Diminution of Chlorpyrifos and Chlorpyrifos Oxon in Tomatoes and Green Beans Grown in Greenhouses

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A simple method based on dichloromethane extraction, gas chromatographic pulse flame photometric detection (GC/PFPD) and GC/MS confirmation has been applied to the analysis of chlorpyrifos and chlorpyrifos oxon residues in both tomatoes and green beans grown in two different types of greenhouses. To establish the influence of environmental conditions on the decrease of chlorpyrifos, several field trials have been carried out in which crops were sprayed at two different doses and in two seasons (spring and winter). Analysis of variance (ANOVA) has been applied to the results obtained, showing that the type of crop and the season are the most relevant factors in the degradation of chlorpyrifos. The oxon was detected the first 5 days after application, but a correlation with the diminution of the parent compound has not been found. The half-life of chlorpyrifos ranged between 4 and 5 days depending on the crop and season.

Keywords: *Chlorpyrifos; chlorpyrifos oxon; diminution; residues; tomatoes; green beans; ANOVA; greenhouses*

INTRODUCTION

The use of pesticides has an important role in agricultural practices. Several international organizations are working on the establishment of regulations for the use, manufacture, handling, and commercialization of pesticides. The Pesticide Residues Expert Group of the World Health Organization (WHO) evaluates toxicological data by estimating the admissible daily intake (ADI) of pesticides (WHO/PCS.94.1, 1993). The Expert Panel of the Food and Agriculture Organization (FAO) of Pesticide Residues (RP) in Foods and the Environment is working on the establishment of good agricultural practices based on the knowledge of chemistry, composition of pesticides, and pesticide residue analytical methodologies and estimations of the appropriate maximum residue levels (MRL) for the different types of food. In the European Union several countries have developed monitoring plans looking at the application of MRLs, as is referred to in the Annual Report of the Working Party of Pesticide Register (1996), Anderson et al. (1996), Klaveren (1996), Gamon (1996), Royal Order 280/1994 (1996), and Order 93/58/EEC (1993).

Information about the persistence of pesticides used and preharvest time is essential for agricultural workers and the quality of productss; therefore, it is interesting to carry out studies in different climatological, application, and crop conditions.

Chlorpyrifos is an organophosphate pesticide that acts as an insecticide−acaricide by ingestion, contact, and inhalation. It has a wide range of application and a high persistence in foliar application and is recommended for the control of aphids, white fly, Leptinotarsa, *Dociostaurus maroccanus*, and other insects in crops such as corn, fruits, and vegetables (Tomlin, 1994; Liñan, 1994).

Multiresidue methods exist for the determination of chlorpyrifos in fruits and vegetables based on acetone as an extracting solvent followed by partitioning with dichloromethane and using flame photometric detection (FPD) or electrolytic conductivity detection as is reported by Luke et al. (1981) and Di Muccio et al. (1988). Mourer et al. (1990) report a method to analyze chlorpyrifos and its metabolite, 3,5,6-trichloro-2-pyridinol (TCP), in dates using acetone as extractant, cleanup with Florisil, and derivatization of TCP using bis-(trimethylsilyl)acetamide. The parent compound is analyzed with nitrogen-phosphorus detection (NPD) and the TCP derivative with a Hall electrolytic conductivity detector. Seiber et al. (1993) report a study of several organophosphate pesticides, including chlorpyrifos and chlorpyrifos oxon, in air, water, and vegetables using ethyl acetate as the extracting solvent.

Dissipation studies of pesticides have also been reported by Cabras et al. (1990, 1995) for pirimicarb and metabolites in lettuces, peaches, and nectarines, accounting for the influence of cultural environment. Martínez Galera et al. (1997) studied the dissipation of fenpropathrin residues in tomatoes and green beans by evaluating the influence of different climatological and growing conditions. A similar study with methomyl was carried out by Gil García et al. (1997).

This study reports the results obtained in different field trials to establish the influence of different growing conditions and application rates in the persistence of chlorpyrifos and chlorpyrifos oxon applied on two different commodities. A previous study of the extraction and analytical method has been performed by matching the results obtained using three different detectors. Finally, analysis of variance (ANOVA) has also been carried out to establish the influence of different factors in the dissipation of chlorpyrifos.

EXPERIMENTAL PROCEDURES

Field Trial Design. The experiments were conducted in two different kinds of greenhouses located in Almeria (Spain) as follows: (1) a flat-roof greenhouse of polyethylene (200-μm

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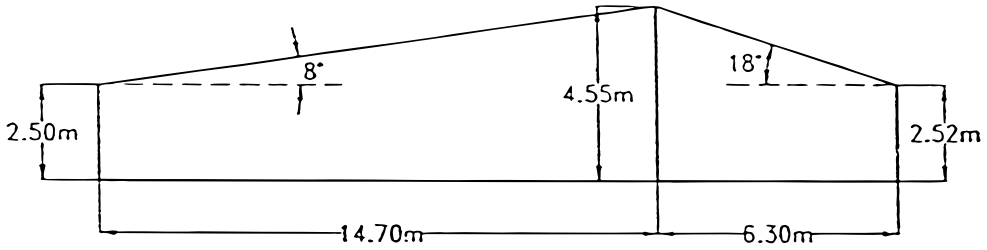

Figure 1. Structure of an asymmetrical roof greenhouse.

thickness) with a lateral window (1.30 × 30 m^2) that is covered with a fine netting; (2) an asymmetrical roof greenhouse of polyethylene (200-μm thickness) with a (1.5 × 30 m^2) window in the roof (Figure 1).

Green beans (cv. Helda) and tomatoes (cv. Daniela) were grown in 0.5-ha plots incorporating 30 000 and 10 000 plants, respectively. Dursban (chlorpyrifos 48% w/w) was applied at 2.0 or 1.0 mL/L and the rate of 1800 or 2000 L/ha in each case, corresponding to full and half doses, respectively. The treatments were carried out in the spring (May 18, 1996) and winter (January 17, 1997). Climatological conditions were monitored and registered during the experiment by using a Jules Richard model 16352.47 thermohygrographer (Argenteuil Cedex, France).

Chemicals. The solvents used were acetone and dichloromethane (residue analysis grade, Panreac, Barcelona, Spain). The chlorpyrifos and chlorpyrifos oxon standards (99% pure, pestanal quality), obtained from Riedel de Haën (Seelze, Germany), were dissolved separately in acetone (0.2 mg/mL) to obtain the primary calibration solution from which solutions of lower concentration were prepared by dilution with acetone or matrix extract when appropriate.

Dursban (chlorpyrifos 48% w/w wettable powder ICI-Zeltia, Madrid, Spain) was used for treating plants in the greenhouses.

Apparatus and Chromatography. To compare the response of three different detectors used for organophosphorus analysis, three gas chromatographs were used: a Hewlett-Packard (Palo Alto, CA) model 5890 gas chromatograph equipped with an NPD and an HP 7673 autosampler; a Perkin-Elmer model 5400 equipped with an FPD; and a Varian Star 3400 CX equipped with a PFPD. A fused silica capillary (HP-1701) column containing 14% methylpolysiloxane as stationary phase [25-m length, 0.25-mm internal diameter (i.d.) and 0.25-μm film thickness] was used for the separation in each GC.

A Hewlett-Packard model 5890 series II gas chromatograph coupled with an HP 5971A mass spectrometer detector and equipped with an on-column injector and an HP 7673 autosampler with HP-UX Chemsystem software was used for GC/MS confirmation purposes. A Chrompak (Middelburg, The Netherlands) CP-Sil 5 capillary column (25-m length, 0.25-mm i.d., and 0.25-μm film thickness) connected to a deactivated fused silica uncoated precolumn (1-m length, 0.53-mm i.d.) was used for the separation.

GC operating conditions were the same for each GC: injector temperature, 250 °C; detector temperature, 300 °C; splitless time, 2 min (min); initial temperature, 105 °C for 2 min, raised at 20 °C/min to 150 °C, and at 10 °C/min to 250 °C, and then held at 250 °C for 5 min. The carrier gas was nitrogen at 1 mL/min.

GC/MS operating conditions: initial oven temperature, 60 °C for 1 min, raised at 10 °C/min to 270 °C (5-min hold); on-column injection was used, the initial injector temperature being 63 °C and then programmed at the same rate as the oven; helium was used as carrier gas with 55-MPa column head pressure. Mass spectrometer settings: electron impact ionization mode with 70-eV electron energy, scan mass range m/z 50−400.

Sampling and Storage. For each vegetable, samples were collected at random at 0, 0.5, 1, 2, 3, 4, 5, 8, and 15 days after application of chlorpyrifos (Dursban 48). Each sample was chopped and divided into four subsamples (50 g), which were stored in individual polyethylene bags at −24 °C until extraction.

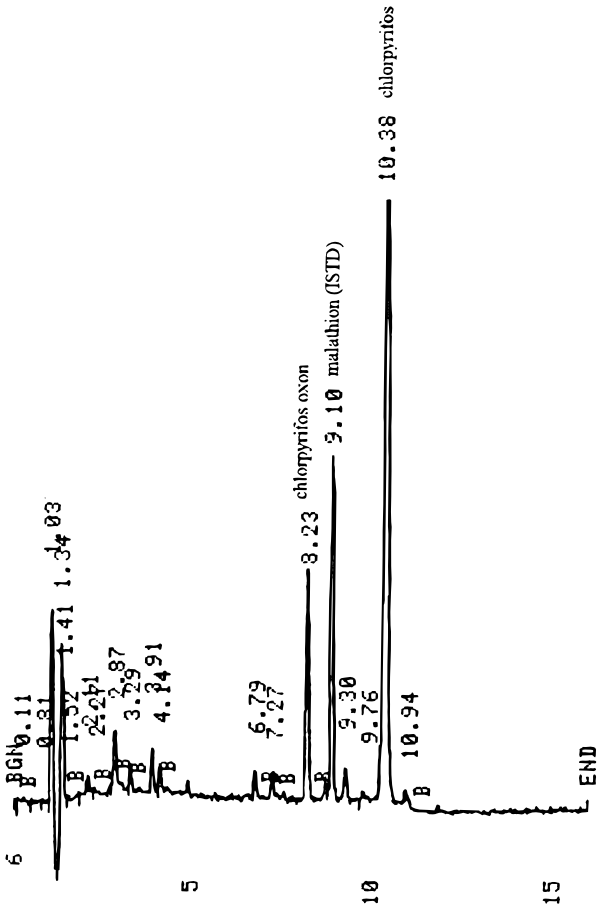

Figure 2. PFPD chromatogram of chlorpyrifos and chlorpyrifos oxon of an acetone extract of green beans spiked with 0.29 mg/kg chlorpyrifos and chlorpirifos oxon and containing the internal standard.

Extraction and Analysis. A sample of chopped tomato or green bean (35 g) and 100 g of anhydrous sodium sulfate was shaken mechanically with dichloromethane (100 mL) for 1 h. The mixture was filtered through a filter paper into a 250-mL round-bottom flask and the cake washed twice with 10 mL of dichloromethane each time. The solvent was removed under vacuum at 40 °C in a rotary evaporator until almost dry and then just to the point of dryness with a slight N_2 stream, after which the internal standard solution (4 μg of malathion) was added and the volume made up to 10 mL with acetone. This solution was injected into the GC/PFPD (1 μL) and GC/MS (5 μL).

Recovery Study. The recovery study was carried out by spiking with 100 μL of chlorpyrifos and chlorpyrifos oxon standard solutions, 35 g of fresh tomato, and green bean samples that had not been treated with the pesticide. The method was assessed at two different spiking levels, 0.29 and 0.05 mg of chlorpyrifdos and chlorpyrifos oxon/kg plant material. After evaporation of the acetone using an air stream, the

Table 1. Calibration Data Obtained with the PFPD

analyte	RTW CPSil-5[a]	RTW HP-1701	detection limit (mg/kg)	quantification limit (mg/kg)	equation	SD slope	SD intercept
chlorpyrifos	9.42−9.47	10.36−10.40	0.003	0.010	$y = 0.679$ (amt ratio) + 0.013	0.03	0.04
chlorpyrifos oxon	7.87−7.92	8.21−8.25	0.012	0.041	$y = 0.111$ (amt ratio) + 0.007	0.04	0.04
malathion		9.08−9.12					

[a] Retention time window obtained with GC/MS operating conditions.

Table 2. RSD (Percent) of the Response Factors

analyte	peak/area NPD	peak/area FPD	peak/area PFPD	peak/height NPD	peak/height FPD	peak/height PFPD
chlorpyrifos	10.9	10.8	7.1	8.9	9.7	11.3
chlorpyrifos oxon	12.1	10.5	8.7	10.2	11.9	12.1

sample was mixed thoroughly and homogenized for 2 min. The samples were then extracted and analyzed. Six replicates of each recovery assay and six blank samples of each vegetable were extracted and analyzed.

RESULTS AND DISCUSSION

Analysis. Figure 2 shows the PFPD chromatogram of chlorpyrifos and chlorpyrifos oxon of an acetone extract of green beans spiked with both compounds at 0.29 mg/kg concentration level and containing the internal standard.

Confirmation of chlorpyrifos residues was carried out by GC/MS under the conditions described above. The predominant ions found were *m/z* 97, 197, 199, and 314 in the case of chlorpyrifos and *m/z* 109, 197, 242, 270, and 298 in the case of the oxon.

Calibration. Table 1 summarizes the retention time window (RTW) determined for chlorpyrifos and chlorpyirifos oxon in the two columns. The RTW is defined as the average of the retention times (eight replicates) plus or minus 3 times the standard deviation (SD) of retention times (RT). The use of three different detectors did not affect the RTW.

A range of calibration solutions (1 μL) containing 0.01−5.0 μg/mL of chlorpyrifos and chlorpyrifos oxon were injected into the three GCs to determine dynamic ranges as described Hsu et al. (1988). It can be seen in Table 2 that the relative standard deviations (RSD) of the response factors (RF, defined as the ratio between the amount of analyte injected and the signal obtained) measured between 5 and 100 times the quantification limit (QL). The best results were 7.1 and 8.7% for both pesticides using PFPD and considering peak/areas, while these values were 11.3 and 12.1% when peak/heights were considered.

The sensitivity of the PFPD was greater than that of the other detectors using peak/areas, but it decreased using peak/heights. This factor is not critical in the rest of the detectors, which showed similar behavior measuring areas or heights. Although the PFPD and FPD are more sensitive, the noise of baselines is higher than in the case of NPD. Detection and quantification limits were calculated as described in Thier et al. (1987) using the sensitivity and the SD of the baseline signal at the RT of the analytes, these limits being lower with the PFPD and similar with the NPD and FPD (Table 1). All calibration solutions used in this study were prepared "in matrix" and calculated for tomatoes and green beans.

The repeatability of the signal response (peak/areas and peak/heights) was also studied by injecting sequentially 10 times 1 μL of a standard solution containing

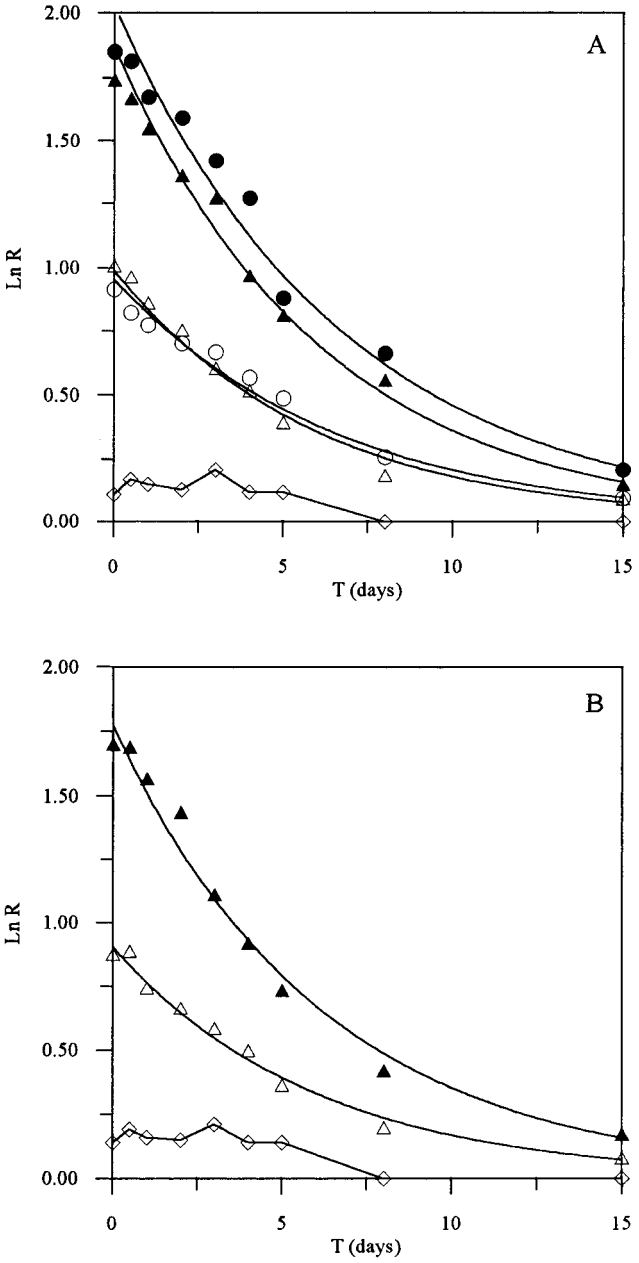

Figure 3. Diminution of chlorpyrifos residues in green beans grown (A) in a flat-roof greenhouse and (B) in an asymmetrical roof greenhouse: (●) winter, full dose; (○) winter, half dose; (▲) spring, full dose; (△) spring, half dose; (◇) chlorpyrifos oxon, full dose, spring.

0.4 μg/mL chlorpyrifos, chlorpyrifos oxon, and malathion. When quantification is carried out without internal standard, the RSDs found for the parent compound using PFPD, FPD< and NPD were 9, 11, and 11%, respectively, in the first case (peak/area) and 12, 11, and 11%, respectively, in the second case (peak/height). However, when the internal standard is taken into account, the RSDs of the ratio analyte/malathion signals decreased dramatically to ∼50% of the values given

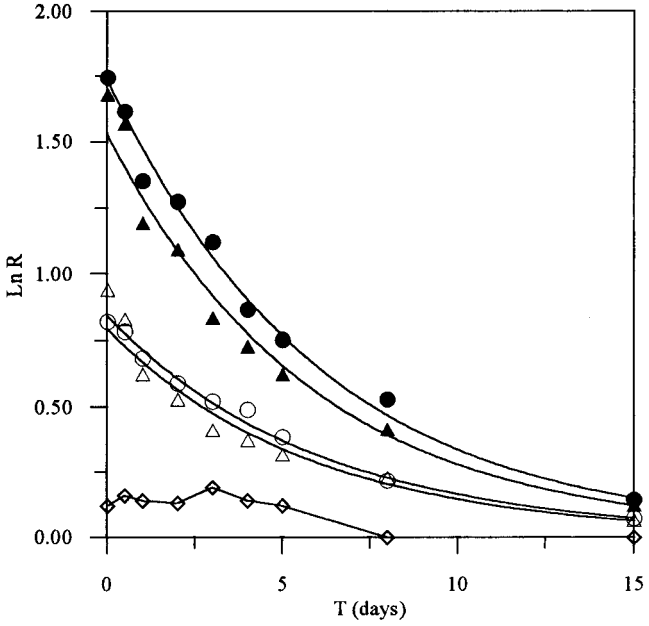

Figure 4. Diminution of chlorpyrifos residues in tomatoes grown in a flat-roof greenhouse: (●) winter, full dose; (○) winter, half dose; (▲) spring, full dose; (△) spring, half dose; (◇) chlorpyrifos oxon, full dose, spring.

above. The behavior of the chlorpyrifos oxon was similar; because of that, PFPD and internal standard calibration were chosen for quantification in the study

Finally, calibration lines (Table 1) were obtained by injecting eight standard solutions prepared in matrixes of green beans and tomatoes that have not been treated with pesticides, in a range of concentrations between 0.04 and 1.0 μg/mL of chlorpyrifos and chlorpyrifos oxon (blanks of these matrixex were also analyzed to check that no contamination from these extracts interfered with the analytes). Internal standard calibration was used by adding to each calibration point 0.4 μg/mL of malathion and plotting signal ratio versus amount ratio. The correlation coefficients obtained, either for chlorpyrifos or chlorpyrifos oxon, were >0.997.

Extraction Procedure and Recovery Study. The extraction procedure described above was used to determine the average recoveries of chlorpyrifos and chlorpyrifos oxon from green beans and tomatoes at the two spiking levels. The values found show that the extraction method is efficient in extracting the residues of both compounds from these vegetables, since the average recoveries of chlorpyrifos were 91.2% (high level) and 90.4% (low level) in tomatoes and 88.3% (high level) and 86.2% (low level) in green beans with RSD < 8.6%. Recoveries of chlorpyrifos oxon were 85.7 and 84.8% at high and low concentration levels, respectively, in tomatoes, and 85.5 and 85.4%, respectively, in green beans, the RSD being <9.7%.

Study of Diminution of Chlorpyrifos and Chlorpyrifos Oxon Residue Levels with Time. Figures 3 and 4 show the rate of loss of chlorpyrifos with time under different conditions. The effects of season and application rate on the diminution of chlorpyrifos residues in green beans grown in flat-roof (Figure 3A) and asymmetrical roof greenhouses (Figure 3B) and in tomatoes grown in a flat-roof greenhouse (Figure 4) can be seen. The highest levels of pesticide were found in green beans and tomatoes, ~1.8 mg/kg, in winter and in the experiment performed at full dose. The average concentration of chlorpyrifos oxon, similar in both species, was ~0.14 mg/kg during the first 5 days, in spring and in the experiment carried out at full dose, the highest level being found the third day (0.21 and 0.19 mg/kg in green beans and tomatoes, respectively). In winter, the chlorpyrifos oxon was also detected in the samples analyzed the first 5 days of the experiment in concentration levels also similar and ~0.09 mg/kg. The highest values were found in spring, when the sunlight and temperature were higher than in winter. This behavior suggests that these factors may facilitate the formation of the oxon compound. Nevertheless, the diminution of chlorpyrifos residue levels with time is not correlated with a similar increase of the chlorpyrifos oxon residue levels, which suggests that this is not the only path in the degradation of the parent compound.

Statistical interpretation of the loss of chlorpyrifos in the experimental plots was performed by assuming that the diminution rate of the residues can be described as a pseudo-first-order reaction according to the equation $R = R_0\, e^{-kt}$ and can be quantified by a linear semilogarithmic regression analysis $\ln R = \ln R_0 - kt$, where R is the residue level at t days after pesticide application, R_0 is the residue level at time $t = 0$, and k is the loss rate constant. The regression coefficients are >0.97 in all cases.

Statistical and chlorpyrifos loss parameters are shown in Table 3. The half-life ($T/2$) range for tomatoes is 4.1−4.3 days and for green beans, 4.1−4.7 days. The tenth-life ($T/10$) is between 13.6 and 14.1 days for tomatoes and between 13.7 and 15.6 days for green beans.

ANOVA was carried out to study the influence on the diminution of chlorpyrifos with the factors species grown (tomatoes or green beans), season (spring or winter), doses (half or full), and type of greenhouse (flat or asymmetrical roof), by using the $T/2$ values obtained in each case (*Statgraphics Reference Manual*, 1995). The results obtained in a previous one-way ANOVA, applied to determine the effect of each parameter individually on the diminution rate of chlorpyrifos, considering a confidence level of 95%, show that the only factor which has an effect is the season, with a significance level of 0.0000. The other factors show significance levels >0.05.

Table 3. Data of Loss of Chlorpyrifos from Treated Crops

	green beans						tomatoes			
statistical	winter (flat roof)		spring (flat roof)		spring (asymmetrical roof)		winter (flat roof)		spring (flat roof)	
parameters	full dose	half dose	full dose	half dose	full dose	half dose	full dose	half dose	full dose	half dose
K (days^{-1})	0.15	0.15	0.16	0.17	0.16	0.17	0.16	0.17	0.17	0.16
R_0 (mg/kg)	2.031	0.955	1.873	0.991	1.777	0.898	1.738	0.842	1.527	0.798
reg coeff	0.9816	0.9894	0.9932	0.9727	0.9887	0.9883	0.9939	0.9956	0.9910	0.9810
$T/2$ (days)	4.68	4.56	4.23	4.11	4.30	4.14	4.25	4.27	4.11	4.10
$T/10$ (days)	15.56	15.16	14.06	13.65	14.28	13.75	14.11	14.13	13.66	13.62
R_{10} (mg/kg)	0.462	0.209	0.364	0.183	0.354	0.168	0.340	0.165	0.283	0.147

Table 4. Average Values of Half-Life Times and Standard Errors for the Treatments

level	av (days) ± SE	level	av (days) ± SE
total	4.30 ± 0.03	full dose	4.36 ± 0.04
green beans	4.40 ± 0.03	half dose	4.25 ± 0.04
tomatoes	4.21 ± 0.04	green beans, winter	4.62 ± 0.05
winter	4.45 ± 0.04	green beans, spring	4.17 ± 0.03
spring	4.15 ± 0.03	tomatoes, winter	4.29 ± 0.05
flat roof	4.28 ± 0.02	tomatoes, spring	4.12 ± 0.05
asymmetrical roof	4.33 ± 0.06		

A multiple ANOVA was also applied, and the results indicated that the factors species grown and season have an influence on the loss of chlorpyrifos (yielding significance levels of 0.0002 and 0.0000, respectively), whereas the factors kind of greenhouse and doses have no significant influence. The cross-effect species grown by season is also significant (significance level = 0.0031), which indicates that the effect of season is different in tomatoes and in green beans. The averages of $T/2$ values and standard errors (SE), either for the total sample or for the different factors in the diminution rate of chlorpyrifos, are summarized in Table 4. It can be seen that chlorpyrifos disappears more rapidly in spring than in winter; the rate of loss is also lower in green beans than in tomatoes, which show the highest diminution rate in spring.

Conclusions. PFPD showed better response than FPD and NPD for the analysis of chlorpyrifos and chlorpyrifos oxon. The metabolite appears the first 5 days after application but at low concentration level, so the loss of the parent compound cannot be ascribed to the transformation in the oxon metabolite. In both crops, after 5 days, only chlorpyrifos is important in residue evaluation; season and crops are the main factors in the loss of this pesticide. A preharvest time can be established for the different conditions studied according to Spanish and EU LMRs for chlorpyrifos in Spain (green beans = 0.2 mg/kg) and EU (tomatoes = 0.5 mg/g). The preharvest time in winter, when chlorpyrifos is applied at full dose and in a flat-roof greenhouse, is 8 days for tomatoes and >15 days for green beans. In spring these data correspond to 7 and 14 days, respectively.

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