Table II shows the results by ACHE assay on the same samples as those shown in Table I. In agreement to Table I, the effect of temperature on the decomposition of the oxon of azinphos-methyl is indicated by a 44% less in the measured amount of GO in panel B than that in panel A. The relative standard derivation of measurements of the oxon of azinphos-methyl by the ACHE method is within 15%, comparable to that of HPLC method, although the rate of formation of the oxon of azinphos-methyl of the A system measured by the ACHE method appears to be 13% greater than those measured by the HPLC method. However, the Student's t test indicates that this difference is statistically insignificant (>0.05). To further test whether there are any acetylcholinesterase inhibitors remaining in the liver homogenates after extraction with ethyl acetate, various volumes of the aliquot portion of A samples (10, 20, 30, 100, and 500 μL) were subjected to the ACHE assay. No response could be detected.

In conclusion, a comparsion between the two techniques indicates that both the ACHE and HPLC methods measure the oxon of azinphos-methyl to the same degree of precision. Since there are no significant difference in the measured amounts of the oxon of azinphos-methyl by both methods, it is likely that the compound may be the sole active metabolite produced in the liver which inhibits acetylacholinesterase. The limit of detection of the oxon of azinphos-methyl by the ACHE method, using the workup procedure described here, is 6 ng while the described procedure for the HPLC method was reliable to 5 ng (Lin et al., 1980). For some purposes of routine assays of the oxon of azinphos-methyl, the ACHE method would be more labor efficient (extraction and cleanup are not required) and more rapid (several metabolic samples can be assayed simultaneously, instead of serially as with HPLC). However, the HPLC method holds the advantage that several metabolites can be measured simultaneously.

Registry No. Azinphos-methyl oxon, 961-22-8; methyl(mercaptomethyl)benzazimide, 85850-18-6; azinphos-methyl, 86-50-0.

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Gas–Liquid Chromatographic Determination of Residue Dissipation of 3,6-Dichloropicolinic Acid in Sugar Beets

Michel P. Galoux,* Jean-C. Van Damme, Albert C. Bernes, and Jean-F. Salembier

Cyronal emulsifiable concentrate was applied at rates of 120, 150, 180, and 360 g of 3,6-dichloropicolinic acid/ha as postemergence herbicide on a sugar beet crop to follow the dissipation of residues in roots, tops, and leaves from the treatment to the harvesting. The method used was a gas–liquid chromatography after derivatization of the acid to form the methyl ester. Results show a light persistence of the pesticide respectively at a rate of 0.11, 0.13, 0.17, and 0.27 ppm in the beets (root plus top).

3,6-Dichloropicolinic acid (3,6-DCP) is a relatively specific, postemergence, growth-regulator herbicide manufactured by Dow Chemical Co. It is intended for use in the control of important phenoxy-tolerant weeds such as thistles which infest Graminaceae, sugar beet, and flax. It is absorbed by roots and leaves and translocated throughout the plant. In susceptible plants, it induces characteristic auxin-type response (Brown et al., 1976; Martin and Worthing, 1977; Jones, 1977).

However, 3,6-DCP is chemically related to another herbicide, picloram (4-amino-3,5,6-trichloropicolinic acid), which is very persistent in crops and soils. Because many problems due to that picloram persistence have been observed, it was interesting to study the dissipation of 3,6-

Ministère de l'Agriculture, Station de Phytopharmacie de l'Etat, 11 rue du Bordia, B-5800-Gembloux, Belgium.

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DCP in sugar beet (Pik and Hodgson, 1976; Pik et al., 1977; Cotterill, 1978).

The present paper states the results of an extended experimentation during 1981, based on previous trials carried out during 1979 and 1980 (Galoux, 1980).

EXPERIMENTAL SECTION

Reagents and Apparatus. (*a*) *Diazomethane.* A total of 120 mg of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (Fluka 68051) in the bottom of the inside tube of a diazomethane generator (millimole size, Chrompack catalog no. 12421) and 500 μL of water was added. In the outside tube 3 mL of ether was poured. The two parts of the apparatus were assembled and the screw cap opening was closed. The sample was allowed to stand overnight in a freezer. The lower part was immersed in an ice bath and 600 μL of 5 M sodium hydroxide was injected through the Teflon rubber septum. The reaction was carried out for 45 min. An efficient fume hood and appropriate safety precautions should always be used when handling diazomethane.

(*b*) *Standard.* 3,6-Dichloropicolinic acid was supplied by Dow Chemical Co. (Midland, MI). Stock solution was prepared by dissolving 0.1000 g of pure 3,6-DCP in ether and making it up to 250 mL with ether. Dilute standards (0.1–10 mg/L) in ether were prepared as required.

(*c*) *Gas Chromatograph.* A Hewlett-Packard 5880 A, level four, equipped with a ^{63}Ni electron capture detector, a split–splitless injector, and an automatic sampler, 7672 A was used. Operating conditions were as follows: injector temperature 250 °C; detector temperature 250 °C; oven program 60 °C for 0.50 min, then at 30 °C/min to 130 °C, and held for 4 min; column cleanup at 220 °C for 10 min; 12 m × 0.20–0.21 mm i.d. wide bore flexible capillary column coated with methyl silicone fluid (Carbowax 20 M deactivated), Hewlett-Packard 19091-60010; carrier gas, high-purity helium at an inlet pressure of 10 psi, flow rate 2 mL/min; auxiliary detector gas, argon–methane (95:5) at an inlet pressure of 30 psi, flow rate 30 mL/min.

Field Treatment. 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, variety Massabel. Treatments were applied in 500 L of spray volume/ha by a Van Der Weij sprayer at a constant pressure of 2.5 kg/cm^2. The crop growth stage varied from 6 to 10 true leaves while thistle growth was between 5 and 20 cm high.

Sampling Procedure. Samples were taken from every replicate plot, first, each week after the treatment during 5 weeks, and each 14 days later, according to the recommended method of sampling for the determination of pesticide residues (Codex Alinorm 79/24, 1979). They were frozen at −30 °C until analysis.

Extraction Procedure. Frozen sugar beets (roots, tops, or leaves) were cut with a food-cutter, "Hobart". A total of 50 g of the homogeneous sample was blended for 5 min at high speed with 150 mL of 0.25 M potassium hydroxide. The mixture was filtered under suction on a 1-cm Celite 545 layer and washed twice with 50 mL of 0.25 M potassium hydroxide and twice with 50 mL of water. The filtrate and washings were collected and combined into a 750-mL separatory funnel. A total of 100 mL of ether, 30 g of sodium chloride, and 70 mL of 4 M sulfuric acid was added. The mixture was shaken and the layers were allowed to separate. The aqueous layer was poured into another separatory funnel and the ether layer into a 250-mL centrifuge bottle. After centrifugation, ether was dried through a 4-cm bed of anhydrous sodium sulfate and it was collected in a 300-mL Erlenmeyer flask. The aqueous layer was again extracted with 50 mL of ether. The ether extract was transferred into the same centrifuge bottle, centrifuged, dried, and combined in the Erlenmeyer flask with the previous ether layer. The ether extract was evaporated to about 5 mL and it was transferred quantitatively into a 25-mL Erlenmeyer flask. This was evaporated to dryness. Seven milliliters of diazomethane, swirled was added, and the solution was allowed to stand for 2 h at 4 °C. Diazomethane was evaporated under a gentle stream of dry air without any heating under a fume hood. The dry residue was kept in a freezer until use. For injection, the residue was dissolved in 10 mL of hexane–ether (70:30) and 1 μL was injected into the gas chromatograph.

Table I. Average and Confidence Limit Residues of 3,6-DCP in Sugar Beets at the Rate of 120 g of a.i./ha[a]

day	roots ± CL, ppm	tops ± CL, ppm	leaves ± CL, ppm
0	0.86 ± 0.34		4.51 ± 0.85
7	0.62 ± 0.23		0.38 ± 0.15
14	0.40 ± 0.07	0.56 ± 0.23	0.23 ± 0.05
21	0.27 ± 0.13	0.31 ± 0.12	0.17 ± 0.07
28	0.20 ± 0.10	0.29 ± 0.12	0.12 ± 0.08
34	0.14 ± 0.07	0.21 ± 0.09	0.12 ± 0.05
49	0.10 ± 0.02	0.14 ± 0.03	0.13 ± 0.04
63	0.08 ± 0.05	0.08 ± 0.04	0.11 ± 0.04
76	0.10 ± 0.05	0.11 ± 0.03	0.13 ± 0.05
91	0.06 ± 0.03	0.07 ± 0.04	0.07 ± 0.02
110	0.07 ± 0.05	0.06 ± 0.02	0.08 ± 0.03
139	0.05 ± 0.02	0.06 ± 0.02	0.10 ± 0.04

[a] Day = sampling day after treatment. CL = confidence limits.

Table II. Average and Confidence Limit Residues of 3,6-DCP in Sugar Beets at the Rate of 150 g of a.i./ha[a]

day	roots ± CL, ppm	tops ± CL, ppm	leaves ± CL, ppm
0	0.85 ± 0.18		4.64 ± 1.58
7	0.99 ± 0.18		0.44 ± 0.11
14	0.52 ± 0.18	0.64 ± 0.49	0.29 ± 0.10
21	0.35 ± 0.13	0.38 ± 0.07	0.20 ± 0.02
28	0.21 ± 0.07	0.27 ± 0.14	0.23 ± 0.08
34	0.22 ± 0.08	0.23 ± 0.09	0.19 ± 0.08
49	0.12 ± 0.04	0.14 ± 0.03	0.13 ± 0.05
63	0.10 ± 0.05	0.10 ± 0.03	0.11 ± 0.02
76	0.12 ± 0.03	0.13 ± 0.10	0.13 ± 0.03
91	0.06 ± 0.02	0.06 ± 0.02	0.09 ± 0.04
110	0.07 ± 0.03	0.07 ± 0.02	0.09 ± 0.03
139	0.06 ± 0.01	0.07 ± 0.02	0.11 ± 0.04

[a] Day = sampling day after treatment. CL = confidence limits.

The principle of the present method is based on a previously reported method for sugar beet (Galoux et al., 1982) with some modifications. Its sensitivity is 0.05 mg/kg 3,6-DCP and its recovery greater than 85%.

Expression of the Results. Results are expressed in mg/kg (ppm) or in μg/sugar beet. Each sample has been analyzed in duplicate and each extract injected twice. The amount of 3,6-DCP has been calculated by comparison with standard references of approximtely the same value injected before and after two samples.

Results given in the tables state the average values from the four replicates of the same treatment.

Confidence limits (CL) are expressed as the bounds of uncertainty about the average caused by the variability of the experiment (Bauer, 1971).

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

where $\bar{X}$ is the average, t is the Student's t value for a degree of confidence of 90%, s is the standard deviation,

Table III. Average and Confidence Limit Residues of 3,6-DCP in Sugar Beets at the Rate of 180 g of a.i./ha[a]

day	roots ± CL, ppm	tops ± CL, ppm	leaves ± CL, ppm
0	0.94 ± 0.34		5.24 ± 0.38
7	1.23 ± 0.32		0.55 ± 0.05
14	0.52 ± 0.20	0.87 ± 0.20	0.35 ± 0.06
21	0.48 ± 0.11	0.48 ± 0.06	0.24 ± 0.06
28	0.32 ± 0.07	0.38 ± 0.08	0.26 ± 0.05
34	0.24 ± 0.08	0.29 ± 0.06	0.23 ± 0.05
49	0.16 ± 0.06	0.18 ± 0.08	0.21 ± 0.06
63	0.11 ± 0.07	0.13 ± 0.05	0.18 ± 0.06
76	0.13 ± 0.01	0.13 ± 0.03	0.15 ± 0.06
91	0.09 ± 0.03	0.09 ± 0.02	0.10 ± 0.03
110	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.02
139	0.07 ± 0.02	0.12 ± 0.10	0.18 ± 0.13

[a] Day = sampling day after treatment. CL = confidence limits.

Table IV. Average and Confidence Limit Residues of 3,6-DCP in Sugar Beets at the Rate of 360 g of a.i./ha[a]

day	roots ± CL, ppm	tops ± CL, ppm	leaves ± CL, ppm
0	1.61 ± 0.51		10.57 ± 0.75
7	2.14 ± 0.90		0.97 ± 0.25
14	1.69 ± 0.87	2.22 ± 0.81	0.70 ± 0.26
21	0.88 ± 0.46	0.95 ± 0.39	0.54 ± 0.36
28	0.60 ± 0.20	0.82 ± 0.22	0.55 ± 0.15
34	0.48 ± 0.18	0.78 ± 0.44	0.57 ± 0.51
49	0.39 ± 0.26	0.37 ± 0.15	0.43 ± 0.24
63	0.25 ± 0.09	0.27 ± 0.09	0.26 ± 0.09
76	0.25 ± 0.21	0.35 ± 0.33	0.52 ± 0.11
91	0.19 ± 0.10	0.19 ± 0.13	0.26 ± 0.18
110	0.10 ± 0.06	0.13 ± 0.07	0.19 ± 0.12
139	0.15 ± 0.12	0.13 ± 0.07	0.31 ± 0.17

[a] Day = sampling day after treatment. CL = confidence limits.

the square root of the variance $V = \sum(X - \bar{X})^2/(n - 1)$, and n is the number of results.

RESULTS AND DISCUSSION

Dissipation of 3,6-DCP residues in sugar beets (roots, tops and leaves) from the first day of the treatment (day 0) to the harvesting (day 139) expressed in mg/kg (ppm) is detailed in Tables I, II, III, and IV for the rates of 120, 150, 180, and 360 g of a.i./ha, respectively.

In the roots, an increase of the amount of 3,6-DCP is clearly stated through the first week following the treatment, especially at high concentration level. At the lower concentration (120 g/ha), this increase is not noticeable. These observations, which confirm those of 1980, show a quick herbicide absorption in the beet; however, the high level, particularly at day 7, is probably due to the washing of the herbicide from the leaves to the tops which are included in the root analysis.

Tops and leaves present a quick decrease of their 3,6-DCP residues level, probably due to rain washings (35 mm for days 0–10, 15 mm for days 11–20, 95 mm for days 21–30).

Between day 91 samples and day 139 samples, no representative changes in residue level were detected.

In addition, these tables state the confidence limits of the results for a degree of confidence of 90%. These confidence limits take into account the variability due to the treatment, the sampling, and the analysis. These tables also show that after 1 month 3,6-DCP residues, expressed in ppm, are similar in roots and tops, which indicates that there is no preferential herbicide accumulation in one or the other part of the plant.

In Tables V and VI, 3,6-DCP residues are expressed in μg/beet respectively in root plus top and in leaves. They show that the herbicide absorption does not slow down

Table V. Amount of 3,6-DCP Residues, in Micrograms per Beet[a] (Root plus Top) after the Various Treatments

	120 g of a.i./ha		150 g of a.i./ha		180 g of a.i./ha		360 g of a.i./ha	
day	DCP, μg	beet, g	DCP, μg	beet, g	DCP, μg	beet, g	DCP, μg	beet, g
0	2.2	2.7	2.3	2.7	2.6	2.8	4.7	2.9
7	9.7	15.3	15.2	15.0	16.4	13.3	30.9	14.6
14	14.4	33.2	17.7	33.7	16.2	26.3	34.7	20.8
21	17.3	62.2	18.0	52.4	32.5	69.2	71.4	80.1
28	27.7	133.4	30.2	136.5	41.1	121.4	85.0	124.5
34	29.7	207.7	48.7	214.0	47.2	229.1	105.2	203.4
49	27.3	261.9	33.2	262.5	57.2	292.1	126.8	336.2
63	33.3	411.8	74.6	682.0	60.5	621.6	164.9	642.1
76	68.2	684.0	123.9	981.2	97.3	748.4	223.0	796.8
91	55.2	1033.3	50.8	894.9	85.2	948.8	170.3	839.8
110	78.2	1179.0	93.2	1310.4	118.3	1298.8	145.9	1338.4
139	74.3	1305.1	83.3	1408.0	109.6	1483.7	147.0	1102.0

[a] Brought back to the mean weight (in g) of a beet.

Table VI. Amount of 3,6-DCP Residues, in Micrograms per Beet[a] (Leaves) after the Various Treatments

	120 g of a.i./ha		150 g a.i./ha		180 g of a.i./ha		360 g of a.i./ha	
	DCP, μg	beet, g	DCP, μg	beet, g	DCP, μg	beet, g	DCP, μg	beet, g
0	189.4	42.4	211.7	45.6	246.7	47.1	555.7	52.6
7	49.1	133.0	58.3	132.8	61.7	112.5	108.9	117.0
14	46.6	201.2	60.8	211.0	51.6	151.7	86.3	128.8
21	57.3	345.3	58.3	291.0	71.3	304.2	162.0	308.8
28	59.8	472.9	103.0	472.5	127.4	495.4	236.7	424.4
34	72.7	645.4	138.1	691.0	135.8	594.4	319.9	548.7
49	84.9	649.5	78.9	606.1	123.2	543.1	292.9	685.4
63	91.2	747.6	82.3	755.0	142.9	814.0	200.5	768.7
76	118.9	966.1	141.9	1126.1	143.3	991.5	543.3	1049.8
91	53.9	739.7	79.7	916.2	79.8	863.0	232.8	853.5
110	69.0	886.3	67.4	784.0	75.3	909.5	161.6	915.5
139	55.7	632.4	62.6	570.5	69.2	597.9	114.9	419.8

[a] Brought back to the mean weight (in g) of a beet.

Table VII. Root Yield, Plant Population, and Sugar Yield of Sugar Beet Crops Treated at Rates of 120, 150, 180, and 360 g of a.i./ha

treatment, g of a.i./ha	root yield, kg/ha	plant population, n/ha	sugar %	sugar kg/ha	sugar purity
120	73 350	71 479	14.40	10 562	87.8
150	79 016	77 405	15.12	11 947	87.8
180	75 646	79 627	15.48	11 710	88.9
360	73 016	73 331	14.62	10 675	86.6
untreated	76 572	77 035	15.00	11 486	88.3

after 15 days as it seems to in Tables I–IV but continues with the plant growth during more or less 2 months. This absorption does not increase proportionally with the beet weight; that is the reason of the ppm profiles decrease after 15 days.

During this experimentation, as it appears in Table VII, it was proved that a 3,6-DCP treatment, even at 3 times the usual dose, does not influence the root yield or the plant population and the sugar percentage in comparison with that of the control.

In conclusion, 3,6-DCP can ensure the protection of the sugar beet crop with a minimum residue level at the harvesting (0.2–0.6 ppm total for treatment rates of 120–360 g of a.i./ha) and has no effect on sugar production. The persistence of the herbicide in soil will be also taken into account in a further study.

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Registry No. 3,6-DCP, 1702-17-6.

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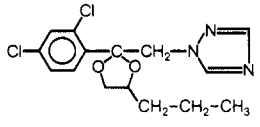
Gas Chromatographic Determination of Propiconazole and Etaconazole in Plant Material, Soil, and Water

Bruno Büttler

Propiconazole and etaconazole are two representatives of a new type of broad spectrum systemic fungicides. They are the active ingredients of Tilt and Sonax or Vangard (trademark used in the United States only), respectively. A method for the determination of each of the two fungicides in various crops, soil, and water is reported. The procedure involves the extraction of the samples with methanol, dilution of the extract with water, partition into dichloromethane, and cleanup on an alumina column. An additional cleanup by gel chromatography is described for straw. The analysis of water samples starts with the partition into dichloromethane. Residues are quantitatively determined by gas chromatography using an alkali flame ionizaton detector operating in the nitrogen-sensitive mode. Recoveries in the range of 76–100% indicate that this procedure is suitable for the residue analysis of these fungicides with detection limits of 0.02 mg/kg in fruit, grain, and soil, 0.05 mg/kg in other plant materials, and 0.001 mg/kg in water. Extraction efficiency of weathered residues was investigated, and results were presented to prove that the proposed procedure is adequate. The specificity of the method was tested with a series of important nitrogen- and/or phosphorus-containing fungicides, herbicides, and insecticides. No interferences were observed.

Propiconazole [1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole, I] and etaconazole



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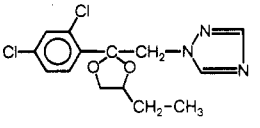

II

[1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]-methyl]-1*H*-1,2,4-triazole, II], developed under the code numbers CGA 64 250 and CGA 64 251, respectively, are two important representatives of a new class of fungitoxic chemicals. Both are broad-spectrum systemic fungicides with activity against powdery mildew, rust, scab, and leaf spot diseases on different crops. Propiconazole is currently used on cereals and in grapes while etaconazole is mainly used in deciduous fruits. The combined or alternative application of these two fungicides is not recommended so that they should not be found simultaneously on the same crop.

The almost identical chemical structure of the two compounds suggested nevertheless the development of a common method which could be used for the residue determination of either of these fungicides in cereals and deciduous fruits. The proposed procedure involves com-

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