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Chlorpyrifos Decline Curves and Residue Levels from Different Commercial Formulations Applied to Oranges

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Residue levels and degradation rates of chlorpyrifos in orange fruits, orange leaves, and soil were investigated by using three different formulation types, that is, emulsifiable concentrate (EC), wettable granules (WG), and microencapsulates (ME). The pesticide degradation was studied for a period 131 days in orange fruits and for 161 days in orange leaves and soil. The experimental data were used to establish a mathematical model for the decline curves of chlorpyrifos residues as a function of time and to determine the relevant parameters describing such a process. Field trials showed a different degradation rate for EC and WG formulations as compared to ME formulation. For the first two formulations, the dissipation of chlorpyrifos in orange fruits was fast during the first phase and became much slower during the later period. Residue levels of chlorpyrifos from ME remained almost constant for ∼65 days and then began to decrease. A similar behavior was observed for the three chlorpyrifos formulations on orange leaves and soil. Although microencapsulation of pesticides leads to improved handling safety, additional risks for the consumers, the agriculture workers, and the environment should be taken into account due to prolonged persistence of high residue levels in fruits as well as in leaves and soil.

KEYWORDS: Chlorpyrifos; pesticide residues; oranges; organophosphates; decline curves

INTRODUCTION

Good knowledge of the pesticide fate in agriculture is necessary to properly assess human exposure and the environmental impact of these contaminants. Chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate] is an organophosphorus insecticide frequently used as a mosquitocide and termiticide. It is a stable compound in neutral and acidic aqueous solutions, although the stability decreases as pH increases. Chlorpyrifos is practically insoluble in water (2 mg/L) and soluble in most organic solvents (i.e., acetone, xylene, and methylene chloride) (*1*). Around 20−24 million pounds of chlorpyrifos is estimated to be applied annually. Approximately 50% of the use of chlorpyrifos is in agricultural settings. Applications of chlorpyrifos also include soil-incorporated/directed uses, bark treatments, and foliar treatments. The U.S. Environmental Protection Agency has assessed hazards including short-, intermediate- and long-term dermal and inhalation endpoints as well as acute oral endpoint (*2*). Chlorpyrifos is used on citrus fruits mainly to control various insects such as grasshoppers, aphids, and fire ants, etc. (*1, 2*). The preharvest interval (PHI) established by the Italian and European legislation for chlorpyrifos is 60 days for orange fruits, and the maximum residue level (MRL) is 0.3 mg/kg with the acceptable daily intake (ADI) equal to 10 mg/kg of body weight/day. As for other organophosphates, cholinesterase inhibition is the mode of action of chlorpyrifos and the cause of potential toxicity in human (*3*).

The compound can pose a hazard to agricultural workers who come into extensive and prolonged contact with treated foliage (*4*−*6*). Moreover, soil contamination results during the spraying operations. Therefore, it is important to assess residue levels of chlorpyrifos on fruits as well as on foliage and soil in order to reduce the risks for human health and to guarantee an acceptable ecotoxicological impact (*3, 7, 8*).

The formulation vehicle for a pesticide can have a significant impact on the stability and performance of the product. In this regard, the crop protection chemical market has seen significant changes in the past decade. The industry is active in developing better products with improved safety for the user, lowered impact on the environment, and more efficient use of products applied in the field. Controlled-release technology has emerged as an approach with potential for solving the problem associated with the application of conventional agrochemicals. With this technology active chemicals are made available to a specific target at a desired rate for a specific period of time. In this system a pesticide or any other bioactive agent is incorporated into a carrier, which is generally a polymeric material. The rate

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of release of the substance is determined by the properties of the polymer itself and also by the associated environmental factors (*9*−*17*).

In consideration of the continuously increasing use of these new-generation formulations, studies are needed to verify residue levels and decline curves of chlorpyrifos in citrus fruits (as well as in leaves and soil) in order to better understand the pesticide behavior after application of different types of formulations.

The aim of this work was to evaluate and compare the degradation rate and residue levels of three commercial formulations of chlorpyrifos on orange fruits, orange leaves, and soil. The typology of formulations investigated were the emulsifiable concentrate (EC), wettable granules (WG), and microencapsulates (ME), which are characterized by different behaviors with respect to the availability of the active ingredient (ai) after treatment. In particular, the first (Clorpiran 40 EC) is a conventional emulsifiable concentrate that makes immediately available all of the active ingredient applied; the second formulation (Dursban 75 WG) releases the active ingredient during a period ranging from 1 to 30 h after treatment in relation to UV exposure, and the third (Pyrinex ME) has a release mechanism depending on the environmental conditions, with particular reference to humidity, and has been introduced to the market to maintain a constant residue level for as long as possible.

MATERIALS AND METHODS

Reagents and Apparatus. *Reagents.* Chlorpyrifos (certified analytical standard, 99.7%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Solvents were of pesticide residue grade (Carlo Erba, Milano, Italy).

Standard Solution Preparation. Chlorpyrifos (10.6 mg) was dissolved in acetone/hexane 1:1 (v/v) and made up to 100 mL. Working standard solutions were prepared by volumetric serial dilutions.

Gel Permeation Chromatography (GPC). A dedicated sample cleanup system (DSCS) model 18L MLS (Lab Service Analytica S.r.l., Bologna, Italy) with 18 loops was used for sample cleanup. The chromatographic column was a 25 mm i.d. glass column with PTFE fittings, packed with 50 g of Bio-Beads SX3 resin, 200−400 mesh, compressed to a bed length of ∼30 cm (Lab Service Analytica S.r.l.). The elution solvent was ethyl acetate/cyclohexane (50:50, v/v) at a flow rate of 5 mL/min. GPC calibration was performed by injecting 5 mL of pesticide standard corresponding to the highest fortification level (1 mg/kg) in order to accurately determine the dump volume (i.e., the eluent volume to be discarded before and after the pesticide collection) and the pesticide collection volume.

Gas Chromatography (GC). An Austosystem Perkin-Elmer (Norwalk, CT) was equipped with a split−splitless injector, two capillary columns [RTX-5, 95% dimethyl−5% diphenyl polysiloxane, 30 m × 0.32 mm i.d, 0.25 μm film thickness; RTX-1701, 14% cyanopropylphenyl−86% methyl polysiloxane, 30 m × 0.32 mm i.d., 0.25 μm film thickness (Restek Corp., Bellefonte, PA)], and two nitrogen−phosphorus detectors. The chromatographic conditions for the analysis of chlorpyrifos were as follows: detector temperature, 350 °C; injector temperature 250 °C; oven temperature program, 2 min at 50 °C, 25 °C/min to 150 °C, 2.5 °C/min to 275 °C, hold for 25 min; makeup, Ar/CH_4 5%; carrier gas, He at 2 mL/min; injection volume, 2 μL, in a splitless mode. Chlorpyrifos certified standard was used for external calibration. The detector response was found to be linear in the studied range of 0.01−5 mg/kg. Under these conditions chlorpyrifos retention times were 24.38 and 27.56 min on the RTX-5 and RTX-1701 columns, respectively.

Field Trials. The experiments were carried out on Navelina cultivar oranges (20-year-old trees; 3.0 m height, 3.5 m diameter) in a citrus grove of the Metaponto area (southern Italy), on a silty clay soil (45.6% clay, 44.0% silt, and 10.4% sand) with a pH of 8.3 and 9.5% active lime. The grove was located 26 m above sea level, 40° 23′ 30″ latitude and 16° 43′ 00″ longitude, covering an area of 4 ha with a plant spacing of 6 m × 6 m. The grove was sprayed with three commercial chlorpyrifos-based products: Clorpiran 40 EC (emulsifiable concentrate), Dursban 75 WG (wettable granules), and Pyrinex ME (microencapsulate) with 14 hL/ha at the doses recommended by the manufacturers (120, 210, and 70 mL/h, respectively). Application rates were 680, 730, and 700 g of ai/ha for EC, WG, and ME formulations, respectively. These levels were selected on the basis of the producer-recommended doses. The three commercial formulations were sprayed on three 0.36 ha fields. Three replicate plots (∼0.12 ha), each consisting of five rows and containing 34 plants, were used for each treatment. Treatments were carried out with a pneumatic sprayer (Agrionica, Policoro, Italy).

Table 1. Climatic Data in Proximity of the Treatment Period

	October 1999					
	26	27	28	29	30	31
precipitation (mm)	0.2	0.0	0.0	0.0	0.0	0.0
daily mean *T* (°C)	20.4	20.0	19.5	17.1	14.4	15.4
daily min *T* (°C)	16.2	14.5	14.9	9.8	7.5	9.9
daily max *T* (°C)	24.2	27.8	26.7	29.7	22.6	23.1
daily mean RH[a] (%)	86.0	76.0	61.0	61.0	74.0	80.0
daily min RH (%)	67.0	49.0	27.0	21.0	48.0	57.0
daily max RH (%)	93.0	94.0	85.0	90.0	91.0	93.0
wind mean speed (m/s)	1.2	1.4	2.5	1.7	1.1	1.1

[a] RH, relative humidity.

Meteorological data were continuously recorded by an automatic weather station of the Basilicata Department of Agriculture. Treatments were performed on October 27, 1999, in the absence of precipitation and wind, with temperatures within the range registered locally during that period of the year (ranging from 15 to 20 °C). The climatic data in proximity of the treatment period and the monthly average of the climatic data during the whole experiment are reported in **Tables 1** and **2**, respectively.

Sampling. *Orange Fruits.* Samples were collected at time 0 (2 h after treatment) and 2, 6, 12, 19, 26, 40, 54, 75, 110, and 131 days after chlorpyrifos application; average fruit sizes at the different sampling dates were 181.2, 183.2, 190.2, 199.0, 199.2, 199.3, 232.3, 245.4, 248.7, 230.7, and 233.9 g, respectively. Fruit samples (30 pieces) were collected from 10 plants (three pieces per plant) in the three internal rows of each plot at ∼ 1.5 m height. The sample size of orange fruits was reduced by cutting with a knife each fruit into four parts and taking two opposite parts.

Orange Leaves and Soil. Leaf samples (300 whole leaves, ∼400 g) were collected randomly, at 1.5 m height, from the same plants (30 leaves per plant) of the treated groves at time 0 (2 h after treatment) and 14, 89, and 161 days after application. Samples were stored in glass jars and delivered to the laboratory immediately after harvesting.

Soil samples (∼1 kg) were collected during the morning of each day when the leaves were collected. At each sampling period, eight soil plugs (5 cm diameter × 5 cm deep) were removed randomly from each plot and mixed in a ceramic bowl.

No more than 2 h passed from sampling of leaves and soil to storage at 0−1 °C; analyses were carried out within 1 day from sampling.

Extraction and Analytical Procedure. *Orange Fruits and Leaves.* The extraction of chlorpyrifos residues from orange samples was carried out according to Steinwandter's procedure (*18*). A brief description of the procedure is as follows. About 1 kg of orange fruits (or 300 g of leaves) was homogenized in a 5 kg food cutter (CU56 Zoppas, Abusson, France). Fifty grams of homogenized sample (25 g in the case of leaves) was weighed into an Omnimixer glass jar; 100 mL of acetone, 75 mL of dichloromethane, and 15 g of sodium chloride were added, and then the mixture was blended at high-speed mode (15000 rpm) for 2 min. Fifty grams of anhydrous sodium sulfate was added to the supernatant after decantation. One hundred milliliters of the extract was evaporated to ∼4−5 mL using a vacuum rotary evaporator (40 °C water bath). The residue was transferred to a 100 mL round-bottom flask after filtration through anhydrous sodium sulfate (20 g). The original flask was rinsed twice with 15 mL of dichloromethane. The latter was combined with the extract after filtration through anhydrous sodium

Table 2. Monthly Average of the Climatic Data during the Experimental Period

	1999			2000					
	Oct	Nov	Dec	Jan	Feb	March	April	May	June
precipitation (mm)	16.2	30.4	34.0	6.2	44.6	22.6	40.4	49.0	9.4
daily max precipitation (mm)	7.8	11.0	27.0	4.2	25.2	9.4	20.8	22.8	4.8
mean *T* (°C)	18.0	12.1	9.4	6.2	7.4	9.7	15.3	19.8	24.0
mean of max *T* (°C)	26.0	19.0	15.7	13.6	14.8	17.2	22.1	27.2	32.2
max *T* (°C)	29.9	25.4	21.8	20.2	20.7	22.5	32.3	32.5	37.4
mean of min *T* (°C)	11.7	6.7	4.5	0.1	1.6	3.3	8.5	13.0	15.8
min *T* (°C)	7.5	–0.2	–2.9	–6.7	–3.5	–1.9	2.2	7.7	11.3
mean RH (%)	86.3	74.8	84.6	71.6	69.3	66.4	72.4	87.8	76.5
mean of min RH (%)	67.7	50.7	66.0	42.8	42.9	37.9	49.6	69.3	54.8
mean of max RH (%)	97.3	93.6	96.8	92.5	92.9	93.2	92.8	97.9	94.2
wind mean speed (m/s)	1.8	2.0	2.7	2.6	2.6	2.3	2.8	2.0	2.4
mean of max wind speed (m/s)	3.7	4.8	6.7	5.9	5.9	4.4	5.3	2.9	4.5
mean of min wind speed (m/s)	1.1	0.8	0.9	1.0	0.9	0.9	1.5	1.3	1.5

sulfate. The eluate was concentrated to ~1 mL using a vacuum rotary evaporator, and the evaporation was completed under a stream of nitrogen. The residue was taken up with 7 mL of ethyl acetate/cyclohexane (1:1) and filtered through a 0.45 μm PTFE filter (Farmitalia Carlo Erba, Milan, Italy).

Soil. Fifty grams of soil was mixed with 10 mL of distilled water and allowed to stand for 2 min; acetone (100 mL) was added, and the mixture was blended for 1.5 min at high-speed mode with an Omnimixer; dichloromethane (75 mL) and sodium chloride (15 g) were added and blended again for ~2 min. The mixture was submitted to ultrasound for 3 min and allowed to stand for 3−4 min. Fifty grams of anhydrous sodium sulfate was added to the organic phase; 100 mL was evaporated to ~5−10 mL using a vacuum rotary evaporator (40 °C water bath). The mixture was collected, and the flask was rinsed twice with 15 mL of dichloromethane; the organic portions were filtered through anhydrous sodium sulfate (20 g). The eluate was concentrated to ~1 mL using a vacuum rotatory evaporator, and the evaporation was completed under a stream of nitrogen. The residue was taken up with 7 mL of ethyl acetate/cyclohexane (1:1) and filtered through a 0.45 μm PTFE filter (Farmitalia Carlo Erba).

Cleanup was performed by GPC as follows. A portion of each extract (ca 6.5 mL) was loaded with a 10 mL glass syringe into 1 of the 18 calibrated 5 mL GPC loops. Then, 5 mL of extract was injected. The eluate was collected in a 250 mL round-bottom flask at 21−33 min of retention time (eluate volume fraction, 60 mL) and evaporated to ~5−10 mL using a vacuum rotatory evaporator (40 °C water bath), and then the evaporation was completed under a gentle stream of nitrogen at 30 °C using an automatic evaporator (Turbovap LV, Zymark Hopkinton, MA). The residue was taken up with 3 mL of acetone/hexane (50:50, v/v). The concentration factor relevant to the full extraction and cleanup procedure was 6.8. Chlorpyrifos was quantified by GC as reported above. The method limit of detection (LOD) (signal-to-noise ratio = 3) was found to be 0.004 mg/kg and the limit of quantitation, 0.01 mg/kg.

SigmaPlot 5.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis and to establish a dissipation model for chlorpyrifos.

Recoveries Assay. Four replicate analyses were performed on orange fruits, orange leaves, and soil at different spiking levels to assess recoveries of the analytical method. Samples of homogenized orange fruits and leaves or homogeneous soil, known to be free of the target active ingredient, were spiked with 0.01, 0.1, 0.5, or 1.0 mg/kg chlorpyrifos. Extraction and analysis were performed as described above.

For each set of analyses at different sampling times, at least two blank samples (chlorpyrifos free) and a chlorpyrifos-spiked sample were included to routinely check the method performance.

RESULTS AND DISCUSSION

Recovery and precision data of the analytical method applied to chlorpyrifos-spiked samples of orange fruits, orange leaves, and soil are reported in **Table 3**. Mean recovery rates ranged from 95 to 103% for orange fruits, from 93 to 101% for orange leaves, and from 82 to 92% for soil.

Table 3. Recoveries of the Analytical Method Used for Chlorpyrifos in Orange Fruits, Orange Leaves, and Soils

sample	fortification level, mg/kg	% recovery ± RSD ($n = 4$)
orange fruits	0.01	103 ± 9
	0.1	95 ± 4
	0.5	99 ± 10
	1.00	100 ± 7
orange leaves	0.01	97 ± 3
	0.1	93 ± 9
	0.5	101 ± 8
	1.00	95 ± 2
soil	0.01	91 ± 12
	0.1	85 ± 8
	0.5	82 ± 9
	1.00	92 ± 7

The residue data were submitted to statistical analysis to evaluate the decline of chlorpyrifos residues as a function of time and to determine the mathematical parameters describing the dissipation process. It was possible to establish a mathematical dissipation model (decline curve) only for EC and WG, whereas for ME formulation chlorpyrifos release remained steady at a well-defined level of active ingredient for a long period after the treatment (up to 65 days for orange fruits and 90 days for orange leaves and soil) before the effective decay of the residue levels could take place.

In particular, the experimental data of chlorpyrifos residues in orange fruits for EC and WG formulations at various times after application showed a biphasic behavior; the active ingredient dissipates rapidly during the first few days, and then the decline becomes slower. Therefore, a two-phase model was used to describe in a more appropriate way the dissipation of chlorpyrifos from these formulations and to calculate its dissipation half-lives according to the equation

$$C_t = C_1 e^{-k_1 t} + C_2 e^{-k_2 t} \quad (1)$$

where C_t is the insecticide residue concentration at time t, C_1 and C_2 are the initial insecticide residue concentrations of the two phases, t is the time after applications in days, and k_1 and k_2 are the dissipation rate constants in days^{-1}.

The dissipation half-lives of chlorpyrifos were calculated from the usual equation:

$$t_{1/2} = \ln 2/k \quad (2)$$

Table 4. Models To Calculate Half-Life Times ($t_{1/2}$) and Correlation Coefficients (R^2) for Decline Curves of Chlorpyrifos in Orange Fruits Treated with Emulsifiable Concentrate (Clorpiran 40 EC) and Wettable Granules (Dursban 75 WG) Formulations

formulation	model	R^2	$t_{1/2}$, days fast phase	$t_{1/2}$, days slow phase
two-phase model	$C_t = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}$			
Clorpiran 40 EC	$C_t = 0.2043 e^{-0.1096t} + 0.2053 e^{-0.0133t}$	0.95	6.3	52.1
Dursban 75 WG	$C_t = 0.1158 e^{-0.3109t} + 0.3623 e^{-0.0132t}$	0.94	2.2	52.5
one-phase model	$C_t = C_0 e^{-kt}$			
Clorpiran 40 EC	$C_t = 0.3682 e^{-0.0282t}$	0.90	24.6	
Dursban 75 WG	$C_t = 0.4156 e^{-0.0162t}$	0.91	42.8	

Table 5. One-Phase Model To Calculate Half-Life Times ($t_{1/2}$) and Correlation Coefficients (R^2) for Decline Curves of Chlorpyrifos in Orange Leaves and Soil after Treatment with Emulsifiable Concentrate (Clorpiran 40 EC) and Wettable Granules (Dursban 75 WG) Formulations

formulation	model $C_t = C_0 e^{-kt}$	R^2	$t_{1/2}$, days
	Orange Leaves		
Clorpiran 40 EC	$C_t = 3.2782 e^{-0.1053t}$	0.98	6.6
Dursban 75 WG	$C_t = 5.1255 e^{-0.1042t}$	0.98	6.7
	Soils		
Clorpiran 40 EC	$C_t = 0.6822 e^{-0.0912t}$	0.99	7.6
Dursban 75 WG	$C_t = 1.0629 e^{-0.1093t}$	0.97	6.3

The nonlinear model from the computer program Sigma Plot 5.0 was used to solve for C_1, k_1, C_2, and k_2 in eq 1. The theoretical dissipation model (together with the dissipation half-lives and the correlation coefficients) for chlorpyrifos established from eq 1 through regression between time after application and the corresponding residues in orange fruits is reported in **Table 4** (two-phase model).

Emulsifiable concentrate and wettable granule chlorpyrifos dissipated very quickly in orange fruits in the faster dissipation phase, with half-lives of 6.3 and 2.2 days, respectively. The dissipation rates of the two formulations during the later period became much slower, with half-lives of ~52−53 days. These data show that WG formulations have higher losses due to residue slough off, whereas later on when the deposits are stabilized on the plant surface and residues are partitioned into the waxy epicuticular layer of the plant surface, the dissipation rate is the same in both EC and WG formulations.

Although the behaviors of the EC and WG formulations appear to be similar (they have indeed similar two phase decline curves), the absolute residue levels obtained with EC and WG treatments were quite different. To explain such different behaviors, the overall residue half-life was determined by a simplified first-order decline curve, although this statistical evaluation does not provide the ideal description of the dissipation behavior. Regression analysis of chlorpyrifos dissipation by the first-order equation

$$C_t = C_0 e^{-kt} \quad (3)$$

where C_t is the insecticide residue concentration at time t and C_0 is the initial insecticide residue concentration, showed that the dissipation half-lives of chlorpyrifos were about 25 and 43 days for the EC and WG formulations, respectively (**Table 5**, one-phase model).

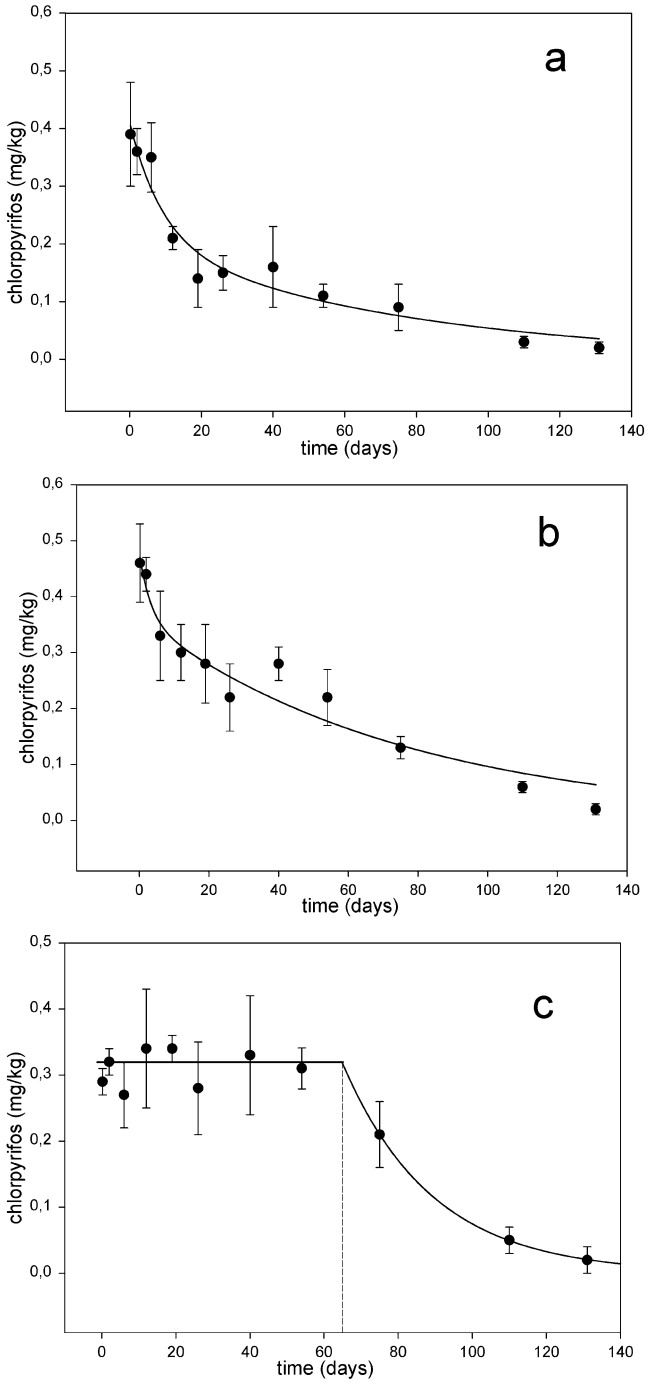

Figure 1. Decline curves in orange fruits of chlorpyrifos from different formulations: (a) emulsifiable concentrate (Clorpiran 40 EC); (b) wettable granules (Dursban 75 WG); (c) microencapsulate (Pyrinex ME). Error bars represent standard deviations of three replicate samples.

In the case of ME, chlorpyrifos residues started to decrease only after 65 days from the treatment. The dissipation behaviors of chlorpyrifos from EC, WG, and ME formulations in orange fruits are described with decline curves in panels a, b, and c, respectively, of **Figure 1**.

The different behaviors among chlorpyrifos formulations can be explained by the immediate release from the EC formulation, whereas WG or ME formulations must first release the active ingredient into the environment from the carrier in order to make it available for physical decay processes such as runoff and degradation.

In particular, the controlled release of WG formulations (occurring during 30 h after the treatment as declared by the

Table 6. Residue Levels of Chlorpyrifos in Leaves after Treatement with Different Formulations

days after application	residue level, mg/kg (SD)		
	Clorpiran 40 EC	Dursban 75 WG	Pyrinex ME
0.2	3.21 (0.45)	5.02 (0.46)	4.87 (0.16)
14	0.75 (0.12)	1.19 (0.07)	3.56 (0.17)
89	0.31 (0.11)	0.50 (0.33)	4.77 (1.42)
161	0.02 (0.01)	0.07 (0.03)	0.75 (0.26)

Table 7. Residue Levels of Chlorpyrifos in Soil after Treatement with Different Formulations

days after application	residue level, mg/kg (SD)		
	Clorpiran 40 EC	Dursban 75 WG	Pyrinex ME
0.2	0.67 (0.09)	1.04 (0.16)	0.41 (0.04)
14	0.19 (0.10)	0.23 (0.09)	0.41 (0.08)
89	0.06 (0.02)	0.13 (0.04)	0.36 (0.05)
161	0.01 (0.01)	0.02 (0.01)	0.07 (0.03)

producer) determines higher residue levels than the EC formulations, probably due to increased partition in the waxy epicuticular and other lipidic layers. Finally, the residue levels in fruits treated with the ME formulation were constant during 65 days (**Figure 1c**). This indicates that the amount of chlorpyrifos released for a long period by the capsules replaces the amount of residue that is continuously degrading; only after such period, that is, after the complete release of the active ingredient, can the residue levels start to decrease.

Another consideration is relevant to the different findings obtained for the initial chlorpyrifos residues found in orange fruits after treatment with EC, WG, and ME, that is, 0.39, 0.46, and 0.29 mg/kg, respectively, although similar levels of absolute amount of active ingredient were applied (application rates ranging from 680 to 730 g/ha active ingredient). The lower residue level observed for ME at the application time with respect to the other two formulations is due to the different manner of residue release, as reported also by the ME producer.

Chlorpyrifos residues found in orange fruits were generally lower than the Italian or European MRL, and the PHI was fully respected for both the EC and WG formulations. In particular, chlorpyrifos residue values in orange fruits treated with Clorpiran 40 EC were below the MRL already at 10−15 days after treatment (DAT), whereas Dursban 75 WG showed a regular and acceptable dissipation in agreement with permitted PHI. On the contrary, Pyrinex ME trials showed chlorpyrifos residues to be very stable during the PHI at levels quite close to the MRL value, indicating that risks of violations are possible when ME formulations are applied. It is necessary to point out that data obtained herein are relevant to a restricted situation in which the climatic effects (low precipitation and absence of irrigation, see **Table 2**) could have given a higher release of the active ingredient from the formulation (encapsulated formulations reduce the release under humid condition). Therefore, it is reasonable to expect high residue levels for a longer time under a normal precipitation situation, with a consequent increased risk for the consumer.

These findings could explain the high residue levels of chlorpyrifos frequently recorded in some recent Italian regional residue monitoring programs (N. Montemurro, unpublished data), unlike previous monitoring programs in which no microencapsulates were used and none of the samples tested in the same area was found to be contaminated by chlorpyrifos at levels higher than the RML (*19*).

Data relevant to orange leaves and soils after treatment with the different chlorpyrifos formulations are presented in **Tables 6** and **7**. In particular, Clorpiran 40 EC and Dursban 75 WG showed a continuous decrease of the residue levels similar to that observed for orange fruits, whereas Pyrinex showed a residue decay only after a long time (∼90 days) of persistent high-residue levels. Due to the limited number of experimental data relevant to leaves and soil, the simplified dissipation model depicted by eq 3 was used. The dissipation half-lives and correlation coefficients for chlorpyrifos obtained by the regression analysis between time after application and the corresponding residues in orange leaves and soil are reported in **Table 5**. These data clearly show a relatively fast decline of chlorpyrifos residues in orange leaves and soil ($t_{1/2}$ between 6 and 8 days) with a very good correlation ($R^2 \geq 0.97$) for both the EC and the WG formulations.

The lower degradation rate found in fruits with respect to leaves may be attributed to the following: first, it is possible that in the fruits the active ingredient is distributed within the waxy epicuticular layer and other lipidic phases, such as essential oils, where the degradation rate is lower; second, the higher surface/volume ratio in the case of leaves may lead to a higher residue slough off.

This work shows that the decline curve and the residue levels in fruits, leaves, and soil could change remarkably if the same active ingredient is used in different formulations.

Concerning the potential risks for agricultural worker contact, recent studies (*2*, *5*) on postapplication exposures of some formulations of chlorpyrifos showed that restricted entry intervals (REIs) for postapplication activities are based on dislodgeable foliar residues (DFRs) using the first part of the decline curve (0−DAT) in which the half-life is 1−1.5 days. Unfortunately, there is still limited information on ME formulations (having different degradation rates) due to the fact that they became commercially available only recently. Although dislodgeable studies are beyond the aim of the present investigation, the finding of high residue levels after treatment with microencapsulates indicates the additional risk for dermal contact during postapplication activities due to the prolonged occurrence of high chlorpyrifos levels on leaves, that is, up to 89 DAT at a persistent residue level of ∼4.40 mg/kg. These levels may overcome the actually accepted REIs for postapplication activities. In light of these results, the potential risks for worker contact should be reconsidered.

Moreover, the ecological risks due to chlorpyrifos are quite high to birds, fish, and mammals as well as to aquatic invertebrates. Most of the available literature on chlorpyrifos is devoted to the study of the environmental impact of this pesticide, whereas there are a limited number of studies on its release from granules. This work provides data on the different behaviors of three types of formulations on soil that may be useful to evaluate their impact on off-target environmental exposures.

In conclusion, although microencapsulation of pesticides leads to improved handling safety, additional risks for the consumers, the agriculture workers, and the environment should be taken into account due to prolonged persistence of high residue levels in fruits as well as in leaves and soil.

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