with bioassay measurements is made indirectly.

CONCLUSION

Experiments cited in this paper indicate that chlorsulfuron and chemically similar compounds in unfiltered soil samples can be detected in nanogram concentrations by ELISA. Further work must be done to optimize the assay. To this time, crude immune serum from one rabbit has been used as the source of antibody. Pooling several animals' sera and even several bleeds from the same animal may enhance the assay's sensitivity. Furthermore, IgG fractions of the rabbit antibody can be isolated with a minimum effort by using commercially available immunoaffinity columns which may further increase sensitivity. The competitive binding assay described in this paper relied on an initial binding which approached saturation of the antibody molecule. Greater flexibility may be achieved if the competition and binding are permitted at the same time since it is more likely to result in a linear dose/response inhibition curve. Preliminary results indicate that chlorsulfuron in soil extracts can be calculated from a standard curve at concentrations as low as 0.1 ng/mL. This corresponds to a concentration of chlorsulfuron in soil of 0.4 ppb. Taken together, these results indicate that the ELISA is a promising immunological tool which may apply directly to herbicide analysis in soil.

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LITERATURE CITED

Bond, W.; Roberts, H. A. *Bull. Environ. Contam. Toxicol.* **1976**, *16*, 431.
Clarke, D. H.; Casals, J. *Am. J. Trop. Med. Hyg.* **1958**, *7*, 561.
Engvall, E.; Perlmann, P. *Immunochemistry* **1971**, *8*, 871.
Ercegovich, C. D.; Vallejo, R. P.; Gettig, R. R.; Woods, L.; Bogus, E. R.; Mumma, R. D. *J. Agric. Food Chem.* **1981**, *29*, 559.
Fatori, D.; Hunter, W. M. *Clin. Chim. Acta* **1980**, *100*, 81.
Hammock, R. R.; Mumma, R. O. In "Pesticide Analytical Methodology"; Harvey, J., Jr., Zweig, G., Eds.; American Chemical Society: Washington, D.C., 1980; p 321.
Hsiao, A. I.; Smith, A. E. *Weed Res.* **1983**, *23*, 231.
Hunter, K. W.; Lenz, D. E. *Life. Sci.* **1982**, *30*, 355.
Kajinuma, H.; Ichikawa, K.; Akanuma, Y.; Kosaka, K.; Kuzuya, N. *Tonyobyo (Tokyo)* **1982**, *25*, 869.
Levitt, T. *Lancet* **1977**, 8033, 358.
Levitt, G. U.S. Patent 4 127 405, 1978.
Levitt, T. *Proc. Anal. Div. Chem. Soc.* **1979**, *16*, 72.
Levitt, G.; Ploeg, H. L.; Weigel, R. C., Jr.; Fitzgerald, D. J. *J. Agric. Food Chem.* **1981**, *29*, 418.
Newsome, W. H.; Shields, J. B. *J. Agric. Food Chem.* **1981**, *29*, 220.
Palm, H. L. *Proc. 1980 Br. Crop Prot. Conf. Weeds* **1980**, *1*, 1.
Rinder, D. F.; Fleeker, J. R. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 375.
Schwalbe, M.; Dorn, E.; Beyermann, K. *J. Agric. Food Chem.* **1984**, *32*, 734.
Vallejo, R. P.; Bogus, E. R.; Mumma, R. O. *J. Agric. Food Chem.* **1982**, *30*, 572.
Van Weeman, B. K.; Schurs, A. H. W. M. *FEBS Lett.* **1971**, *15*, 232.
Wie, S. I.; Hammock, B. D. *J. Agric. Food Chem.* **1982**, *30*, 949.
Wie, S. I.; Sylvester, A. P.; Wing, K. D.; Hammock, B. D. *J. Agric. Food Chem.* **1982**, *30*, 943.
Zahnow, E. W. *J. Agric. Food Chem.* **1982**, *30*, 854.
Zahnow, E. W. *J. Agric. Food Chem.* **1985**, *33*, 479.

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Gas Chromatographic Determination of 3,6-Dichloropicolinic Acid Residues in Soils and Its Application to the Residue Dissipation in a Soil

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Residues of 3,6-dichloropicolinic acid (3,6-DCP) were extracted from soils with sodium hydroxide, partitioned in ether, methylated by diazomethane, and determined by GLC-^{63}Ni ECD with a fused silica capillary column. Recoveries from four types of soil range from 84.5 to 94.3% for fortified samples at 0.05–2.5 mg/kg. The method was applied to the determination of residue dissipation in a soil. 28 days after the field treatment at a rate of 360 g of 3,6-DCP/ha the limit of detection (0.05 mg/kg) was achieved.

INTRODUCTION

Previous papers have described a method for the determination of 3,6-dichloropicolinic acid (3,6-DCP) in sugar beets by gas-liquid chromatography (Galoux et al., 1982) and the residue dissipation of this herbicide after a field treatment in 1981 on a sugar beets crop (Galoux et al., 1983). Results have showed a light persistence of the pesticide at a rate of 0.11, 0.13, 0.17, and 0.27 mg/kg in the beets when Cyronal emulsifiable concentrate was applied at rates of 120, 150, 180, and 360 g of 3,6-DCP/ha.

The present paper explains the modification to the analytical procedure to use it for the determination in soil with a sensitivity of 0.05 mg/kg. It was applied on four types of soil, in laboratory conditions; but also to determine the residue dissipation of 3,6-DCP in soil during the extended experimentation in 1981 and to finish off the previous work on sugar beet crop of 1981.

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EXPERIMENTAL SECTION

Reagents. They are very similar to those described in the previous papers of 1982 and 1983. (a) Diazomethane. Prepared by reaction of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine with sodium hydroxide and absorption in cool ether. An efficient fume hood and appropriate safety precautions should always be used when handling diazomethane and ether. (b) Standard. 3,6-Dichloropicolinic acid (purity >99%) was supplied by Dow Chemical Co. (Midland, MI.). Reference standard solutions for GLC (0.1–10 mg/L) were prepared in ether from a stock solution of 0.1000 g of standard 3,6-DCP in 250 mL of ether. For

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Table I. Types of Soil

type	origin	organic matter
sandy silt loam	Roelers	1.8
sandy loam I	Meldert	1.9
clay soil	St Pieter	2.3
sandy loam II	Tienen	1.2

Table II. Recovery of 3,6-DCP from Dry Soils Fortified at Various Levels

samples	% recovery (blank deducted) when samples fortified at		
	2.5 mg/kg	0.5 mg/kg	0.05 mg/kg
sandy silt loam	92.7	86.0	84.5
sandy loam I	93.0	90.0	91.2
clay soil	91.8	94.3	85.0
sandy loam II	92.5	91.3	90.1

Table III. Recovery of 3,6-DCP from Wet Soils Fortified at Various Levels

samples	% recovery (blank deducted) when samples fortified at		
	2.5 mg/kg	0.5 mg/kg	0.05 mg/kg
sandy silt loam	92.2	86.5	84.9
sandy loam I	93.4	90.1	90.9
clay soil	92.0	93.7	85.6
sandy loam II	93.0	91.7	90.2

sample fortification, solutions of 3,6-DCP (0.1–10 mg/L) were prepared in water from a stock solution of 0.4000 g of standard 3,6-DCP in 1000 mL of distilled water.

Apparatus. A Hewlett Packard 5880 A, level four gas chromatograph equipped with a ^{63}Ni electron capture detector, a split–splitless injector, and an automatic sampler was used. The column was a 12 m × 0.20–0.21 mm i.d. wide bore flexible capillary column coated with methyl silicone fluid.

GLC Analysis. The operating conditions were the same as those previously described except for the oven program which follows: 60 °C for 0.50 min, then 30 °C/min to 120 °C, and held to 5 min; column cleanup at 220 °C for 10 min.

Soils. Four typical Belgian soils were used. Their localization in Belgium and their organic matter content are given in Table I. The first three soils were never treated with 3,6-DCP. The last (Tienen) comes from the 1981 experimentation, where Cyronal emulsifiable concentrate at 100 g of acid equiv/L of monoethanolamine salt of 3,6-DCP was applied at rates of 120, 150, 180, and 360 g of active ingredient/ha in four replicated plots of 5.4 m × 11 m on a sugar beet crop. Analysis of this soil were performed on samples providing from untreated plots to determine the recovery in the laboratory and on samples taken from every replicated plot (fractions 0–10 and 10–20 cm of depth), first each week after the treatment during 5 weeks, and each 14 days later for the residue dissipation study.

Sample Preparation. After sampling, the soils were dried in air at ambient temperature on aluminum foil and sieved to pass a 1-mm screen. They were stored in sealed containers at −30 °C until analysis.

Fortification Procedure. For the recovery determination, aliquots of 20 g of untreated soils were weighed into capped centrifuge tubes and fortified separately at 2.5, 0.5, and 0.05 mg/kg (ppm) with 10 mL of the appropriate fortifying solution of 3,6-DCP. The soil samples were mixed thoroughly during a night.

This fortification was also applied on soils equilibrated in water.

Extraction Procedure. A 20-g sample of soil was extracted with 100 mL of 0.25 M sodium hydroxide in a 250-mL capped centrifuge tube, shaken for 30 min, and allowed to stand at ambient temperature for a night (±16 h). After that, it was shaken 30 min and centrifuged for 5 min at 2.900*g*. The extract was transferred in a 500-mL separatory funnel, and a second extraction of 10 min was performed with 50 mL of 0.25 M sodium hydroxide. The two aqueous extracts were combined, acidified with 70 mL of 4 M sulfuric acid, and extracted twice with 50 mL of ether as described in the previous paper (Galoux et al., 1983) for sugar beets.

The ether extract was evaporated to about 5 mL, transferred quantitatively (with no more than 4 mL of ether) into a 25-mL Erlenmeyer flask, and diazotated by adding 1 mL of diazomethane solution and allowing the solution to stand for 2 h at 4 °C. Diazomethane was evaporated. The dry residue was dissolved in an appropriate volume of hexane–ether (70:30) depending on the expected amount of 3,6-DCP in the residue and 1 μL was injected into the gas chromatograph.

Expression of the Results. Quantification was based on average peak area of the external standards which were injected before and after each sample.

Recovery. The determination of the recovery coming from four replicate analysis injected twice is expressed in percent recovery, blank value deducted. The results give also the confidence limits (CL) of the determination, expressed in μg. $\bar{X}$ is the average, t is the student's t value

$$CL = \bar{X} \pm ts/n^{1/2}$$

for a degree of confidence of 90%, s is the standard deviation square root of the variance $V = \sum (X - \bar{X})^2/(n - 1)$, and n is the number of the results (Bauer, 1971).

Residue Dissipation. The determination of 3,6-DCP residue dissipation in the soil of Tierer is expressed in mg/kg (ppm), blank value deducted. The confidence limits are determined as above.

RESULTS AND DISCUSSION

Residue Recovery from Soils. The method as described ensures a 3,6-DCP residue recovery in soil better than 85% at the level of 0.05 mg/kg (Table II). For the sandy loam soils, the recovery can even reach 90%.

These results and the following arise from dry samples fortified with standard 3,6-DCP in solution. Meanwhile, no differences were observed on the recovery when the fortifying is done on wet soil samples as shown at Table III.

When the residue level increases, the recovery is still better because of the reduction of the noise level against

Table IV. Amount and Confidence Limits Residues of 3,6-DCP in Soils Foritified at Various Levels

samples, 20 g	μg (CL) of 3,6-DCP[a] recovered from samples fortified with			
	50 μg	10 μg	1 μg	untreated
sandy silt loam	47.95 ± 1.34	10.20 ± 0.60	2.44 ± 0.29	1.65 ± 0.10
sandy loam I	48.10 ± 0.65	10.65 ± 0.32	2.51 ± 0.14	1.59 ± 0.10
clay soil	47.08 ± 0.69	10.62 ± 0.71	2.05 ± 0.35	1.20 ± 0.09
sandy loam II	46.95 ± 0.60	9.83 ± 0.61	1.60 ± 0.25	0.70 ± 0.05

[a] CL = confidence limits.

Tables V. Amounts of Diazomethane Solution Required for Derivatization of 3,6-DCP in Soil Extracts

samples	fortified at mg/kg	3,6-DCP % recovery with mL diazomethane 0.1	0.5	1	4	7	10
sandy silt loam	2.5	70.2	91.8	92.7	92.8	81.4	79.6
	0.05	63.5	84.9	84.5	85.0	80.2	78.4
sandy loam I	2.5	69.4	93.1	93.0	92.7	81.8	78.6
	0.05	65.6	90.8	91.2	91.0	80.9	79.1
clay soil	2.5	69.8	91.0	91.8	90.8	82.4	80.1
	0.05	65.7	85.4	85.0	84.9	79.6	79.0
sandy loam II	2.5	71.4	92.0	92.5	92.3	83.7	78.9
	0.05	67.2	89.6	90.1	90.3	81.5	77.9

Table VI. Stability of 3,6-DCP in Dry Soils Fortified at 0.5 mg/kg Stored at -30 °C

samples	3,6-DCP % recovery after 1 month	6 months	12 months	18 months
sandy silt loam	86.0	85.8	86.4	86.2
sandy loam I	90.2	89.9	90.1	90.2
clay soil	93.9	94.2	94.5	94.2
sandy loam II	91.2	91.5	90.9	91.4

the 3,6-DCP GLC signal. However, as it appears in Table IV, the determination at 0.05 mg/kg is a limit in these conditions because the blank value gives generally a noise larger than 1 μg expressed in 3,6-DCP for 20 g of soil.

The organic matter seems not to have much effect on the noise level and on the recovery.

Table IV details the true values determined by GLC with their confidence limits and confirms the good reproducibility and accuracy of the method to the range of studied concentrations.

Concerning the application of the method which is very similar of those for sugar beets, the critical steps are the extraction and the diazotation. For the extraction, trials were done with potassium hydroxide in place of sodium hydroxide, and the extractions were conducted from 0.5 to 16 h. With potassium hydroxide, we have never obtained as good reproducibility as with sodium hydroxide. Similar observations were done when the extraction time is lower than 10 h. For this reason and for the laboratory commodity, the extraction time was fixed for a night. To explain that, further works will be done later to study the adsorption–desorption equilibrium.

The amount of soil to be extracted was also studied. In our conditions, it is possible to use up to 50 g of soil. However, at this level, the emulsion is very important during the ether partition and the GLC background is higher. For these reasons we prefer to use only 20 g of soil.

Diazotation was conducted with 1 mL of diazomethane prepared as previously described. Trials were done with 0.1–10 mL of diazomethane solution. A recovery larger than 85% in the four types of soil was observed as shown at Table V with 0.5–4 mL of diazomethane; with lower or higher volumes, the recovery quickly decreases due to either incomplete derivatization or methyl ester degradation.

Residue Dissipation in Soil. The second part of this paper is the application of the present method to the study of residue dissipation of 3,6-DCP in a sandy loam soil after various field treatments. After verification of the 3,6-DCP residues stability in dry soils stored at -30 °C (Table V), this dissipation was followed from the first day of the treatment (day 0) to the harvesting day (day 139), expressed in mg/kg in the upper 10 cm of the soil. These results finish off those obtained on sugar beets (Galoux, 1983) and are detailed at Table VI.

At the treatment, a good concordance is observed between the treatment rates and the soil residue levels. Progressively, the residue levels decrease in relationship with the time probably due to the sugar beet absorption but principally due to the percolation and the soil dilution, to achieve a not detectable level after 21 days for low rates and 28 days for the highest; at the same time the herbicide absorption does not slow down in the sugar beet but continues during more or less two months to point out the 3,6-DCP presence.

Analysis was also done in the 10–20 cm soil fraction but it has never been possible to detect 3,6-DCP.

In conclusion, 3,6-DCP residues can be detected by the present method at a level of 0.05 mg/kg. As it appears in the field conditions, the residual level in soil decreases very quickly and 3,6-DCP seems not to be persistent. The question of interest is the determination, but it is not easily answered because, as we have previously observed, 3,6-DCP, even at a not detectable level, is always present in soil since it can be absorbed by the crop and a possible accumulation cannot be excluded. Further work is presently being conducted to determine the adsorption–desorption of 3,6-DCP, its persistence, its percolation, and its degradation. It will be published later.

Table VII. Average (in ppm) and Confidence Limits (CI) of Residues of 3,6-DCP in the Upper 10 cm of the Sandy Loam Soil II after the Various Treatment in Relation to the Amount of 3,6-DCP Residues in μg/Beet

day	120 g a.i./ha beet, μg	soil, ppm	150 g a.i./ha beet, μg	soil, ppm	180 g a.i./ha beet, μg	soil, ppm	360 g a.i./ha beet, μg	soil, ppm
0	2.2	0.13 ± 0.02	2.3	0.20 ± 0.07	2.6	0.22 ± 0.08	4.7	0.50 ± 0.11
7	9.7	0.14 ± 0.02	15.2	0.19 ± 0.07	16.4	0.18 ± 0.07	30.9	0.39 ± 0.10
14	14.4	<0.05	17.7	0.05 ± 0.02	16.2	0.07 ± 0.02	34.7	0.18 ± 0.09
21	17.3	a	18.0	a	32.5	a	71.4	0.05 ± 0.02
28	27.7	a	30.2	a	41.1	a	85.0	a
34	29.7	a	48.7	a	47.2	a	105.2	a
49	27.3	a	33.2	a	57.2	a	126.8	a
63	33.3	a	74.6	a	60.5	a	164.9	a
76	68.2	a	123.9	a	97.3	a	223.0	a
91	55.2	a	50.9	a	85.2	a	170.3	a
110	78.2	a	93.2	a	118.3	a	145.9	a
139	74.3	a	83.3	a	109.6	a	147.0	a

[a] Not detected.

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LITERATURE CITED

Bauer, E. L. "A statistical manual for chemist"; Academic Press: New York, 1971.

Galoux, M. P.; Van Damme, J. C.; Bernes, A. *J. Chromatogr.* **1982**, *242*, 323-330.

Galoux, M. P.; Van Damme, J. C.; Bernes, A. *J. Agric. Food Chem.* **1983**, *31* (4), 759-762.

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UV-A Photooxidation of β-Carotene in Triton X-100 Micelles by Nitrodiphenyl Ether Herbicides

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Photooxidation of β-carotene in Triton X-100 micelles was stimulated by lipophilic nitrodiphenyl ether herbicides at concentrations as low as 5 μM after 15 min in UV radiation (UV-A between 315 and 400 nm). Bleaching of β-carotene by acifluorfen-methyl [methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate] was proportional to UV-A intensity and independent of pH. White light (400–700 nm) alone was without effect. At pH 6.5, 100 μM acifluorfen [sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate], a water-soluble nitrodiphenyl ether, stimulated photooxidation of β-carotene after 15 min in UV-A radiation. Activity of 200 μM acifluorfen was enhanced at pHs between 3.5 and 6.5. The chlorodiphenyl ether analogue of acifluorfen-methyl, methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-chlorobenzoate, exhibited little activity at 200 μM and 200 μM phenyl ether was without effect. Activation energy for acifluorfen-methyl stimulated β-carotene photooxidation near 20 and 30 °C was 40.3 and 5.6 kJ mol^{-1}, respectively. Subsequent to UV-A exposure and placement into darkness no further bleaching of β-carotene was detected, indicating that reactive species were generated only in light and consumed quickly in darkness.

INTRODUCTION

Orr and Hess (1982a,b) proposed a model for the mechanism of action of the nitrodiphenyl ether (nitroDPE) herbicide acifluorfen-methyl (AFM). They suggested that light absorbed by carotenoids "activates" the AFM molecule. The carotenoid involved is destroyed subsequent to activation of the herbicide. The light-activated form of the molecule is then involved in the initiation of a free-radical chain reaction through the abstraction of a hydrogen atom from the divinylmethane structure present in the polyunsaturated fatty acid moieties of membrane lipids. This fairly stable radical reacts with molecular oxygen to form a lipid peroxide. These reactions propagate readily throughout the hydrophobic matrix of the membrane. The perturbations that follow result in a loss of the membrane's selective permeability characteristics, thereby leading to cell death.

Although the model proposed (Orr and Hess, 1982a,b) is consistent with much of the DPE data published previously (Fadayomi and Warren, 1976; Gorske and Hopen, 1978; Kunert and Boger, 1981; Matsunaka, 1969; Orr and Hess, 1981; Orr and Hess, 1982a,b; Pritchard et al., 1980; Vanstone and Stobbe, 1977; Vanstone and Stobbe, 1979; Yih and Swithenbank, 1975) and with information on the chemistry of lipophilic radical reactions in vivo (Mead, 1976; Pryor, 1978) the evidence accumulated thus far in support of the model is strictly circumstantial. The exact nature of the light-activating mechanism is unknown and direct proof for the involvement of free radicals is lacking. The purpose of experiments reported here was to characterize the first reported interaction in vitro between nitroDPEs and a carotenoid.

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MATERIALS AND METHODS

β-Carotene (β-car) solutions were prepared by first adding 60 mg β-car to 50 mL of acid-free chloroform in a boiling-flask. After the solution was dried in vacuo, 250 mL of 0.5% (v/v) Triton X-100 was added, the solution stirred, and β-car scraped from the sides of the flask with a spatula. The solution was then placed in a 70 °C water bath (heating increased solubility of β-car into Triton X-100), stirred for 2 min, removed, stirred an additional 2 min at 25 °C, and then stirred and cooled rapidly on ice to 25 °C. The solution was filtered and diluted with 0.5 M potassium phosphate buffer and water to 11.76–16.34 nmol of β-car mL^{-1} of 0.375% (v/v) Triton X-100 in 50 mM potassium phosphate buffer. Unless indicated otherwise pH was 6.5. All manipulations were done under N_2 and in dim laboratory light [less than 5 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)]. Solutions were prepared immediately prior to use.

Isolated carotenoids in solution are sensitive to cis–trans isomerization in light and upon heating (Britton, 1983). Although they are stabilized when present in association with protein or lipid, and presumably detergent, we were concerned that heating of the β-car/Triton X-100 solutions may have had additional indirect effects on the outcome of our experiments. Therefore, solutions were also prepared without heating by adding β-car directly to Triton X-100, diluting with 50 mM potassium phosphate buffer to 0.375% (v/v) Triton X-100, and filtering before use. Results using solutions prepared in this manner were not different from those using solutions prepared as described above. This latter method was discarded, however, since initial β-car concentration was low.

After addition of herbicide and mixing to aerate solutions, reactions were initiated by placing in light. Unless

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