

# Simultaneous Determination of Carbaryl, Chlorpyrifos, and Its Metabolite 3,5,6-Trichloro-2-pyridinol (TCP) by Derivative Spectrophotometry. Direct Determination of the Degradation Grade of a Pesticide Formulation by Measurement of TCP

Anunciación Espinosa-Mansilla,\* Francisco Salinas, and Angel Zamoro

Department of Analytical Chemistry, University of Extremadura, 06071 Badajoz, Spain

The degradation grade (DG) of a commercial pesticide formulation composed of carbaryl and chlorpyrifos has been directly determined by taking the first-derivative signal at 339 nm originated by 3,5,6-trichloro-2-pyridinol (TCP), which is the major breakdown product of chlorpyrifos. Detection and determination limits of  $1.3 \times 10^{-6}$  and  $4.4 \times 10^{-6}$  M, respectively, have been determined by using a commercial undegraded formulation (previously 1:50 diluted). Simultaneous determination of carbaryl, chlorpyrifos, and TCP can be performed in commercial formulations up to DG values of 25%.

**Keywords:** Carbaryl; chlorpyrifos; 3,5,6-trichloro-2-pyridinol; pesticides; derivative spectrophotometry

## INTRODUCTION

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) and carbaryl (1-naphthyl methylcarbamate) are pesticides frequently formulated together. They are widely employed as insecticides and fungicides. These commercial formulations lose activity in the solid product with time due to the degradation process of the chlorpyrifos. Hence, while the carbaryl remains undegraded during for long times, chlorpyrifos is degraded, under the same storage conditions, to give rise mainly to the toxin 3,5,6-trichloro-2-pyridinol (TCP). This breakdown product can present a toxicological effect upon livestock (Allender and Keegan, 1991). In soil, chlorpyrifos is initially degraded to TCP and subsequently to organochlorine compounds, while carbaryl is degraded to 1-naphthol (Worthing, 1987).

Both pesticides have been examined as residues in many substrata and also in commercial formulations. Chromatographic techniques have been the most extensively applied (Allender and Keegan, 1991; Husain et al., 1991; Bogus et al., 1990; Luke et al., 1981; AOAC, 1984; Changes in Methods, 1986).

The presence of hydrogen peroxide increases the rate of degradation of chlorpyrifos to detectable TCP; in contrast, the degradation product of carbaryl (1-naphthol), which is instantaneously formed in alkaline medium, is unstable in the presence of hydrogen peroxide (Espinosa-Mansilla et al., 1994a,b).

Most papers do not report the analysis of the metabolites. In the AOAC recommended method for chlorpyrifos formulation analysis, addition of small amounts of acetic acid to the mobile phase (acetonitrile-water) is suggested to suppress nonreproducible ionization of TCP which might otherwise interfere (AOAC, 1984). Recently, two chromatographic methods have been proposed to determine TCP in the presence of chlorpyrifos in green dates (Mourer et al., 1990) and in aged formulations (Allender and Keegan, 1991). In the last mentioned paper the results show that appreciable amounts of TCP had accumulated in old and poorly stored chlorpyrifos formulations.

Derivative techniques are often applied with the aim

of eliminating spectral interferences due to band overlapping when the interfering band is either known or constant, and then the zero-crossing technique can be employed (O'Haver and Green, 1976; O'Haver, 1979). In a recently published paper (Espinosa-Mansilla et al., 1992), the abilities of the derivative spectrophotometry and partial least-squares (PLS) methods in the resolution of binary mixtures of carbaryl and chlorpyrifos have been reported. It can be concluded that similar results are obtained by both methods and also that derivative methods are simpler to apply to binary mixtures for analysis of relatively simple matrices such as pesticide formulations.

In this work, a derivative method was applied to the direct determination of TCP in aged chlorpyrifos formulations, which contain undegraded carbaryl, with the object of obtaining a simple relationship between degradation grade of the commercial formulation and derivative signal, easily applicable in routine analysis. Also, the possibility of the simultaneous determination of the three components present in the degraded formulation was studied.

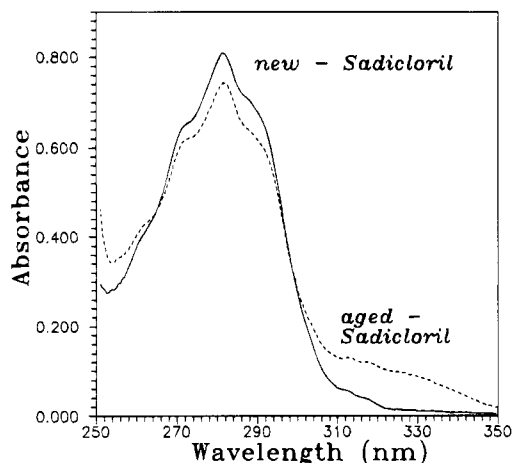
## EXPERIMENTAL PROCEDURES

**Apparatus.** A Beckman DU-50 spectrophotometer connected via an RS-232 to an IBM PS-2 microcomputer has been used for all absorption measurements. Beckman Data Leader Software, version 3.0, was used for spectra acquisition, storage, manipulation, and analysis of the spectrophotometric data. Differentiation was performed according to the simplified least-squares procedure of Savitzky and Golay (1964).

**Reagents.** All experiments were performed with analytical grade chemicals. Standard solutions ( $500 \mu\text{g mL}^{-1}$ ) of chlorpyrifos, carbaryl (Chem Service), and TCP (Dow Chemical) were prepared by dissolution in dimethylformamide (DMF). Sadicloril (Sadisa, Spain) stock solution was prepared by placing about 0.15 g (exactly weighed) of solid product in a 50-mL calibrated flask and diluting to the mark with dimethylformamide (an ultrasonic bath was used to dissolve the commercial product); the solution was centrifuged if necessary.

The use of gloves and a hood is essential when pesticides are handled.

**Procedures.** *General Procedure for Determining 3,5,6-Trichloro-2-pyridinol (TCP).* Into a 25-mL volumetric flask



**Figure 1.** Absorption spectra of new Sadicloril and aged Sadicloril.

were introduced an aliquot of the sample solution containing between 12.5 and 350  $\mu\text{g}$  of TCP, 5 mL of acetic acid/sodium acetate buffer (pH 4.7), and an adequate volume of DMF so as to give a final concentration of 40% (v/v) after dilution to the mark with water. Absorption spectra were recorded between 250 and 350 nm against a blank solution. The first-derivative spectra were calculated with a bandwidth of 8 nm. The value of the first-derivative signal at 339 nm was taken as the analytical signal. Calibration graphs are constructed by using TCP solutions of known concentration.

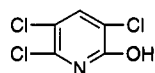
**Procedure for Determining TCP in Sadicloril.** Sadicloril is a solid commercial formulation with the following stated composition [determined by high-performance liquid chromatography (HPLC)] chlorpyrifos, 24%; and carbaryl, 35.2%.

Aliquots of the Sadicloril stock solution were placed in a 25-mL calibration flask, and the samples were then analyzed as described under the general procedure. The degradation grade (DG) was determined by using a calibration graph based upon the total dilution applied to Sadicloril samples.

**Procedure for Simultaneous Determination of Carbaryl, Chlorpyrifos, and TCP in Sadicloril.** Aliquots of the Sadicloril stock solution were placed in a 25-mL calibration flask; 5 mL of acetic acid/sodium acetate (pH 4.7) buffer solution and a sufficient volume of DMF to give a DMF-water mixture 40 + 60 (v/v) were added, and the solution was diluted to the mark with water. Absorption spectra were recorded between 250 and 350 nm against a blank solution, and the first-derivative spectra ( $\Delta\lambda = 8$  nm) were obtained. The carbaryl content was determined from the first-derivative signal at 290 nm, chlorpyrifos at 280.5 nm, and TCP at 339 nm and by comparing the values with the appropriate calibration graphs.

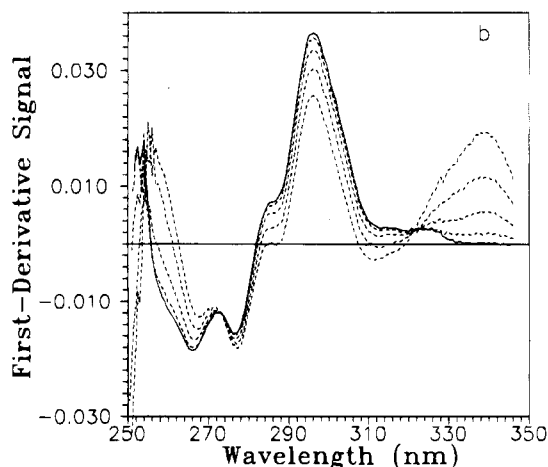
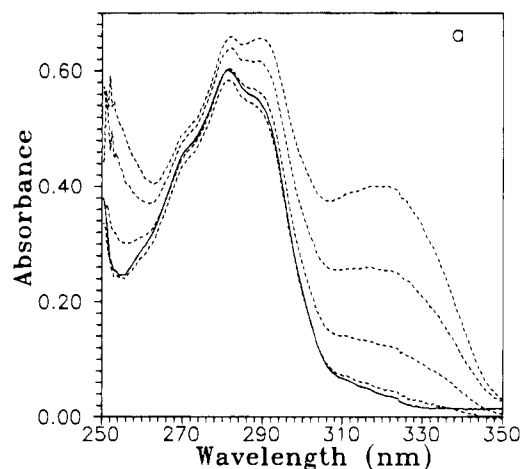
## RESULTS AND DISCUSSION

Chlorpyrifos both as a solid and in solution gives rise slowly to 3,5,6-trichloro-2-pyridinol (TCP); however, carbaryl as a solid remains undegraded during a long time, but in basic solution gives instantaneously 1-naphthol.



3,5,6-trichloro-2-pyridinol

Sadicloril is a commercial formulation (solid product) containing carbaryl and chlorpyrifos. Two different Sadicloril formulations have been employed for analysis: a recently formulated Sadicloril (analyzed by HPLC, new Sadicloril) and a 1-year-aged Sadicloril (aged Sadicloril). By HPLC the absence of 1-naphthol in the aged Sadicloril was confirmed. In Figure 1 the absorption spectra obtained for the two different Sadicloril formu-

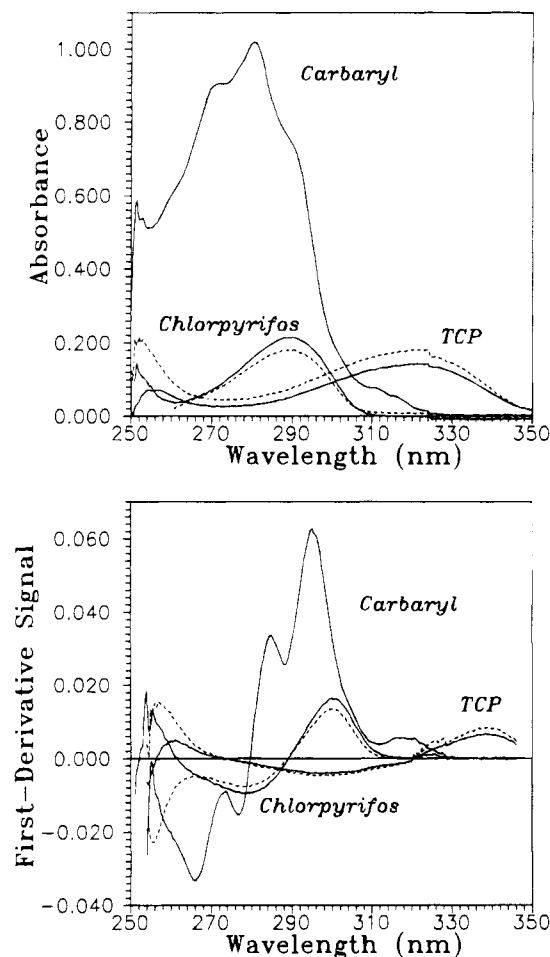


**Figure 2.** Artificial samples containing chlorpyrifos ( $3.8 \times 10^{-5}$  M), carbaryl ( $6.7 \times 10^{-5}$  M), and different amounts of TCP (between 0 and  $6.6 \times 10^{-5}$  M): (a) absorption spectra; (b) first-derivative spectra.

lations are shown. The aged Sadicloril formulation exhibits notable absorption at wavelength greater than 320 nm, in contrast with the new Sadicloril absorption spectrum. Also, a decrease in the absorbance value in the wavelength range between 280 and 300 nm is observed.

In a previous paper (Espinosa-Mansilla et al., 1992) we have studied the optimum conditions for simultaneous determination of carbaryl and chlorpyrifos by derivative spectrophotometry. Hence, in the mentioned established conditions, artificial samples containing chlorpyrifos ( $3.8 \times 10^{-5}$  M), carbaryl ( $6.7 \times 10^{-5}$  M), and different amounts of TCP (between 0 and  $6.6 \times 10^{-5}$  M) have been prepared. Their absorption (a) and first-derivative (b) spectra are shown in Figure 2. An increase in the absorbance can be observed in all ranges of wavelength studied with a new absorption maximum located at 320 nm. Hence, two facts can be observed: first, TCP exhibits an absorption maximum located at 320 nm, in accordance with the spectral changes observed in the aged Sadicloril; second, the presence of TCP interferes in the absorption spectra of carbaryl and chlorpyrifos. These two subjects have been experimentally confirmed.

**Determination of TCP by Derivative Spectrophotometry.** Individual samples have been prepared containing a concentration ratio between carbaryl, chlorpyrifos, and TCP similar to those theoretically present in 40% and 50% degraded Sadicloril. Absorption and first-derivative ( $\Delta\lambda = 8$  nm) spectra have been recorded



**Figure 3.** Samples containing a concentration ratio between carbaryl, chlorpyrifos, and TCP similar to those theoretically present in 40% (—) and 50% (- -) degraded Sadicloril: (a, top) absorption spectra; (b, bottom) first-derivative spectra.

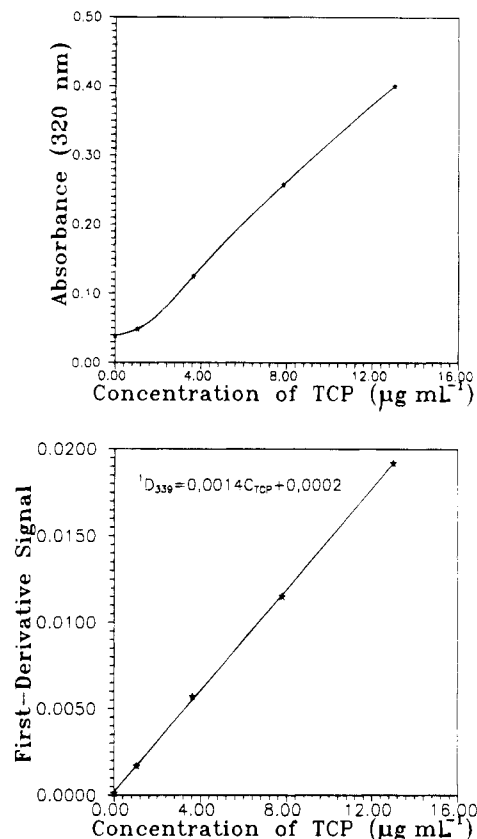
(Figure 3). From the results obtained by using the conventional spectra, an overlapping between TCP and carbaryl at the absorption maximum for TCP can be observed (this effect is most notable for a low DG). At wavelengths larger than 310 nm, chlorpyrifos does not absorb. In the first-derivative spectra it is possible to measure the derivative signal at 339 nm specifically for TCP.

Calibration graphs were obtained from the absorbance ( $A_{320}$ ) (Figure 2a) and first-derivative signal ( ${}^1D_{339}$ ) (Figure 2b) measured for standard solutions containing between 0 and  $6.6 \times 10^{-5}$  M of TCP, in the presence of a constant  $3.3 \times 10^{-5}$  M of carbaryl and chlorpyrifos, by using both conventional and first-derivative spectra, respectively. In Figure 4, both calibration graphs are represented. For TCP concentration lower than  $3 \mu\text{g mL}^{-1}$ , a curved relationship is observed due to the presence of carbaryl. For this reason low degradation grades could not be determined by conventional absorption. However, by measuring the derivative signal at 339 nm, a linear relationship was observed in all ranges of TCP concentration assayed, the linear equation being

$${}^1D_{339} = 278[\text{TCP}] + 0.0002 \quad (1)$$

and the linear regression coefficient 0.9993, being [TCP] expressed in moles per liter.

**Determination of the DG in Sadicloril.** By taking into account the stated composition for Sadicloril (HPLC



**Figure 4.** Calibrations graph: (a, top) absorbance ( $A_{320}$ ) vs concentration of TCP; (b, bottom) first-derivative signal ( ${}^1D_{339}$ ) vs concentration of TCP.

**Table 1. Linear Equations and  $F$  Factor Calculated between Derivative Signal and Degradation Grade (DG) for Different Dilution Factors for a Commercial Aged-Sadicloril Formulation**

dilution	eq	linear correl coeff	$F$
1:125	${}^1D_{339} = 4.65 \times 10^{-5}[\text{DG}]$	0.9990	21.50
1:83	${}^1D_{339} = 6.96 \times 10^{-5}[\text{DG}]$	0.9990	14.37
1:62	${}^1D_{339} = 9.30 \times 10^{-5}[\text{DG}]$	0.9990	10.75
1:50	${}^1D_{339} = 1.15 \times 10^{-4}[\text{DG}]$	0.9990	8.70
1:25	${}^1D_{339} = 2.29 \times 10^{-4}[\text{DG}]$	0.9990	4.37

established), the amount of Sadicloril present in the stock solution employed, and the calibration graphs obtained for TCP (eq 1) and by using the first-derivative signal at 339 nm, several calibrations,  ${}^1D_{339}$  vs DG, can be established for the different dilution factors. In Table 1, the equations obtained and the linear correlation coefficients are summarized. Also, the following simple linear relationship can be directly applied to determine the degradation grade:

$$\text{DG} (\%) = {}^1D_{339}F \quad (2)$$

$F$  is a value depending on the dilution factor. The calculated  $F$  values are also summarized in Table 1.

By using eq 2, the aged Sadicloril has been analyzed. For that, from the Sadicloril stock solutions prepared according to the procedure, four sets of samples with different dilutions were prepared. Each set was composed of six independent samples using the same dilution. In Table 2, the DG values obtained for aged Sadicloril are summarized.

The limits of detection ( $[\text{TCP}] = 3\sigma/m$ , where  $\sigma$  is the standard deviation of the derivative signal corresponding to an undegraded Sadicloril and  $m$  is the slope of

**Table 2. Results Obtained in the Determination of the Degradation Grade of an Aged Sadicloril**

dilution	DG <sup>a</sup>	RSD <sup>b</sup> (%)	dilution	DG <sup>a</sup>	RSD <sup>b</sup> (%)
1:125	34	7	1:62	35	6
1:83	34	7	1:50	34	5

<sup>a</sup> Mean of six independent samples. <sup>b</sup> RSD, relative standard deviation.

**Table 3. Limits of Detection and Determination for DG of an Aged Sadicloril**

dilution	DG <sup>a</sup> (%)		dilution	DG <sup>a</sup> (%)	
	C <sub>L</sub>	C <sub>Q</sub>		C <sub>L</sub>	C <sub>Q</sub>
1:125	9	29	1:50	3	11
1:83	5	17	1:25	2	5

<sup>a</sup> C<sub>L</sub>, limit of detection. <sup>b</sup> C<sub>Q</sub>, limit of determination.

**Table 4. Compositions of the Artificial Ternary Mixtures**

sample	[carbaryl] (M × 10 <sup>5</sup> )	[chlorpyrifos] (M × 10 <sup>5</sup> )	[TCP] (M × 10 <sup>5</sup> )	equivalent to DG (%)
1	15.4	6.84	0	0
2	15.4	6.15	0.55	8
3	15.4	5.47	1.13	17
4	15.4	4.79	1.67	26
5	15.4	4.10	2.25	35
6	15.4	3.42	2.80	45

**Table 5. Simultaneous Determination of Carbaryl, Chlorpyrifos, and TCP in Artificial Ternary Mixtures**

sample	recovery (%)			
	carbaryl	chlorpyrifos	TCP	equivalent to DG (%)
1	109	100	0	0
2	98	102	103	8
3	99	108	103	17
4	94	115	103	26
5	95	123	108	35
6	92	133	104	45

the adequate calibration graph) and the limit of determination ([TCP] = 10σ/m) have been calculated. In Table 3 are summarized these results.

**Simultaneous Determination of Carbaryl, Chlorpyrifos, and TCP.** In a previous paper, the simultaneous determination of carbaryl and chlorpyrifos in artificial binary mixtures and in an undegraded Sadicloril (new Sadicloril) has been performed by using the following equations:  ${}^1D_{280.5} = -249C$  for chlorpyrifos and  ${}^1D_{290} = 140C$  for carbaryl ( $C$  is expressed in mol L<sup>-1</sup>). Six artificial ternary mixtures have been prepared simulating the ratio concentration present in the new Sadicloril and in different aged Sadicloril formulations with DG values between 0 and 45%. In Table 4, the compositions of the artificial ternary mixtures employed are shown.

First-derivative absorption spectra have been recorded and by using the eqs 1, 3, and 4 the contents of TCP, chlorpyrifos, and carbaryl have been calculated.

From the results summarized in Table 5 can be concluded that the recovery values for TCP and carbaryl are adequate for all DG values; however, for DG values larger than 25%, elevated recovery values are obtained for chlorpyrifos.

By taking into account the DG, calculated previously from the aged Sadicloril analyzed in this paper (34%), it was decided to analyze simultaneously only carbaryl and TCP.

**Simultaneous Determination of Carbaryl and DG in Aged Sadicloril.** Six sets of samples (individually weighed) have been prepared. Each set is composed

**Table 6. Determination of Carbaryl and DG in an Aged Sadicloril**

stock Sadicloril (g/50 mL)	dilution	carbaryl content <sup>a</sup> (%)	DG (%)
0.1498	1:25	37.08	28
	1:83	35.60	30
	1:62.5	35.80	31
	1:50	35.70	33
0.1498	1:125	32.90	34
	1:83	32.20	36
	1:62.5	32.90	36
	1:50	33.30	36
0.1067	1:125	38.80	37
	1:83	36.80	35
	1:62.5	35.00	36
	1:50	34.90	37
0.1493	1:125	36.10	37
	1:83	35.30	33
	1:62.5	35.20	36
	1:50	34.90	32
0.1483	1:125	34.90	32
	1:83	34.20	33
	1:62.5	34.50	33
	1:50	33.80	32
0.1496	1:125	33.88	34
	1:83	32.50	33
	1:62.5	32.30	36
	1:50	32.50	35

carbaryl (%) = 34.60    DG (%) = 34  
σ = 1.67                    σ = 2.34

<sup>a</sup> 35.20% in new Sadicloril (HPLC).

of four samples with different dilution factors. In Table 6, the results obtained show that the carbaryl effectively does not exhibit a degradation process and the chlorpyrifos is strongly degraded to give TCP (34% of DG).

**Conclusions.** TCP can be determined directly in the presence of carbaryl and chlorpyrifos by using the first-derivative signal at 339 nm. A simple equation has been established between the degradation grade and the first-derivative signal at 339 nm with the object of easy application to routine analysis of a commercial formulation (Sadicloril). Carbaryl and TCP can be simultaneously analyzed in an aged formulation up to 45% DG; however, chlorpyrifos could be determined in the presence of TCP only for DG less than 25%.

#### ACKNOWLEDGMENT

This work was supported by the DGICYT of the Ministry of Education and Science of Spain (Project PB91-0856).

#### LITERATURE CITED

- Allender, W. J.; Keegan, J. Determination of Chlorpyrifos and its Major Breakdown Products in Technical Formulations. *Bull. Environ. Contam. Toxicol.* **1991**, *46*, 313–319.
- AOAC. *Official Methods of Analysis*; AOAC: Arlington, VA, 1984; Section 29.
- Bogus, E. R.; Watschke, T. L.; Mumma, R. O. Utilization of Solid-Phase Extraction and Reversed-Phase and Ion-Pair Chromatography in the Analysis of Seven Agrochemicals in Water. *J. Agric. Food Chem.* **1990**, *38*, 142–144.
- Changes in Methods. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 365 (Section 29).
- Espinosa-Mansilla, A.; Muñoz de la Peña, A.; Salinas, F.; Zamoro, A. Simultaneous Determination of Pesticides by Multivariate Spectral Analysis and Derivative Spectrophotometry. *Anal. Chim. Acta* **1992**, *258*, 47–53.

- Espinosa-Mansilla, A.; Salinas, F.; Zamoro, A. Kinetic Study of the Degradation of Chlorpyrifos by Using a Stopped-Flow FIA system. Semiautomatic Determination in Commercial Formulations. *Talanta* **1994a**, *41* (5), 651–657.
- Espinosa-Mansilla, A.; Salinas, F.; Zamoro, A. Simultaneous Kinetic Determination of Chlorpyrifos and Carbaryl based on Differential Degradation Processes in Alkaline Oxidative Medium. *Mikrochim. Acta* **1994b**, *113*, 9–17.
- Husain, S.; Sarma, P. N.; Swami, G. Y. S. K.; Narsimha, R. Determination of Chlorpyridine Isomers by Gas-Chromatography and High-Performance Liquid Chromatography in Chlorpyrifos Process Development. *J. Chromatogr.* **1991**, *540*, 331–341.
- Luke, M. A.; Froberg, J. E.; Doose, G. M.; Masumoto, H. T. Improved Multiresidue Gas-Chromatographic Determination of Organophosphorus, Organonitrogen, and Organohalogen Pesticides in Produce, using Flame Photometric and Electrolytic Conductivity Detectors. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1187–1195.
- Mourer, C. R.; Hall, G. L.; Whitehead, W. E.; Shibamoto, T. Gas-Chromatographic Method for Determination of Chlorpyrifos and its Metabolite 3,5,6-trichloro-2-pyridinol (TCP) in Dates. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 294–297.
- O'Haver, T. C. Derivative and Wavelength Modulation Spectrometry. *Anal. Chem.* **1979**, *51*, 91A–100A.
- O'Haver, T. C.; Green, G. L. Numerical Error Analysis of Derivative Spectrometry for Quantitative Analysis of Mixtures. *Anal. Chem.* **1976**, *48*, 312–318.
- Savitzky, A.; Golay, M. J. E. *Anal. Chem.* **1964**, *36*, 1627.
- Worthing, C. R., Ed. *The Pesticide Manual, a World Compendium*, 8th ed.; The British Crop Protection Council: Bracknell, U.K., 1987.

Received for review June 16, 1994. Accepted October 20, 1994.\*

JF940326P

---

\* Abstract published in *Advance ACS Abstracts*, December 1, 1994.