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Residues of Dicloran in Clingstone Peaches After Pre- and Postharvest Application

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Trials were carried out over a period of several years in order to test the efficacy of pre-and postharvest treatments against fungi causing postharvest rots of stored clingstone peaches destined to canning. Among the fungicidestested,dicloran(2,6-dichloro-4-nitroaniline) was applied against *Botrytis cinerea, Monilia cinerea* and *Rhizopus stolonifer.* The purpose of this work was to determine the residues of this fungicide in fresh, washed, peeled and canned fruits. No relevant data are reported in the open literature to our knowledge. Some residue data, most of them produced by the manufacturer of the compound, were evaluated by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) and are summarized in the 1974 and 1977 monographs (FAO/WHO 1975 and 1978).

MATERIALS AND METHODS

The study site was situated in the North of Greece amd the trees were of the cultivar ANDROS. The main trials were carried out for two consecutive years (1988 and 1989). Four experimental plots were established each year with four replicates of three trees each. In 1988 one plot was treated with dicloran (BOTRAN W 75% w/w) at 0.13% a.i. one day before harvest and triforine (SAPROL 16% EC) at 0.02% a.i. 15 days before harvest. The second plot was treated with dicloran at 0.13% a.i. one day before harvest. The third plot was treated with triforine at 0.02% a.i. 15 days before harvest and the last plot received no treatment (control plot). In 1989 the first plot was treated with dicloran at 0.13% a.i. one day before harvest and iprodione (ROVRAL 50% WP) at 0.05% a.i. 15 days before harvest. The second plot was treated with dicloran at 0.13% a.i. one day before harvest. The third plot was treated with iprodione at 0.05% a.i. 15 days before harvest and the last was the control plot.

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The chemicals were applied by a common orchard spraying equipment until runoff. Confirmatory applications were made in 1990 and 1991, when three trees were treated with dicloran at the application rate of 0.13% a.i. 1 day before harvest. From the harvested fruits of each replicate samples consisting of 18 fruits (6 from each tree, according to FAO/WHO recommendations) (FAO/WHO 1987) were randomly collected. In 1988 the number of samples taken from each plot and analyzed for dicloran residues was six, in 1989 three and in 1990 four, while in 1991 20 fruits collected from the treated plot were homogenized and analyzed as one sample. A large number of fruits from each plot was forwarded to the Institute of Technology of Agricultural Products of the National Agricultural Research Foundation in Lycovrissi, Attica and were subjected to postharvest handling and treatment.

Half of these fruits were dipped for 3 min in an aqueous suspension of BOTRAN at a concentration of 0.13% a.i. and the other half in water, then dried under air stream at 28°C. From the dicloran-treated fruits as well as from the fruits dipped in water (washed fruits) samples were collected for residue analysis at time zero (just after drying of fruits). In 1988, three samples of six fruits each were randomly taken for each case from each batch corresponding to each field plot. In the following years two samples from each plot were taken consisting of 6 fruits each in order to ascertain the effect of this type of washing on the level of residues.

In the 1988 and 1990 trials, part of the fruits was stored for 20 (1988) and 15 and 20 (1990) days in a cold room at 4° C and 90-95% relative humidity. Samples of six fruits each (three in 1988 and two in 1990) were collected after the storage periods and analyzed for dicloran residues.

In 1988 after 15 days of storage the fruits were canned using a simulated industrial technique. For that purpose the stones were removed with a knife and the peaches were peeled by alkali and heating (2% NaOH at 95° C for 50 sec) . Samples of peeled peach parts, weighting ca 1 kg each, were taken. The peeled fruits were canned with the addition of sugar syrup(=50:50) and the cans were stored in the laboratory. Three cans from each plot were randomly taken after eight months of storage in the laboratory and analyzed for dicloran residues. The content of cans (fruit+syrup) was homogenized and the analysis was carried out to the homogenized material.

Before analysis of field-treated fruits, the weight of

each sample was taken and the stones were removed with a knife and their weight noted. In this way it was possible to calculate the concentration in the whole fruit (pulp+stone) from the concentration determined in the pulp (flesh+peel) of fruits. This was necessary because in general the maximum residue limits (MRLs) set by international organizations apply to whole fruits (FAO/WHO 1986, EEC 1990) and not only to the edible part. For postharvest treated fruits the results are given for the pulp only, because of the high quantity deposited, which made the difference between concentration in the pulp and concentration in the whole fruit insignificant. The fruits were quartered and one set of opposite quarters of each peach were homogenized. The analytical samples were kept in a freezer (-20°C) until analysis.

All solvents and chromatographic adsorbents were of high purity and suitable for pesticide residue analysis. Dicloran standard of 100% purity was kindly provided by the Upjohn Company, USA. The method used for the determination of dicloran residues was that recommended by the manufacturer, with some minor modifications concerning the analytical sample size (20 instead of l00-200 g) and the addition of Hyflo Super Cel (diatomaceous earth) during extraction to facilitate filtration. According to this modified method, 25-50 g analytical sample was extracted in an Omni-Mixer with 100 ml acetone in the presence of 20 g Hyflo Super Cel for two min, followed by filtration on Buchner funnel and Whattman paper filter. The cake was reextracted with 150 ml dichloromethane and filtered on the same funnel. The filtrate was transferred in a separatory funnel of 500 ml where 250 ml water and 10 g NaCl were added. The content was shaken vigorously for 30 sec and left to equilibrate. After equilibration, the lower phase was collected in a 500 ml round bottom flask through anhydrous sodium sulphate, then 50 ml dichloromethane were added in the separatory funnel and the cleanup procedure was repeated. The solvent was rotary evaporated to dryness and made up in a volumetric flask with hexane. The extracts from cans were made up with a mixture of petroleum ether and dichloromethane 70:30 w/w and were subjected to a further cleanup by column chromatography. For that purpose glass columns of 18 mm diameter containing 10 g of florisil 60-100 mesh (activated at 180°C for 48 h and deactivated with 3% water) and 2-3 g anhydrous sodium sulphate were prepared. The columns were washed with 100 ml petroleum ether, then the extract was deposited on the top of the column and eluted with 250 ml of the mixture of solvents. This column cleanup was not necessary for the fruit samples, because their high concentration of residues necessitated a great dilution of extracts before gas chromatography resulting to very clean chromatograms. Indeed, after dilution, the extracts corresponded to

0.008 g fruit/ml extract for field-treated samples, and to 0.0005 g fruit/ml extract for postharvest treated samples.

For recovery efficiency studies known quantities of dicloran dissolved in hexane were added to 25 g samples previously tested to be dicloran free. These dicloran-free fruits were taken from another field, since the control fruits of the field trials were contaminated with dicloran . These control samples were fortified at the 0.005, 0.05 and 5 mg/kg level. Three samples for each fortification level were extracted as above and analyzed. The recoveries varied from 85 to 95% and the coefficients of variation from 5 to 10%.

For gas chromatographic analysis a Varian Aerograph 3700 and a Perkin Elmer Sigma 2 gas chromatographs equipped with ^{63}Ni electron capture detectors were used under the conditions given in Table 1. As mentioned, the extracts of fresh fruits were greatly diluted, because the detectors' responses were linear only over the range of 0.01 to 0.1 ng. Under all the gas chromatographic conditions used, the sensitivity was very high due to the two atoms of chlorine in the molecule of dicloran. Thus, the concentration of 0.001 mg/kg was estimated to be a reliable limit of determination. All analyses were carried out in duplicate.

RESULTS AND DISCUSSION

The residues determined on and in peaches harvested 1 day postapplication ranged from year to year between 3.40 and 5.71 mg/kg in the pulp and between 3.21 and 5.43 mg/kg in the whole fruit (pulp plus stone) (Table 2). Control samples contained 0.06-0.25 mg/kg dicloran in the pulp (0.05-0.22 mg/kg in the whole fruit), due to unavoidable contamination by drifting. The residues resulting from the preharvest treatment were lower than the limit of 15 mg/kg set by Codex Alimentarious as maximum admissible concentration (FAO/WHO 1994).

The washing of fruits in the way described above, removed great part of the residues, estimated to 40% of the residues present before washing (Table 2). Data on the effect of washing on dicloran residues on peaches are not available. However trials with other crops, such as Brussels sprouts and plums/prunes showed considerable reduction due to washing (FAO/WHO 1978).
The residues after postharvest treatment determined in 1988 (57.33 mg/kg on the average in the pulp of fruits pre- and postharvest treated and 49.5 in the pulp of

fruits only postharvest treated) (Tables 5 and 4, were deemed surprisingly high, given that the effect;;; concentration recommended by the manufacturer postharvest use on peaches is 20 mg/kg. For that reason the trials were repeated in 1989, 1990, and 1991. The

Table 1. Gas chromatographic conditions for the determination of dicloran residues.

Column	Temperature	Carrier gas	Retention time
	Instrument	Varian 3700	
5% OV-17 on Gas Chrom Q 80/100 mesh 1m x2mm	injector: 230°C detector: 250°C oven: 200°C	nitrogen 30 ml/min	11 min
3% OV-101 on Chrom WHP 100/120 treated with 6% Carbowax M 0.95mx2mm	injector: 210°C detector: 300°C oven: 250°C	nitrogen 30 ml/min	13 min
	Instrument Perkin Elmer Sigma 2		
1.5% 0V-101+1.95% QF1 on Gas Chrom Q 100/120 mesh 0.95mx2mm	injector: 210°C detector: 250°C oven: 180°C	nitrogen 30 ml/min	7 min
4% SE-30+ 6% OV-210 on Chrom P 80/100 mesh 2mx2mm	injector: 190°C detector: 250°C oven: 170°C	nitrogen 35 ml/min	10 min

concentrations determined during these trials were more reasonable and as expected, ranging from 13.50 and 22.10 mg/kg in pre- and postharvest treated fruits and between 15.50 and 20.00 in only postharvest treated fruits (Tables 3 and 4). This suggests that probably the high concentration in 1988 was due to technical error during application of the chemical. However, the relative data are reported here, because provide useful information about the fate of residues during processing.

Table 2. Residues (mg/kg) of dicloran in unwashed and washed clingstone peaches[a] sprayed in the orchard with dicloran at 0.13% a.i. and collected 1 day postapplication.

Treatments recieved	Unwashed fruits		Washed fruits[b]		% Loss
	pulp	whole fruit	pulp	whole fruit	
Year 1988					
dicloran+ triforine	4.63[c] ±0.36	4.32± 0.33	N.A.[d]	N.A.	
dicloran	4.13[c] ±0.39	3.88± 0.34	N.A.	N.A.	
control	0.25[c] ±0.05	0.22± 0.04	N.A.	N.A.	
Year 1989					
dicloran+ iprodione	3.40[e] ±1.32	3.21± 1.24	2.00[e] ±0.10	1.90 ±0.10	≈40
dicloran	3.43[e] ±0.73	3.22± 0.65	2.00[e] ±0.11	1.90 ±0.05	≈40
control	0.06[e] ±0.01	0.05± 0.00	0.03[e] ±0.05	0.02 ±0.05	≈50
Year 1990					
dicloran	5.71[f] ±0.46	5.43± 0.46	3.60[f] ±0.41	3.41 ±0.30	≈40
Year 1991					
dicloran	4.20[g]	3.86	2.55[g]	2.34	≈40
control	0.07[g]	0.06	0.04[g]	0.03	≈40

a. cultivar ANDROS
b. washing by dipping for 3 min in tap water
c. mean ± standard deviation of 6 samples of 18 fruits each from 3 trees
d. Not Analyzed
e. mean ± standard deviation of 3 samples of 18 fruits each from 3 trees
f. mean ± standard deviation of 4 samples of 6 fruits each from 3 trees
g. one sample of 20 fruits from 3 trees

Table 3. Residues (mg/kg) of dicloran in tne pulp or fresh. cold-stored, and canned clingstone peaches[a] after pre[b]-and postharvest[c] treatment.

Year	Fresh fruits at PHI[d] 1 day, time 0	Fruits cold-stored[e] days of storage		Peeled fruits[f]	Canned fruits[g] after 8 months of storage[h]
		15	20		
1988	56.33[i] ± 0.57	N.A.[j]	52.66[i] ± 0.57	2.18[i] ± 0.42	0.006-0.008[k]
	58.33[i] ± 2.88	N.A.	55.65[i] ± 5.13	1.91[i] ± 0.35	0.006-0.008[k]
1989	13.50[l]	N.A.	N.A.	N.A.	N.A.
	15.00[l]	N.A.	N.A.	N.A.	N.A.
1990	22.10[l]	21.50[l]	20.85[l]	N.A.	N.A.
1991	19.00[l]	N.A.	N.A.	N.A.	N.A.

a. cultivar ANDROS
b. spraying with dicloran at 0.13% a.i.
c. dipping for 3 min in a suspension of 0.13% a.i. dicloran
d. Preharvest Interval
e. temperature 4°C, relative humidity 90-95%
f. chemical peeling by 2% NaOH at 95°C for 50 sec
g. homogenized fruits plus syrup
h. storage in the laboratory (≈22°C)
i. mean ± standard deviation of three samples of ≈l kg
j. Not Analyzed
k. concentration range in 3 cans
1. mean of 2 samples of 6 fruits each

The residues on and in the pulp of fruits cold-stored for 15 or 20 days were not significantly different from those determined before storage.

Considerable reduction of residues was observed after chemical peeling of fruits. As shown in Tables 3 and 4, the percentage reduction was of the order of 96% for fruits pre- and postharvest treated and 97-98% for fruits only postharvest treated. These results, supported by the results on the effect of washing on the magnitude of residues, show that most of the residue remains on or in

Table 4. Residues (mg/kg) of dicloran in the pulp of fresh, cold-stored, and canned clingstone peaches after postharvest treatment[a].

Year	Fresh fruits PHI 1 day time 0	Fruits cold-stored for 20 days[b]	Peeled fruits[c]	Canned fruits[d] after 8 months of storage[e]
1988	51.00[f] ± 5.29	51.33[f] ± 5.13	0.91[f] ± 0.35	0.007-0.008[g]
	48.00[f] ± 7.93	47.33[f] ± 6.11	1.31[f] ± 0.35	0.006-0.007[g]
1989	15.50[h]	N.A.[i]	N.A.	N.A.
	16.00[h]	N.A.	N.A.	N.A.
1991	17.10[h]	N.A.	N.A.	N.A.
	20.00[h]	N.A.	N.A.	N.A.

a. dipping for 3 min in a suspension of 0.13% dicloran
b. temperature 4°C, relative humidity 90-95%
c. chemical peeling by 2% NaOH at 95°C for 50 sec
d. homogenized fruits plus syrup
e. storage in the laboratory (≈22°C)
f. mean ± standard deviation of 3 samples of ≈1 kg
g. concentration range in 3 cans
h. mean of 2 samples of 6 fruits each
i. Not Analyzed

the skin, and are attributable to the fact that dicloran is practicably insoluble in water, therefore there is very limited translocation of the fungicide into the interior of the fruit.

In spite of the high quantity of dicloran deposited on the fruits after the postharvest application the cans, after storage for 8 months, contained very low residues of dicloran (0.006 - 0.008 mg/kg) (Tables 3 and 4). These low concentrations in the cans indicate that apart from the expected reduction of ≈50% caused by dilution due to the addition of syrup, the residues are reduced further by metabolism of dicloran to other compounds. Trials with peaches for canning (pre- and postharvest applications) carried out by the manufacturer in California were evaluated by the 1977 JMPR (FAO/WHO 1978). According to these data, none of the samples of commercially canned peaches from 16 separate trials contained any detectable residue of dicloran when analyzed by microculorimetric

gas chromatography with a limit of determination of 0.01 mg/kg.

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