 nm, 280 nm; chart speed, 1 cm/min.

By adding 2-propanol to the mobile phase, a more rapid stabilization of the base line at the end of the chromatogram was achieved. Detection at 280 nm permitted a more definite identification of DNOP, since the extinction coefficient is higher at this wavelength. The detection limit

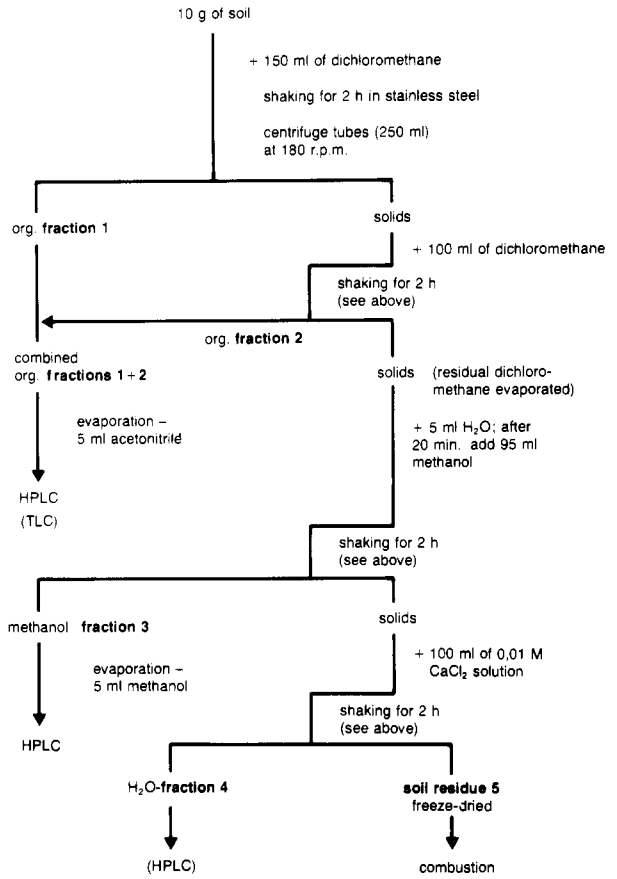

Figure 2. Degradation of [*ring-U*-^{14}C]dinocap in soil: scheme of soil extraction.

is about 40 ng, for a limiting concentration of about 220 ng/mL of extract.

The separation of the aqueous phase was possible without any further cleanup if the radioactivity was sufficiently high (≥5000 dpm/mL). In that case 250–500 μL of the filtered aqueous phase was injected and eluted by using a gradient elution of 35 acetonitrile/65 water/0.2 acetic acid or 3 acetonitrile/97 water/0.2 acetic acid/0.04 2-propanol to 70 acetonitrile/30 water/0.2 acetic acid/1 2-propanol.

The advantage of HPLC over TLC in this case is that quantitative separation could be achieved more quickly. In addition, the separation efficiency could be constantly monitored.

Determination of Radioactivity. All absorbing solutions (NaOH, ethylene glycol monomethyl ether), extracts as well as TLC and HPLC fractions, were counted in a liquid scintillation spectrometer (LSC, Packard 460C). The total ^{14}C activity in the experimental soil, as well as that in the soil remaining after the extractions, was determined by combusting 0.5 g in the Packard sample oxidizer (306).

RESULTS AND DISCUSSION

Mineralization of [*ring-U*-^{14}C]Dinocap to $^{14}CO_2$. In the standard soil Neuhofen Neu the accumulated mineralization of the ring carbon of the [*ring-U*-^{14}C]dinocap amounted to 5.0% of the applied radiocarbon at a constant temperature of 25 °C over a period of 100 days (Figure 3). The total mineralization was at 5.1% only slightly higher with the addition of alfalfa at the same temperature. The two degradation curves are about parallel after the 21st day, which becomes more evident when the daily rates of $^{14}CO_2$ evolution are compared (Figure 4). Only during the first 3 weeks can a distinct effect of the organic amendment

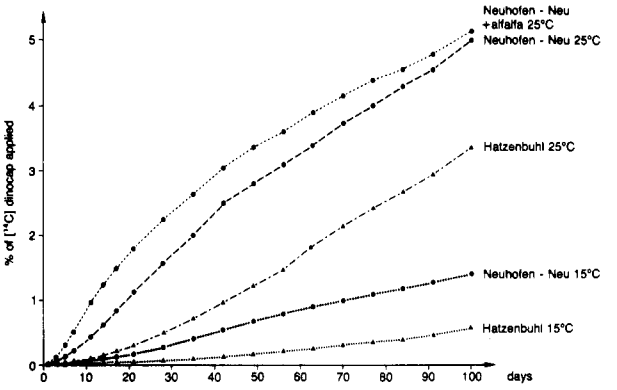

Figure 3. Degradation of [*ring-U*-^{14}C]dinocap in the two standard soils: accumulation of $^{14}CO_2$ produced.

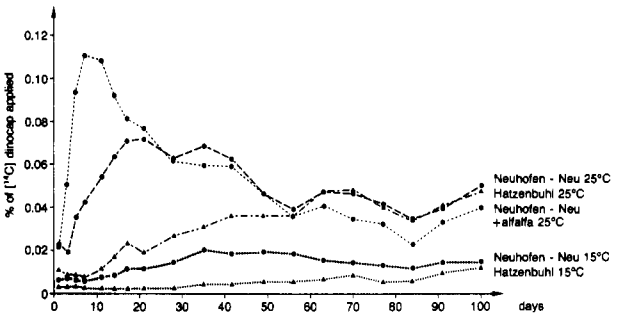

Figure 4. Degradation of [*ring-U*-^{14}C]dinocap in the two standard soils: daily $^{14}CO_2$-evoluation rates.

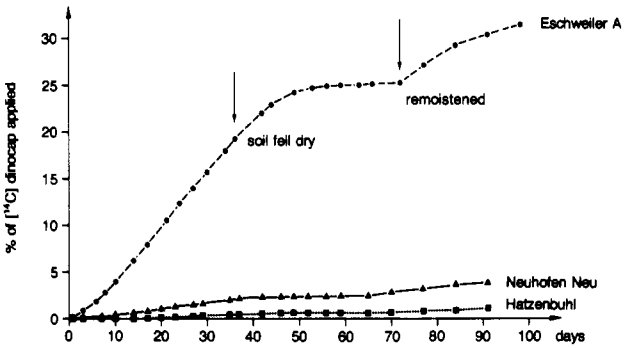

Figure 5. Degradation of [*ring-U*-^{14}C]dinocap in three soils at 25 °C: accumulation of $^{14}CO_2$ produced.

on the degradation rate of [*ring-U*-^{14}C]dinocap be observed. It is known from experiments with ^{14}C-labeled green manure that the mineralization of such materials is most pronounced during the second and third week after addition to the soil (Sauerbeck, 1968; Oberländer and Roth, 1980). This provides additional energy for the microbial degradation process of pesticides, as has been confirmed by results given in the literature (Cheng et al., 1975).

The degradation rates are much lower at 15 °C, so that after 100 days with the Neuhofen Neu soil a mineralization of only 1.4% of the applied radioactivity is recorded (Figure 3). Dinocap is degraded even more slowly in the Hatzenbühl soil: after 100 days the total mineralization at 25 °C is 3.3% and at 15 °C only 0.6% of the applied radioactivity.

In spite of equilibration before the start of the experiments, designed to allow regeneration of microorganisms in the two standard soils, obviously not enough biomass had been formed to degrade dinocap to the extent that was found in fresh farm soil (Figure 5). In the Eschweiler soil, the degradation of about 32% of the applied radioactivity was, after 98 days, about 6–8 times that found in the standard soil Neuhofen Neu and about 10–28 times higher

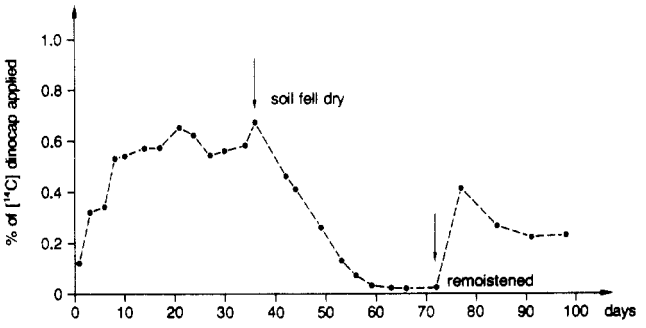

Figure 6. Degradation of [*ring-U*-^{14}C]dinocap in three soils at 25 °C: daily $^{14}CO_2$-evolution rates.

than the degradation in the Hatzenbuhl soil. This mineralization of the ring carbon is about the same as found by Fisher (1971) in his experiments with a silt loam soil (Hagerstown). Additional experiments would be necessary to examine the formation and course of the biomass in order to clarify the discrepancy in the different degradation results of compounds in these standard soils. The low mineralization rate is certainly not due to the spectrum of microorganisms that are present in the soil because the addition of a fresh garden soil suspension had no effect on the mineralization rate (results are not listed).

The curves of dinocap degradation in the Eschweiler soil (Figures 5 and 6) show that the mineralization of [*ring-U*-^{14}C]dinocap stopped drastically as the soil moisture decreased to about 5% of the maximum water holding capacity of the soil. Bacteria are especially sensitive to drying (Rose, 1976; Domsch et al., 1983), and the microbial population is frequently reduced to less than 50%. The recovery time after remoistening the soil was in the range of 3–5 days, this being registered by the $^{14}CO_2$ production (Figure 6). After this interruption, the rates of degradation no longer attained the values that had been registered previously. Without this interruption of the microbial activity, the total degradation would presumably have been higher than the measured value of about 32%. Overall, this experiment shows that soil moisture obviously plays an important role in the degradation of dinocap, which also suggests that the degradation of dinocap is primarily regulated by microbial processes. The degradation values at 15 °C are only $^1/_4$ to $^1/_6$ of those found at 25 °C, which can usually be considered optimal for the mixed population of bacteria in the soil. Thus, [*ring-U*-^{14}C]dinocap is comparable to other compounds that were examined by Führ and Mittelstaedt (1979) regarding their degradation pathways at different soil temperatures in the Eschweiler soil. Apparently, the intensity of soil biomass development is decisive for the degradation of dinocap as long as it is not fixed irreversibly by the soil.

Desorption of Radioactivity with a Simulated Soil Solution. Immediately after being mixed into the soil, the sorption behavior of [*ring-U*-^{14}C]dinocap in the Neuhofen Neu and Eschweiler soils was about the same, about 28 and 34%, respectively, were desorbed with 0.01 M $CaCl_2$, whereas, in the Hatzenbuhl soil, dinocap is obviously more strongly adsorbed, so that only 11% of the applied radioactivity was released during five desorption steps. The detailed data are not presented here. However, during the course of degradation more polar compounds were formed, which were increasingly found in the first desorption step. A comparison of the two standard soils shows that especially the Hatzenbühl soil adsorbs dinocap or, with progressive degradation, also dinocap metabolites in increasing amounts, so that the portion that can be desorbed is reduced to half of that found in the Neuhofen

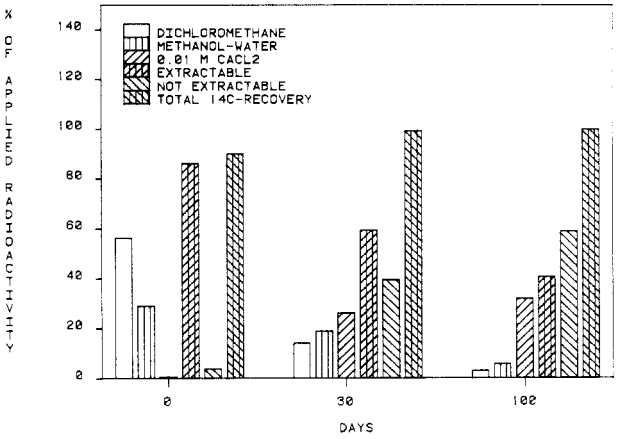

Figure 7. Extraction of radioactivity from Neuhofen Neu soil after application of [*ring-U*-^{14}C]dinocap. Degradation at 25 °C and 50% of the maximum water holding capacity.

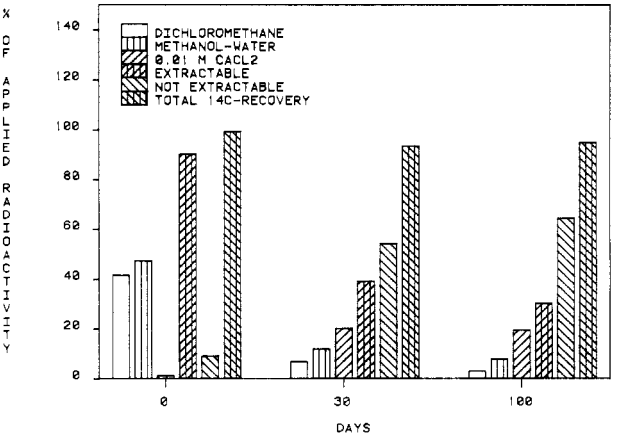

Figure 8. Extraction of radioactivity from Neuhofen Neu soil plus alfalfa after application of [*ring-U*-^{14}C]dinocap. Degradation at 25 °C and 50% of the maximum water holding capacity.

Neu soil. In this way, there is less dinocap available in the soil solution for microbial reaction, which may in part explain the low mineralization rates (Figure 4).

The addition of alfalfa meal caused a considerable reduction in the desorbable portions of radioactivity of up to 20% of the applied radioactivity. This indicates that additional fixation reactions are taking place. While the turnover of the alfalfa components leads to the formation of new soil organic substances, dinocap or metabolites are obviously increasingly adsorbed and possibly also transformed into more stable organic compounds of the soil.

Extraction and Metabolite Spectra. It becomes clear that as the experiment progresses in the Neuhofen Neu soil the portion of organic-soluble compounds decreases greatly in favor of water-soluble metabolites, while the portion of nonextractable radioactive material increases concurrently from 40% at 30 days to 59% after 100 days (Figure 7). In Figures 7–9 the total ^{14}C recovery includes the mineralized $^{14}CO_2$ trapped in NaOH solution.

The addition of alfalfa meal to the Neuhofen Neu soil led to a temporarily increased intensity of the degradation processes (Figure 4), which is also reflected in the distribution of the extractable and the nonextractable radioactivity (Figure 8). The portion of extractable radioactivity decreased with a corresponding increase in the radioactivity in the "bound residue" fraction. The bound residue fraction already represented 54% of the applied radiocarbon after 30 days and increased to almost 65% after 100 days. Thus, it can be concluded that if energy-supplying organic compounds (e.g., alfalfa) are available, as

Table II. HPLC Separation of Extracted ^{14}C Compounds from Eschweiler Soil Treated with [*ring*-U-^{14}C]Dinocap (100%) at 25 °C

extraction step	^{14}C extracted	polar compounds	characterization by HPLC (increasing elution volume →)					
			unk. I	DNOP	unk. II	dinocap	unk. III	unk. IV
30 days								
CH_2Cl_2	25.2	6.3	1.9	5.1	1.3	10.2	1.1	1.1
methanol/H_2O	3.7	1.8		0.4	0.4	0.8	0.1	0.2
total	28.9	8.1	1.9	5.5	1.7	11.0	1.8	1.3
98 days								
CH_2Cl_2	5.1			0.9		4.0		0.4
methanol/H_2O	3.4	1.6		0.6		1.2		0.3
total	8.5	1.6		1.5		5.2		0.7

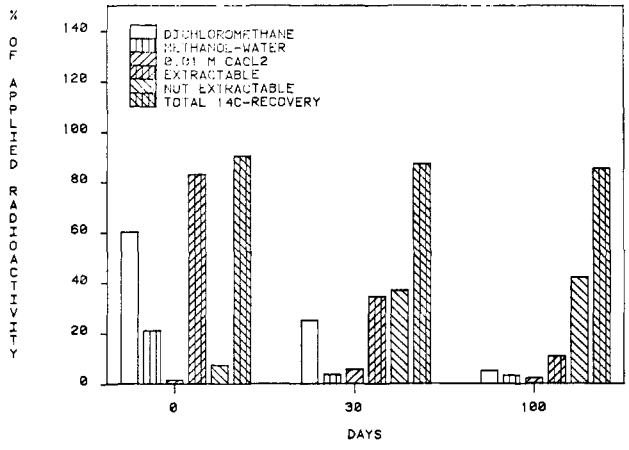

Figure 9. Extraction of radioactivity from Eschweiler soil after application of [*ring*-U-^{14}C]dinocap. Degradation of 25 °C and 50% of the maximum water holding capacity.

a result of the intense reaction processes, dinocap and dinocap metabolites are drawn up into the turnover of plant residues and are partially withheld from a final microbial degradation, at least during the period of time when the experiments were conducted.

In the fresh farm soil Eschweiler the intensive mineralization (Figure 5) is reflected in the extraction data (Figure 9). The radioactivity extracted with either methanol/H_2O or 0.01 M $CaCl_2$ is reduced to about one-fourth of that found in the soil Neuhofen Neu at 30 and 100 days, respectively (Figure 7). Already after 30 days only about 4 and 6% could be dissolved in the respective solvents. These were apparently the dinocap and dinocap metabolite fractions, which were attacked more intensively by microbial degradation in the Eschweiler soil, so that after 98 days in total only 11% of the applied radioactivity could be extracted.

Intensive HPLC separation of the extraction fractions 1 and 2 (CH_2Cl_2) and 3 (methanol/water) was conducted. The individual values are average values of the analyses of three replicated samples from each experiment. The variation coefficient of the average value is between 10 and 50%. The tabulated data (Table II) are arranged according to increasing retention time and identical columns also mean identical retention times. In the column "polar compounds", all polar compounds with retention times of up to 3 min are combined.

In the Eschweiler soil (Table II) after 30 days of degradation at 25 °C, about 11% of the applied radioactivity was characterized by cochromatography as unchanged dinocap, which is comparable to the results of the Neuhofen Neu soil (not listed). The DNOP portion represented 5.5%. After 98 days dinocap still represented 5% and the DNOP portion dropped to 1.5% of the radioactivity applied.

The HPLC results (Table II) permit the conclusion that under suitable microbial reaction conditions, dinocap was first transformed to DNOP. In the organic phase soil extracts four additional metabolites could be separated, but any single product never amounted to more than 2% of the applied radioactivity. After 98 days, all of these metabolites decreased to less than 1%. This indicates that these compounds were not stable and will not accumulate in the soil. The more polar compounds in the organic extracts behaved similarly: during the intensive reaction in the Eschweiler soil, they temporarily (day 30) represented about 8% of the applied radioactivity (Table II), but by the end of the reaction after 98 days, they decreased to 1.6%. Under the metabolism conditions found in the Ap horizon of the parabraunerde Eschweiler soil, there was obviously a rapid degradation of dinocap to DNOP and further to more polar metabolites.

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Registry No. Dinocap, 39300-45-3.

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