Effect of a Pilot Washing System on Dicofol Levels in Orange Matrix

Maria L. Ribeiro,*,† João R. Amador,‡ Luciana Polese,† Elaine F. G. Jardim,† Elizabeth V. Minelli,† and Olavo C. P. de Cordis§

Departamento de Química Orgânica, Instituto de Química, UNESP 14800-900, CP355, Araraquara, S.P., Brazil; FMC Food Tech. do Brasil Ind. e Com. Ltda., Araraquara, S.P., Brazil; and COMAPA—Cooperativa Mista da Agro-Pecuária de Araraquara, S.P., Brazil

An efficient analytical method is described for the analysis of dicofol residues in pulp and orange peel. Samples are mixed with Celite and transferred to chromatographic columns prepacked with silica gel. Dicofol is eluted with ethyl acetate, and the extracts are analyzed by gas chromatography with electron capture detection. Mean recoveries for dicofol at levels of 0.5, 2.0, 5.0, and 10 mg/kg ranged from 87 to 95% with relative standard deviation values between 2.6 and 9.0%. To investigate the effect of a pilot washing system on dicofol residues in oranges, the analytical procedure was applied to samples submitted to different treatments with commercial formulations under field and laboratory conditions. The orange samples with and without washing were analyzed in duplicate, and the results indicated that washing under the described conditions did not allow a complete removal of dicofol residues from orange peel.

Keywords: *Dicofol residues; gas chromatography; orange; washing*

INTRODUCTION

Brazil plays an important role in the world production of oranges. Brazilian citriculture produces fruits for both processing and fresh fruit market purposes (Gravena, 1997). São Paulo State, located in southeastern Brazil, is the biggest orange producer, and ∼74% of the orange production was destined for industrial processing between 1996 and 1997 (ABECITRUS, 1998).

Citrus processing generally includes the following steps: fruit unloading, fruit storage, fruit washing, juice and oil extraction, finishing, and evaporation. Fruit washing before extraction is important in juice production that does not employ heat treatment because it can remove excessive microbiological contamination. Also, the fruits coming from the field are usually dirty and may contain sand and residues of pesticides undesirable to the processed products (Kimball, 1996).

Processing studies (FAO/WHO, 1993) showed that residues of dicofol, a nonsystemic acaricide, are concentrated in the peel and citrus oil but are extremely low in the juice and pulp. As orange peel and its components are largely employed as raw material in the food, pharmaceutical, cosmetics, and animal food industries, it is important to investigate the fate and levels of dicofol and other pesticides in this matrix.

The aim of this study was to evaluate the effect of a pilot washing system on dicofol levels in orange samples. Dicofol is employed for the control of spider mites and soft-bodied mites in fruits, vegetables, and hops (Gillespie et al., 1994). The agricultural use of dicofol in Brazil is restricted to the application in cotton, apple, and citrus cultures (ILSI, 1995).

* Corresponding author (fax +55 16 2227932; telephone +55 16 2016600; e-mail mlucia@iq.unesp.br).

† UNESP.

‡ FMC Food Tech. do Brasil Ind. e Com. Ltda.

§ COMAPA.

MATERIALS AND METHODS

Apparatus. A Varian gas chromatograph (GC; model 3300) equipped with a 200 cm × 2 mm i.d. glass column packed with 1.5% OV-17/1.95% QF-1 on 100−120 mesh Chromosorb WHP, a constant current ^{63}Ni electron capture detector (ECD), and a Varian (model 4290) integrator were used. The operating conditions were as follows: injector temperature, 230 °C; column temperature, 190 °C; detector temperature, 300 °C; nitrogen flow rate, 40 mL/min; ECD range, 10; ECD attenuation, 4; and chart speed, 0.5 cm/min.

Reagents. *n*-Hexane and ethyl acetate (Mallinckrodt) were of pesticide grade. Silica gel 60 (70−230 mesh ASTM, Merck) was heated at 130 °C for 24 h before use. Celite (Reagen) was of analytical grade. Reference standard of dicofol [2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol] was obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC). Standard solutions were made up in *n*-hexane and stored at a temperature <−18 °C.

Preparation of Laboratory Sample and Fortification. Orange samples were peeled and chopped with a stainless steel knife. Peel and pulp were triturated separately using a household blender, homogenized, and stored in closed glass flasks at a temperature <−18 °C.

Fortified orange samples (peel or pulp) were prepared by adding 0.5 mL of each standard solution to 0.5 g (± 0.001) of sample.

Analytical Procedure. An analytical sample (peel or pulp) of 0.5 g (± 0.001) was mixed with 0.25 g of Celite by using a glass rod and transferred to the top of a glass chromatographic column (35 cm × 10 mm i.d.) prepacked with 1.5 g of silica gel. The elution was processed with 20 mL of ethyl acetate at 2 mL/min. The eluate was collected in a 100 mL modified round-bottom flask and concentrated in a rotary evaporator (45 °C). The final extract was reconstituted to an appropriate volume with *n*-hexane and analyzed by GC-ECD.

GCh Analysis. Suitable aliquots of sample extracts and standard solutions were injected into the gas chromatograph. The percentages of recoveries were calculated by comparing the average chromatographic peak areas of the standard, fortified samples and those of the unfortified samples. Quantification of dicofol in orange samples was performed using a

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Table 1. Recoveries of Dicofol in Fortified Orange Samples

fortification level (mg/kg)	recovery (%, mean[a] ± RSD) [range] peel	pulp
0.5	87 ± 5.7 [82−92]	90 ± 4.8 [85−95]
2.0	94 ± 5.0 [88−99]	93 ± 9.0 [85−101]
5.0	91 ± 7.6 [85−99]	95 ± 5.4 [87−98]
10.0	87 ± 5.7 [81−92]	93 ± 2.6 [91−96]

[a] Four analyses.

external standard calibration method. Calibration graphs were constructed by plotting peak areas versus concentrations.

Fruit Treatments with Dicofol. To investigate the effect of a pilot washing system on dicofol residue removal from oranges, field and laboratory experiments were achieved before the fruit washing.

Field Experiments. (1) Samples were taken from an orange grove ~40 days after the last application. (2) A field experiment was carried out in an orange grove of a farm located near Araraquara, in the State of São Paulo, Brazil. The commercial formulation Kelthane 480 (480 g/L dicofol, Rohm Haas) was applied in July 1996 according to the dosage (1.5 kg/ha) and application procedure recommended by the manufacturer. Treatment was accomplished in ~100 trees. Orange samples were collected 3 days after the dicofol application.

Laboratory Experiment. (3) Orange samples (~120 kg) obtained from the same farm as experiment 2 were soaked during 15 min in a Kelthane 480 solution prepared according to the instructions of the manufacturer (77 mL of Kelthane/100 mL of water).

Fruit Collection. Two hundred and forty kilograms of fruit of each treatment with dicofol was collected and distributed in two lots of ~120 kg by random sampling. One lot was washed, the other was not. A representative random sample (16 fruits, ~2 kg) of each treatment (with and without washing) was delivered to the laboratory and analyzed in duplicate as described above.

Fruit Washing. Washing experiments were carried out at FMC Food Tech. do Brasil Ind. e Com. Ltda. using a pilot mechanical system with nozzles and special brushes. A nozzle pressure of 450 psi was attained by adjusting special pumps. The exposure period of each fruit to the washing system was 5 s. After washing, the fruits were left to dry at room temperature (28 °C) for 12 h, and they were not exposed to sunlight.

RESULTS AND DISCUSSION

The analytical procedure is a modification of a method described by Torres et al. (1995) for the determination of organochlorine and organophosphorus pesticide residues in fruits and vegetables. In this method, after being blended with octadecylsilica (C_{18}), the samples were transferred to chromatographic columns filled with silica gel and the pesticides were eluted with ethyl acetate.

In the present study the orange samples were mixed with Celite in place of C_{18} silica and transferred to columns prepared as described previously.

The efficiency of the method was evaluated by means of recovery analyses with samples fortified at four different levels. The fortification levels were selected according to the maximum residue limits (MRL) established by different countries (FAO/WHO, 1993; ILSI, 1995). Table 1 shows the recovery and precision expressed as relative standard deviation (RSD). Mean recoveries ranged from 87 to 95% with RSD values between 2.6 and 9.0%. These data are in good agreement with those obtained by Torres et al. (1995) and confirm the efficiency of this methodology. The detection limits

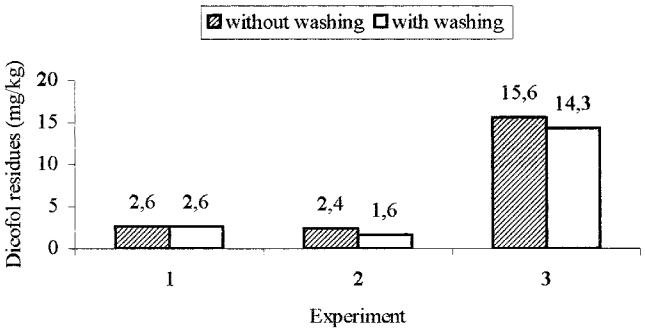

Figure 1. Effect of washing on dicofol levels in orange peel (washing conditions: pressure, 450 psi; time, 5 s).

were 0.07 and 0.08 mg/kg for pulp and peel, respectively, and the quantification limit was 0.5 mg/kg for both matrixes. They were determined according to the procedure described by Thier and Zeumer (1987). The gas chromatograms of unfortified samples used in the recovery analysis were free of dicofol residues and interfering compounds. The linear dynamic range of the ECD response for the dicofol was checked. A good linearity was achieved in the range from 25 to 500 pg injected on-column, and the average correlation coefficient was 0.9995. The retention time for dicofol was, under the advised conditions, ~3.9 min.

To evaluate the influence that the fruit washing may have on dicofol levels, the proposed method was applied to samples submitted to different treatments with commercial formulations under field and laboratory conditions. One representative sample of each treatment (with and without washing) was analyzed in duplicate, and the differences between the data of each duplicate did not exceed 6.7%. The main results are presented in Figure 1.

For the first experiment, no reduction in dicofol level (2.6 mg/kg) was obtained for washed oranges. In the second experiment, fruit washing decreased residue levels from 2.4 to 1.6 mg/kg (33%). When the dicofol residues from the unwashed oranges (2.6 and 2.4 mg/kg) were compared, no significant differences were noted for both experiments. Despite the fact that the trials were not carried out at the same field conditions, these data suggest that the residue levels declined slowly after 3 days from the dicofol application.

One additional laboratory experiment (3) was conducted for determining the effect of washing in fruits pretreated with dicofol under drastic conditions. The samples were soaked in a dicofol solution. The concentration values were c.a. ~8% lower in washed (14.3 mg/kg) than in unwashed fruits (15.6 mg/kg). These values are greater than the MRL established for citrus fruits (FAO/WHO, 1993; ILSI, 1995), so it is important to point out that dicofol is not indicated for postharvest treatments (FAO/WHO, 1993). Therefore, this experiment reproduced an incorrect use of dicofol.

Samples of orange pulp were analyzed separately, and residue values lower than 0.5 mg/kg were obtained for all samples. Similar results were achieved in experiments conducted in Japan and the United States and confirm that dicofol is a nonsystemic acaricide and that residues are predominantly found in orange peel (FAO/WHO, 1993).

Confidence in the results of this investigation was supported by the random sampling strategy employed and the accuracy and precision of the analytical meth-

odology that did not require specific optimizations for all analyzed samples.

On the basis of the results obtained in this study, it was concluded that fruit washing under the described conditions does not allow a complete removal of dicofol residues from orange peel; however, it did promote reduction in dicofol levels in the samples submitted to treatments 2 and 3.

Because fruit washing was performed at the same operating conditions, further investigations are necessary to evaluate the efficiency of the pilot washing system employed under different conditions for removal of dicofol and other pesticides from oranges.

ABBREVIATIONS USED

GC, gas chromatography; RSD, relative standard deviation; ECD, electron capture detector; MRL, maximum residue limit; C_{18}, octadecyl.

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