

Pesticide Residues in Lettuce. 1. Influence of the Cultivar

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The residual behavior of three pesticides, administered in granulated formulation (carbofuran) and by spraying (chlozolinate and parathion methyl), on two different outdoor lettuces (crisp and cos) was studied. The residue content in the edible leaves is heavily affected by the shape of the lettuce only when pesticides are administered by spraying, and not when adsorbed from the soil. Dilution effect due to plant growth is the most important factor in the decrease in residue content in the edible leaves. At harvest time the residues of all active ingredients (AI), determined via HPLC, show very low values or none at all. Toxicological problems, connected with possible high residues and duration of safety times, are evaluated.

The pesticide doses (g/hl, hl/ha) normally used to control a certain pest are the same for different cultures. Thus, if in a certain culture the edible portion is only a part of the whole treated surface (e.g., tomato), the residue amount deposited will be much lower than that deposited in a plant in which surface and edible portion are more or less coincident. This occurs in lettuce, which is an entirely edible plant, except the external leaves, which are generally discarded at harvest for commercial aesthetic reasons. This character is of particular interest in that residue levels of the different pesticides used on this type of culture could be very high in comparison with other cultures.

In a previous work, Meloni et al. (1984) studied this topic and reported that residues much higher than those admitted by Italian Laws were found in the external leaves of greenhouse-grown lettuce, which are usually discarded before commercialization, while the internal ones did not contain traces of the studied pesticides. This fact was related to the type of lettuce (crisp) in which the external leaves completely cover the internal ones, protecting them as a true shell.

Thus, we thought of studying the residual behavior of three pesticides in a completely different open-shaped cultivar (cos) in which the external leaves do not cover or protect the internal ones. The pesticides chosen were carbofuran, parathion methyl, and chlozolinate. The first is a systemic insecticide, able to control *Pemphigus bursarium*, an insect that infects the roots and causes the plant to parch, and which in the last few years has produced great damage to the farmers in South Sardinia. Parathion methyl is the most used contact insecticide, because of its wide range of action and lower cost in comparison with other insecticides with similar activity but lower toxicity. Chlozolinate is a new dicarboximide fungicide, active against *Botrytis cinerea*.

EXPERIMENTAL SECTION

Materials and Methods. The trial was carried out in alluvial soil using the cultivars Salina (Asgrow), type crisp, and Valmaine (Peto Seed), type cos.

Transplantation was carried out on May 3, 1986, in double rows, containing plants linearly spaced at 0.70 m with 0.40 m between the rows, for a density of 7.5 plants/m².

A random-block scheme was used, with four replications, and each block measured 10 m² (20 m × 0.50 m).

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Table I. Rate of Pesticides Used in Experiments

trademark	AI	formulation rate (SD)		
		kg/ha	hl/ha	mg AI/m ²
Curater Gr.	carbofuran	15.0		75
Metyphon 20	parathion methyl	1.0	10	19
Serinal	chlozolinate	1.5	10	50

Carbofuran was sown broadcast on May 16, 1986, in granulated formulation, and then earthed, whereas the other two pesticides were applied with portable mechanical sprayers on June 3, 1986. Doses were exactly in accordance with the manufacturers recommendations (SD; Table I) and double strength (DD).

Sampling started on June 3 and continued weekly until commercial ripening.

The external leaves (first and second layers) were removed and analyzed separately.

Apparatus and Chromatography. A Spectra-Physics SP 8700 solvent delivery system, equipped with a UV/vis variable-wavelength SP 770, a Valco AH 20 injector (loop 50 μ L), and a Hewlett-Packard 3390 A reporting integrator, was employed.

Partisil-10 C₈ columns (250 × 4.6 mm (i.d.), 10 μ m, Whatman) were employed with a 45:55 mixture of water and acetonitrile as the eluent, at a flow rate of 1.0 mL/min. The detector was set at 200 nm, the best wavelength for simultaneous detection of the pesticides under study. In these analytical conditions 0.001 ppm of carbofuran, 0.003 ppm of paraoxon methyl, and 0.005 ppm of parathion methyl and chlozolinate could be detected.

The standard curve of each pesticide was constructed by plotting peak areas (external standard method) vs. concentrations. Good linearity was achieved in the range 0-10 ppm for chlozolinate, 0-2.5 ppm for parathion methyl, and 0-1 ppm for paraoxon methyl and carbofuran.

Chemicals. Water was distilled twice and filtered through a Millipore apparatus before use (0.45 μ m). Acetonitrile was HPLC-grade solvent while cyclohexane and benzene were pesticide-grade solvents (all from Carlo Erba, Milan, Italy). Chlozolinate was supplied by Farmoplant S.p.A. (Milan, Italy), and all other analytical standards were purchased from Siegmund-Ehrenstorfer (Augsburg, FRG).

Extraction Procedure. Each sample, two to five tufts depending on the size, was frozen immediately after sampling and then triturated with a mincing knife and homogenized. A 50-mL portion of a cyclohexane/benzene (50:50, v/v) solution was then added to 20 g of homogenized leaves; the mixture was shaken with a flask shaker (Stuart Scientific Co. Ltd) for 10 min. After centrifugation and filtration (Millipore, 0.45 μ m), 2.0 mL of the organic

Table II. Residues (ppm \pm SD) of Chlozolinate

irrigation syst	days after spraying	residues				av wt cabbage-lettuce, g
		external leaves		edible leaves		
		SD	DD	SD	DD	
Outdoor Crisp Lettuce						
sprayer	0	6.78 \pm 1.91	12.26 \pm 1.50	1.75 \pm 0.32	1.96 \pm 0.52	100
	7	3.29 \pm 1.03	8.72 \pm 1.23	0.66 \pm 0.26	0.69 \pm 0.11	162
	14	0.07 \pm 0.04	0.17 \pm 0.09	<0.005	<0.005	239
	21	0.03 \pm 0.01	<0.005			298
dripper	0	10.81 \pm 1.94	20.14 \pm 2.30	1.65 \pm 0.79	2.22 \pm 0.60	108
	7	5.22 \pm 0.56	15.42 \pm 1.19	0.97 \pm 0.50	0.80 \pm 0.35	234
	14	0.67 \pm 0.46	1.16 \pm 0.32	<0.005	<0.005	348
	21	<0.005	<0.005			354
Outdoor Cos Lettuce						
sprayer	0	2.48 \pm 1.08	7.02 \pm 1.14	6.18 \pm 0.98	15.21 \pm 3.13	135
	7	1.22 \pm 0.42	6.68 \pm 1.02	1.47 \pm 0.36	3.72 \pm 0.94	370
	14	0.62 \pm 0.07	3.15 \pm 0.33	0.22 \pm 0.18	0.98 \pm 0.15	743
	21	0.25 \pm 0.09	0.64 \pm 0.46	<0.005	0.39 \pm 0.06	836
dripper	0	2.16 \pm 0.31	5.27 \pm 1.50	10.85 \pm 1.52	21.71 \pm 1.79	159
	7	1.77 \pm 0.67	3.54 \pm 1.28	2.83 \pm 0.49	8.60 \pm 0.72	444
	14	1.02 \pm 0.42	1.92 \pm 0.41	0.19 \pm 0.04	1.16 \pm 0.80	792
	21	0.14 \pm 0.07	0.88 \pm 0.63	<0.005	0.02 \pm 0.02	767

layer in a 25-mL beaker was evaporated to dryness under reduced pressure (60 mmHg, 40 °C) in a stove. The residue was then recovered with 1 or 2 mL (depending on AI concentration) of the eluting mixture (H₂O/CH₃CN, 45:55, v/v). Recoveries obtained with this method on blank lettuce, fortified with known amounts of the studied pesticides, were satisfactory (88.9–103.6%). Fortification was made by adding pesticide amounts similar to those commonly found in outdoor lettuce.

RESULTS AND DISCUSSION

Chlozolinate. This new fungicide is registered in Spain, but only so far as regards flower cultures in Italy, while its registration is in process for other cultures in several countries.

From the data reported in Table II it was noted that residue values in the external leaves of outdoor crisp lettuce are very high immediately after administration (6.78–10.61 ppm in SD samples and 12.26–20.14 ppm in DD samples), whereas those in internal leaves are much lower (1.65–1.75 ppm in SD and 1.96–2.22 ppm in DD samples). This is due to the fact that, when treated, the plants were still young and the external leaves did not completely cover the internal ones. On the other hand in the outdoor cos lettuce, because of the particular calix shape, the internal leaves partially cover the external ones and the residual behavior will therefore be opposite that observed for the outdoor crisp lettuce.

Residues greatly decrease with time, both because of the dilution effect due to the plant growth (the weight of outdoor crisp lettuce increases 3-fold and that of outdoor cos lettuce 5–6-fold) and because of AI degradation. The main factor inducing the residue decrease is the plant growth effect.

When the influence of irrigation systems on the residue content was analyzed, it was noted that in the internal leaves the sprayer system did not produce any reduction due to washing away as compared to the drip-localized system. These analyses were made on data previously corrected for dilution due to plant growth, whereas for the external leaves this comparison was not possible because their growth was not determined.

The edible part of outdoor crisp lettuce did not contain chlozolinate residues 2 weeks after administration, while the outdoor cos lettuce contained high residues immediately after administration, which decreased below de-

tectable values within 3 weeks in the blocks treated with SD.

Parathion Methyl. Most European countries (Austria, France, Greece, FRG, Italy) fixed a residue legal limit of 0.15 ppm for vegetables, while in Denmark this limit is fixed at 0.20 ppm. In Italy this residue value includes also that of the main metabolite paraoxon methyl because of its high toxicity. In lettuce this metabolite is produced in such low amounts (1–4% of the starting parathion methyl; Mollhoff, 1968) that it was never found in this research.

The AI residues found in both internal and external leaves immediately after administration show values behaving similarly as for chlozolinate (Table III). The external leaf residues in the outdoor crisp lettuce are much higher than those found in the internal, and vice versa for the outdoor cos lettuce. This behavior is explained as for chlozolinate above.

One week after administration, the residues completely disappeared in both the internal and external leaves of the two types of lettuce. This is in accordance with the data reported in the FRG where a $t_{1/2}$ of 1–1.5 days was determined (FAO/WHO, 1969) and ratifies the decision made by the European countries of fixing safety times of between 2 and 4 weeks in order to avoid any toxicological risk in lettuce.

Carbofuran. This pesticide is highly toxic and must be used with particular care. For this reason, those countries allowing the use of carbofuran in edible cultures have fixed very long safety times: Belgium, 5 months; Austria, 8 weeks; England, 6 weeks; FRG, 70 days. Moreover, in granulated formulations, its administration is allowed immediately before sowing or transplantation (Portugal, The Netherlands, Denmark).

Extremely low maximum residual limit (MRL) was also fixed for vegetables in most European countries: 0.0 ppm in Belgium and in The Netherlands; 0.1 ppm in Switzerland; 0.5 ppm in France (European Directory of Agrochemical Products, 1984).

Carbofuran is allowed in Italy only for sugar beet at MRL 0.1 ppm (Italian Health Department Act, 1985).

From the data reported in Table IV it can be noted that, after the first sampling, residues show a completely different behavior as compared to that observed for the other studied pesticides. In fact, neither the shape of the lettuce nor the doses, nor even the irrigation systems, affect the

Table III. Residues (ppm \pm SD) of Parathion Methyl

irrigation syst	days after spraying	residues				av wt cabbage-lettuce, g
		external leaves		edible leaves		
		SD	DD	SD	DD	
Outdoor Crisp Lettuce						
sprayer	0	1.07 \pm 0.18	1.60 \pm 0.13	0.36 \pm 0.11	0.57 \pm 0.21	100
	7	<0.005	<0.005	<0.005	<0.005	162
	14					239
	21					298
dripper	0	2.07 \pm 0.30	2.99 \pm 0.65	0.60 \pm 0.06	0.48 \pm 0.14	108
	7	<0.005	<0.005	<0.005	<0.005	234
	14					348
	21					354
Outdoor Cos Lettuce						
sprayer	0	0.43 \pm 0.08	0.92 \pm 0.26	1.19 \pm 0.30	2.19 \pm 0.41	135
	7	<0.005	<0.005	<0.005	<0.005	370
	14					743
	21					836
dripper	0	0.70 \pm 0.20	0.83 \pm 0.34	1.63 \pm 0.64	2.72 \pm 0.30	159
	7	<0.005	<0.005	<0.005	<0.005	444
	14					792
	21					767

Table IV. Residues (ppm \pm SD) of Carbofuran

irrigation syst	days after administr	residues				av wt cabbage-lettuce, g
		external leaves		edible leaves		
		SD	DD	SD	DD	
Outdoor Crisp Lettuce						
sprayer	18	0.125 \pm 0.041	0.201 \pm 0.034	0.038 \pm 0.040	0.072 \pm 0.012	100
	25	0.047 \pm 0.017	0.113 \pm 0.047	0.031 \pm 0.020	0.081 \pm 0.038	162
	32	0.046 \pm 0.018	0.038 \pm 0.014	0.024 \pm 0.003	0.023 \pm 0.021	239
	39	<0.001	0.010 \pm 0.005	0.008 \pm 0.007	0.010 \pm 0.005	298
dripper	18	0.157 \pm 0.071	0.139 \pm 0.013	0.062 \pm 0.019	0.058 \pm 0.018	108
	25	0.088 \pm 0.024	0.041 \pm 0.017	0.043 \pm 0.021	0.028 \pm 0.005	234
	32	0.043 \pm 0.009	0.031 \pm 0.005	0.014 \pm 0.005	0.014 \pm 0.004	348
	39	0.008 \pm 0.002	0.007 \pm 0.003	0.006 \pm 0.004	0.005 \pm 0.002	354
Outdoor Cos Lettuce						
sprayer	18	0.157 \pm 0.067	0.166 \pm 0.109	0.105 \pm 0.018	0.110 \pm 0.060	135
	25	0.047 \pm 0.029	0.130 \pm 0.078	0.057 \pm 0.028	0.116 \pm 0.039	370
	32	0.006 \pm 0.003	0.005 \pm 0.003	0.018 \pm 0.003	0.011 \pm 0.008	743
	39	0.006 \pm 0.003	0.005 \pm 0.001	0.009 \pm 0.004	0.004 \pm 0.002	836
dripper	18	0.122 \pm 0.081	0.153 \pm 0.055	0.143 \pm 0.024	0.077 \pm 0.007	159
	25	0.095 \pm 0.066	0.073 \pm 0.021	0.051 \pm 0.032	0.044 \pm 0.020	444
	32	0.022 \pm 0.014	0.053 \pm 0.034	0.021 \pm 0.011	0.028 \pm 0.006	792
	39	0.016 \pm 0.012	0.016 \pm 0.004	0.014 \pm 0.012	0.014 \pm 0.004	767

residue content, because the AI is adsorbed from the soil through the roots.

The highest residue values were detected at the first sampling, the highest amount being in the external leaves, probably because the older the leaves the greater the absorption.

In the following days residues decrease to very low values and to procedure sensitivity limits, in both external and internal leaves.

The influence of the plant growth on the residue decrease could not be evaluated because while the plant weight and the AI degradation increased, a new amount of AI was being absorbed from the soil through the roots.

At harvest, carbofuran residues in edible leaves are much lower than the maximum limit of 0.1 ppm recommended by FAO/WHO (1977).

CONCLUSIONS

Parathion methyl shows such a high degradation rate that the operator is safe from any toxic effect even if the 20-day safety interval is disregarded.

On outdoor cos lettuce, chlozolinate residues were found to be very high on edible leaves at spraying time. A remarkable decrease in concentration observed in the same

leaves was mainly due to a dilution effect produced by the plant growth.

Thus, administration of chlozolinate just before harvesting, when growth slows down, can lead to dangerous residual contents in the market lettuce.

On the other hand, it does not occur in outdoor crisp lettuce because the external leaves completely cover the internal ones and protect them from any contamination.

Carbofuran, administered in granulated formulations, is absorbed from the soil through the roots, and neither the shape of the lettuce nor the doses, nor even the irrigation system, can affect its residue content in the plant.

Following our experimental schedule and considering the residue found in commercial lettuce and the maximum acceptable daily intakes (ADI = 0.003 ppm) suggested by the FAO/WHO (1983), carbofuran can be used safely.

The residual behavior of the studied pesticides showed that the shape of lettuce greatly affects the residue amount in the edible leaves, when the AI are applied by spraying. On the other hand, no shape influence was determined when the AI were absorbed from the soil through the roots.

Furthermore, absence of the studied pesticide residues in lettuce at harvest, or even presence in very low concentrations, confirms that respect of safety times is an

important safeguard of public health.

Registry No. Carbofuran, 1563-66-2; chlorozolinate, 72391-46-9; parathion methyl, 298-00-0.

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O-Acylation as a Novel Conjugation Pathway for Cinmethylin in Rats

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A complex degradation pattern of [*phenyl*-¹⁴C]cinmethylin (1) in laboratory rats following oral administration has been reported earlier. In addition to the undegraded 1, 10 metabolites were detected. In the urinary organic extractable fraction, two minor metabolites (each accounting for less than 1% of the administered radioactivity) were identified as *o*-(acetoxymethyl)benzoic acid and 9-(acetoxymethyl)- α -carboxycinmethylin. They were the corresponding *O*-acetyl analogues of *o*-(hydroxymethyl)benzoic acid and 9-hydroxy- α -carboxycinmethylin, the principal metabolites of 1. A detailed description of the isolation and identification of these two novel *O*-acylation conjugates is presented.

The metabolic fate of [*phenyl*-¹⁴C]cinmethylin (1) [Cinch herbicide, 7-oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-, *exo*], a novel cineole herbicide, in laboratory rats has been reported (Lee et al., 1986). Following a single oral dose, the major route of elimination was via urinary excretion. Approximately 75-85% of the administered radioactivity was eliminated during the initial 48-h postdosing. A complex degradation pattern of 1 was observed. In addition to the undegraded 1 (recovered only in the feces) 10 metabolites were isolated from the urine and feces as both organic extractable and conjugated products. The metabolic pathways of 1 involved hydroxylation and oxidation at the benzyl and cineole portion of the parent molecule, conjugation (with glucuronic acid and glycine), and ether cleavage (Figure 1).

During the analysis of the urine from animals administered a single dose of 450 mg/kg, two minor organic extractable metabolites (each accounting for less than 1% of the administered dose) were isolated. They were identified as the *O*-acetyl analogues of *o*-(hydroxymethyl)benzoic acid (6) and 9-hydroxy- α -carboxycinmethylin (4). Their identification represented a discovery of a novel conjugation pathway involving the acylation of the hydroxyl moiety. To the best of our knowledge, this is the first reported example of *O*-acylation of xenobiotics. Due to the potential significance of this finding, this report describes the isolation and identification of these two *O*-acetyl conjugates.

EXPERIMENTAL SECTION

A detailed description on the synthesis of radiolabeled test material and reference standards, treatment of test animals, and the analysis of excreta has been reported (Lee et al., 1986). A brief summary of the experimental design

is presented in the following outlines.

Test Compound. The radiochemical purity of [U-*phenyl*-¹⁴C]cinmethylin (1) was greater than 99% as determined by two-dimensional thin-layer chromatography (TLC) and liquid scintillation system (LSC). Radioactive impurities greater than 0.5% were not detected.

Test Animals. Male and female rats (Fischer 344, 10-16 weeks of age, weighing from 150 to 200 g each) were obtained from the Simonsen's Laboratories, Gilroy, CA.

Route of Administration. [¹⁴C]Cinmethylin was formulated in propylene glycol. An appropriate dose (450 mg/kg) was administered to the test animals by stomach intubation at a constant volume dose of 1.7 mL/kg. The final specific activity of the [¹⁴C]cinmethylin treatment solution was 0.16 μ Ci/mg. Animals were sacrificed 4 days postdosing.

Urine Sampling and Analysis. Urine from the [¹⁴C]cinmethylin-treated animals was collected daily. For the qualitative characterization of urinary degradation products, day 1 and day 2 excreta from each animal were combined for analysis. The pH of the combined urine sample was adjusted to pH 3 with 6 N HCl and partitioned three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous sodium sulfate, concentrated, and analyzed by two-dimensional TLC.

Separation of the organic extractable metabolites was carried out by preparative TLC prior to chromatographic and spectroscopic characterization. Confirmation of structure was obtained by direct comparison with an authentic standard.

Radioassay and Analytical Procedures. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution in a Packard Model 2660 or 300 liquid scintillation system. Extractable radioactivity was analyzed by TLC (silica gel F-254, 0.2 mm, E. Merck). The following solvent combinations were used: (1) toluene-2-propanol-acetic acid (150:20:1.5); (2) hexane-2-propanol-acetic acid (120:30:1); (3) toluene-ethyl acetate (17:3). Radioactivity on the TLC plate was visualized by autoradiography on

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