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Study of the Decay of Diazinon and Chlorpyrifos in Apple Samples, Using Gas Chromatography

J. Sanz Asensio, C. Sáenz Barrio, M. T. Galarreta Juez & J. Galbán Bernal

Analytical Chemistry Department, University College of La Rioja, University of Zaragoza, Logroño-26001, Spain

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ABSTRACT

*This paper presents a study of the decay of diazinon and chlorpyrifos in samples of apple. After an initial study of the optimum method for extracting the two pesticides, and for determining them by gas chromatography (using a capillary column and NPD detector), the decay study itself was performed both in the laboratory (*in vitro*) and on the trees (*in vivo*). Samples of apple were treated with commercial products containing each compound, and these were measured at intervals. These two pesticides are found to degrade much more quickly* in vivo *than* in vitro.

In no case was either pesticide observed to penetrate inside the apple.

INTRODUCTION

The analytical chemistry of pesticides in solid samples involves two basic problems: (1) to find reliable, reproducible methods for getting them into solution, and (2) to use sufficiently sensitive and selective techniques to detect not only the pesticide itself, but also its known metabolites, and to characterize new metabolites.

The methods for solubilizing and pretreating diazinon and chlorpyrifos are similar to those for other pesticides, and consist basically of a solid–liquid extraction (with acetonitrile (Guinivan *et al.*, 1981; Skelly *et al.*, 1981), acetone (Simonaitis *et al.*, 1981; Kirkbride, 1987), ethyl acetate (Sultatos *et al.*, 1982), or mixtures of solvents (López-Avila *et al.*, 1985), followed by conditioning in the liquid phase, which can consist simply of the

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removal of residual water (with a desiccating agent such as calcium chloride (Petrova & Andreev, 1980) or sodium sulphate (anhydrous), evaporation or column purification (Guinivan *et al.*, 1981; or of longer and more laborious procedures (Simonaitis *et al.*, 1981; Kirkbride, 1987), depending on the complexity of the sample and the selectivity of the determination method to be used.

As with most pesticides, the first determination techniques used with diazinon and chlorpyrifos were spectrophotometry and fluorimetry, which are still used today (Irudayasamy & Kamala, 1975; Poziomek *et al.*, 1981) Since the 1960s, most work in this field has used chromatographic techniques such as TLC, HPLC and, above all, GC. The use of TLC was developed using silica gel as support, and different organic solvents (hexane–acetone (Petrova & Andreev, 1980), or cyclohexane–acetone–chloroform (Bargnoux *et al.*, 1978). The plate is normally developed by fluorescent labelling (Potti *et al.*, 1975; Federici & Paul, 1986). Another chromatographic support used has been Silofol (Belashova *et al.*, 1983; Belashova, 1984).

The commercialization of new HPLC systems has led to an increased use of this technique to determine pesticides in real samples. Noteworthy references in this context are Skelly *et al.* (1981) and Sultatos *et al.* (1982) for the determination of chlorpyrifos, and Trujillo *et al.* (1984) and Farran & de Pablo (1987) for diazinon.

GC is even more widely used than either HPLC or TLC. The methodology for determining diazinon and chlorpyrifos by this technique has evolved towards the use of ECD detectors, such as tritium (Chapman & Harris, 1980), ^{63}Ni (Cochrane *et al.*, 1979; Chuguenot *et al.*, 1980; Petrova & Andreev, 1980; Inman *et al.*, 1981), ^{90}Sr (Cochrane *et al.*, 1979), or NPD (Osterloh *et al.*, 1983; Kirkbride, 1987), although conventional FID (Lawrence & Iverson, 1975; Dishburger *et al.*, 1977; Bargnoux *et al.*, 1978; Inman *et al.*, 1981; Poziomek *et al.*, 1981; Simonaitis *et al.*, 1981; Cainus *et al.*, 1985; Karr, 1985) is still used. These detectors enable these pesticides to be determined simultaneously in mixtures of other pesticides, but their determination of metabolites of these and other pesticides is limited. To use any of these conventional detectors to analyse metabolites requires the use of complex protocols; examples are the excellent work by Guinivan *et al.* (1981) on the joint determination of chlorpyrifos and trichloro-pyridine-2-ol (its main metabolite), and by Lawrence & Iverson (1975) on the determination of diazinon and its metabolites. These types of studies, and the detection and characterization of new metabolites, are normally done with GC/MS coupled systems (Sovocool *et al.*, 1981; Cainus *et al.*, 1985). Most studies have been and are done in packed columns, although the use of capillary columns is increasing because of their higher resolution.

Aside from the detection and determination of new metabolites, another important aspect of dealing with pesticides is their decay on samples which are destined for human consumption. This paper presents a study of the decay of diazinon and chlorpyrifos on apple samples, both on the tree (*in vivo*) and in the laboratory (*in vitro*), using GC.

MATERIALS AND METHODS

Apparatus

All measurements were made with a Hewlett–Packard HP5890 gas chromatograph, with a HP Ultra 2 capillary column (Crosslinked 5% phenyl-methyl silicone gum phase), a NPD detector, and a HP3390A integrator. The working conditions found to be optimum are shown in Table 1.

A Mettler H10 balance, Hamilton 7002-n 2 μl microsyringes, and 100-ml Soxhlet extraction apparatus were also used.

Reagents

Pesticide standards (diazinon and chlorpyrifos) were 99·9% pure, and obtained from Riedel-de-Haen (Seelze, FRG). Iso-octane of HPLC quality was obtained from Lab-Scan (Dublin, Ireland), and calcium chloride from Merck (Darmstadt, FRG).

Solutions

Pesticide solutions, 1·000 μg/ml, were prepared by dissolving the standards in iso-octane. More dilute solutions were prepared by dilution with iso-octane.

TABLE 1
Instrument Conditions Used in the Gas Chromatograph

Detector temperature (°C)	300
Column temperature (°C)	225
Injector temperature (°C)	275
Carrier gas flow rate (nitrogen, ml/min)	0·6
Purge flow rate (ml/min)	3
Split (ml/min)	100
Flow rates for NPD detector	
Auxiliary gas (nitrogen, ml/min)	30
Air (ml/min)	70
Hydrogen (ml/min)	4
Sample volume injected (μl)	2

RESULTS AND DISCUSSION

Sample extraction and treatment method

A study was performed to find the optimum procedure for extracting the two pesticides (diazinon and chlorpyrifos) from apple samples. Three different procedures were considered, and tested on samples which were free of both pesticides.

Liquid–liquid extraction (Method A). The apple sample is crushed with 50-ml bidistilled water and 1-ml pesticide solution. Extraction is performed using 50-ml of different organic solvents (chloroform, iso-octane, and mixtures of these with acetone). The results obtained by injecting the organic phase were not satisfactory, and pesticide signals (non-quantitative) were only obtained after extraction with iso-octane/acetone (10:1 v/v).

Solid–liquid extraction by mechanical stirring (Method B) or *Soxhlet* (heating in a water/propylene glycol (1:1 v/v) bath) (Method C). The sample

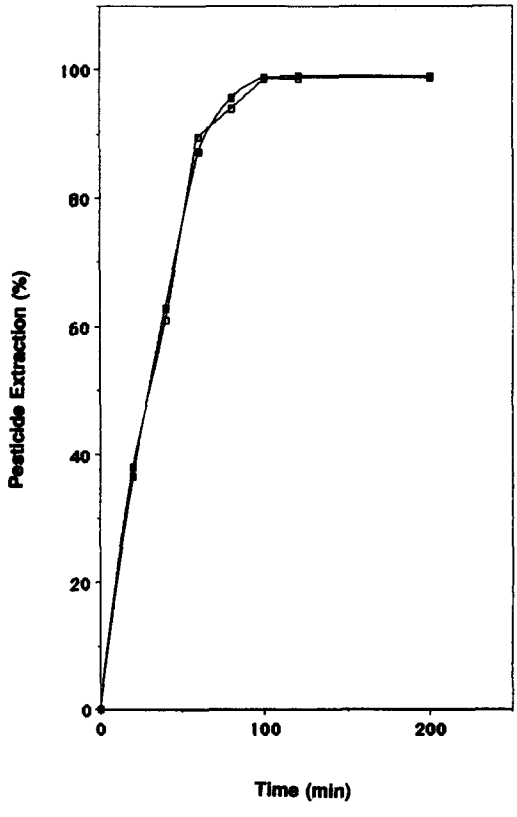

Fig. 1. Amount of pesticide extracted (%) versus shaking time. (□) Diazinon, (■) chlorpyrifos.

is crushed with 1-ml solution of one of the pesticides, and the mixture is extracted with iso-octane (20 ml). In both methods, the amount of pesticide extracted was found by withdrawing 10-ml aliquots of the extract (replacing with equal volumes of pure solvent) at intervals. These aliquots were dehydrated (calcium chloride) for 15 min, after which 50-μl internal standard were added, and the solution was injected into the chromatograph. The recovery studies for the two pesticides using Method B are shown in Fig. 1. Clearly, after 2 h, either of the pesticides is quantitatively extracted, with a recovery of the order of 99%. Similar results are obtained with Method C, recovery being 98% for the two pesticides, and optimum extraction times being of the order of 100 min.

In view of these results, Method B (solid–liquid extraction by mechanical stirring) was adopted for the rest of the study, since it is not only simpler, but also avoids the risk of thermal decomposition of the pesticides.

The choice of desiccating agent and internal standard deserves some comment. Three desiccating agents were tested: calcium chloride, sodium sulphate (anhydrous) and sulphuric acid. The best results were obtained with calcium chloride. Sulphuric acid almost completely suppressed the signal (due probably to solvolysis or oxidation of the pesticides). Sodium sulphate (anhydrous) gave good results, but posed certain practical difficulties (the solid decants slowly and is easily resuspended). With regard to the internal standard, with the retention times obtained, it is possible to use diazinon as the internal standard for chlorpyrifos and vice versa, since the studies of the two pesticides were performed independently.

Chromatographic characteristics

Using this extraction procedure, the only peaks observed in the chromatogram were those for the pesticides, so that the extract did not require any further purification.

In the conditions shown in Table 1, the linear response range is 0·3–100 ng for diazinon and chlorpyrifos. The relative standard deviation (RSD) (the method's precision) is 2·5% ($n = 10$) for both pesticides. The retention times for diazinon and chlorpyrifos were 2·63 and 5·34 min, respectively.

Decay study

This study was performed with two objectives: to find experimentally the decay time of the two pesticides, both *in vitro* and *in vivo*, and to check that the pesticides did not penetrate inside the fruit, in both situations.

For the study of decay *in vitro*, two groups of apples were considered, and were treated with an aqueous suspension (0·3% v/v) of a commercial

preparation of either diazinon or chlorpyrifos. The suspension was applied in such a way that each of the apple samples received a similar amount (in excess of real levels), and were then maintained free of contact with any surface to avoid losses. The residual pesticide was determined by taking samples periodically. For the study of decay *in vivo*, four apple trees (which had not been treated with these or any other pesticides) were treated with the aqueous suspension as described before, two with a suspension of diazinon and two with a suspension of chlorpyrifos. Samples were determined periodically.

In order to perform the pesticide penetration study at the same time, five different solutions were prepared of each apple by the following procedure:

(1) The apple (weight 90 ± 10 g) is washed twice with $10 + 10$ ml iso-octane. The washings are collected and labelled 'fraction 1'.
(2) The apple is left to dry and peeled. The weight of the peeling is about 10% of the total weight and the thickness is about 1 mm. The skin is extracted with 20-ml iso-octane, using the procedure described before. The extract is 'fraction 2'.
(3) The rest of the apple is washed twice with $10 + 10$ ml solvent. The washings are collected and labelled 'fraction 3'.
(4) The sample is left to dry and a spherical shell, about 4 mm thickness and 35% weight of the apple, is cut from the apple, peeled and then extracted with 20-ml iso-octane. This extract is 'fraction 4',
(5) The centre of the fruit is extracted with 20-ml solvent. This extract is 'fraction 5'.

Each of these five fractions was dehydrated, the internal standard added, and solution volume was made up to 25 ml. The solution (2 μl) was injected into the chromatograph. This procedure was repeated with four different apples (two from each tree in the in-vivo experiment), each time samples were taken. Five replicate determinations were made with each solution, and the RSD was less than 3% in all cases.

Chromatography signals of the pesticides were only obtained with fractions 1 and 2 in all cases (both *in vivo* and *in vitro*), which indicates that the penetration into the fruit is low, probably due to the impermeable structure of the skin.

With regard to the decay study itself, clear differences were observed between the fall in the amount of pesticide present, both *in vivo* and *in vitro*, and between the decay curves for the first and second fractions.

Figure 2 shows the decay curves for the two pesticides *in vivo* (in the tree). The amount of pesticide extracted in fraction 1 (Fig. 2(a)) falls quickly with time. In fact the curves fit exponential functions. In contrast, in fraction 2

TABLE 2
Equations Obtained by Least Squares for the Decay Curves (*in Vivo*)

	Diazinon	*Chlorpyrifos*
Fraction 1	$X = 2{\cdot}53 \times 10^{-0{\cdot}075t}$ ($r = 0{\cdot}999$)	$X = 3{\cdot}17 \times 10^{-0{\cdot}043t}$ ($r = 0{\cdot}999$)
Fraction 2	$X = 0{\cdot}24 - 0{\cdot}011t$ ($r = 0{\cdot}993$)	$X = 1{\cdot}05 - 0{\cdot}020t$ ($r = 0{\cdot}998$)
Total	$X = 2{\cdot}52 \times 10^{-0{\cdot}064t}$ ($r = 0{\cdot}999$)	$X = 4{\cdot}58 \times 10^{-0{\cdot}033t}$ ($r = 0{\cdot}998$)

(Fig. 2(b)) the decay is slower and the concentration falls linearly. Since the concentration of pesticides in fraction 1 is much higher than in fraction 2, the overall pesticide concentration falls exponentially with time (Fig. 2(c)), with functions very similar to those for fraction 1. Table 2 gives the mathematical expressions which relate the amount of pesticide found to the time elapsed, by using the least squares method.

One interpretation of the results is that the pesticides are bound to the surface of the apple by two different types of bonds. One group is retained on active surface groups, and a second group forms a layer on top of this first layer, by simple adhesion. The concentration of pesticide found in the solution 1 corresponds to the weakly bound pesticide, and that found in solution 2 corresponds to the strongly bound molecules. These two groups are affected differently by environmental decay factors; the weakly bound pesticide is decayed both by physical agents (air) and by chemical agents (atmospheric oxidation, and photodecomposition) in the atmosphere, while the strongly bound pesticide is only affected by chemical agents.

TABLE 3
Equations Obtained by Least Squares for the Decay Curves (*in Vitro*)

	Diazinon	*Chlorpyrifos*
Fraction 1	$X = 2{\cdot}77 \times 10^{-0{\cdot}066t}$ ($r = 0{\cdot}995$)	$X = 1{\cdot}36 \times 10^{-0{\cdot}016t}$ ($r = 0{\cdot}994$)
Fraction 2	$X = 1{\cdot}46 - 0{\cdot}025t$ ($r = 0{\cdot}994$)	$X = 1{\cdot}04 - 0{\cdot}014t$ ($r = 0{\cdot}997$)
Total	$X = 4{\cdot}26 \times 10^{-0{\cdot}088t}$ ($r = 0{\cdot}994$)	$X = 2{\cdot}38 \times 10^{-0{\cdot}029t}$ ($r = 0{\cdot}994$)

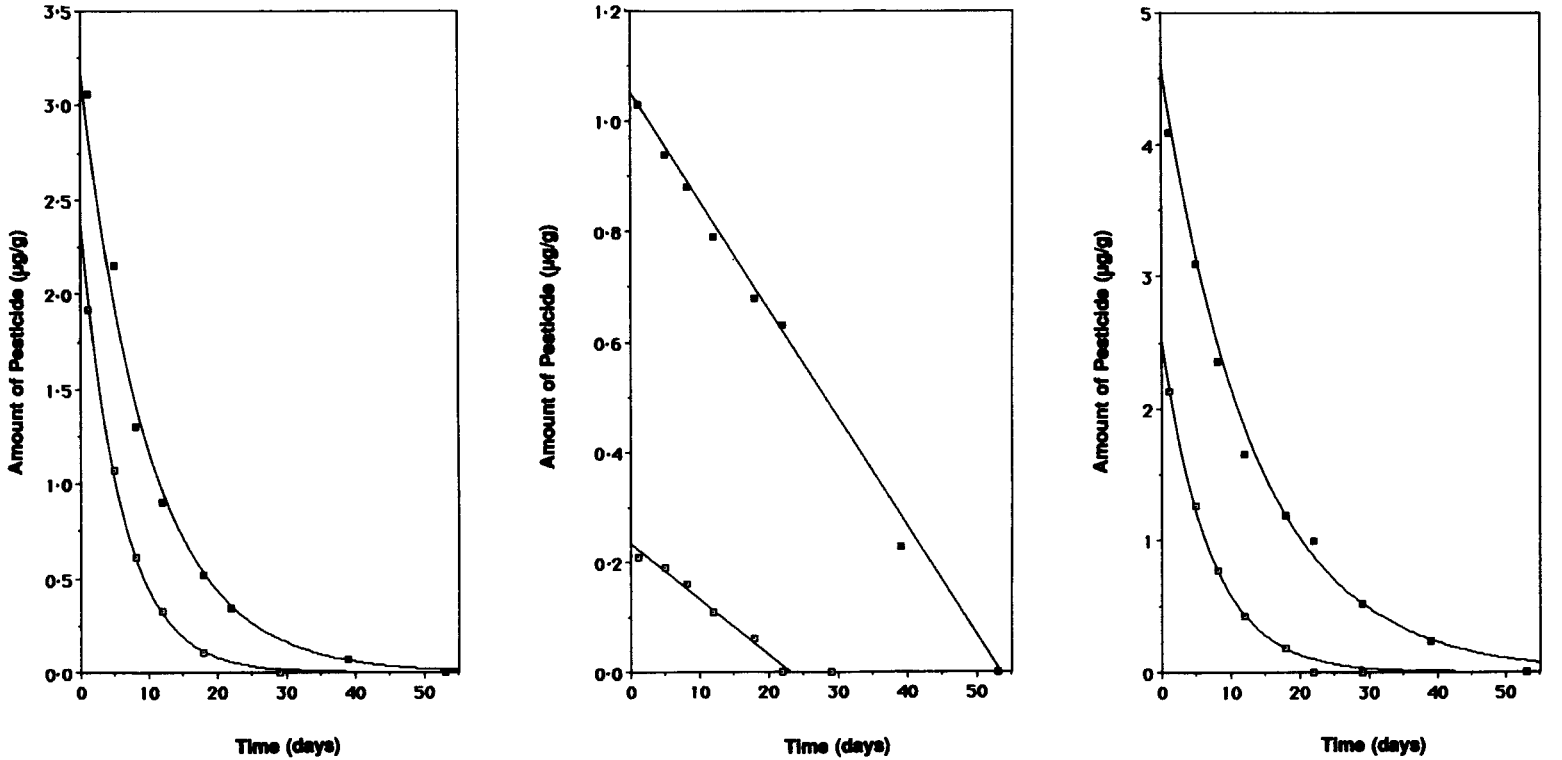

Fig. 2. Variation with time with amount of pesticide found, *in vivo*. (a) First fraction (□) diazinon, (■) chlorpyrifos; (b) second fraction (□) diazinon, (■) chlorpyrifos; (c) overall (□) diazinon, (■) chlorpyrifos.

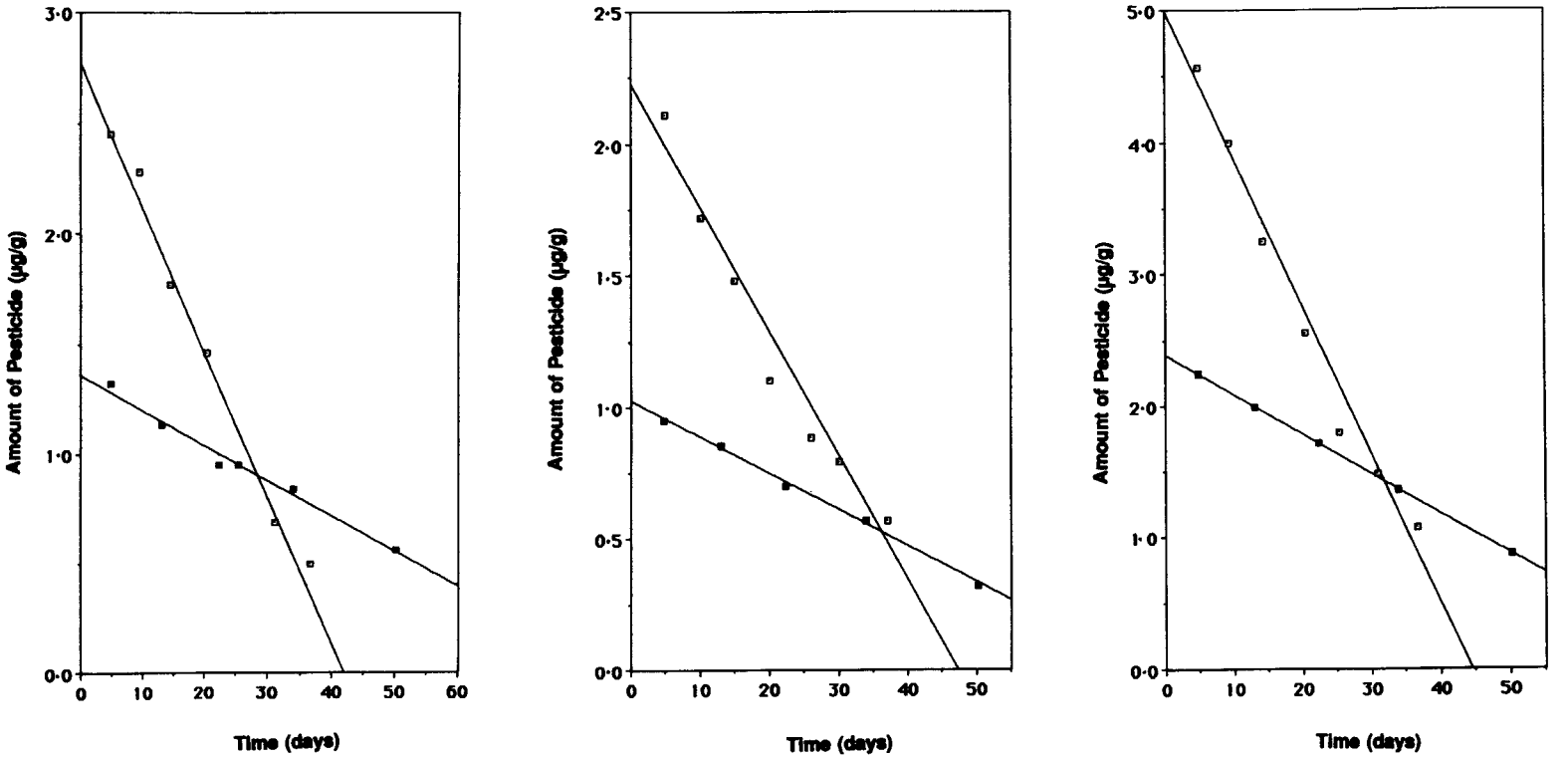

Fig. 3. Variation with time of amount of pesticide found. *in vitro*. (a) First fraction (□) diazinon, (■) chlorpyrifos; (b) second fraction (□) diazinon, (■) chlorpyrifos; (c) overall (□) diazinon, (■) chlorpyrifos.

The results of decay *in vitro* (in the laboratory) are shown in Fig. 3. The concentration of pesticide in fraction 1 (Fig. 3(a)) is found to fall linearly with time, and not exponentially, while the decay in fraction 2 is linear (Fig. 3(b)). In this case, the overall pesticide concentration falls linearly with time (Fig. 3(c)). Table 3 shows the decay equations found by least squares.

Comparing the results from the two studies, the following conclusions can be drawn:

(1) The time required to totally eliminate both pesticides differs in the two studies. While in the case of chlorpyrifos the differences are relatively small, in the case of diazinon, the total decay time is much greater in the laboratory than in the field. This shows that it is necessary to perform decay studies in real situations, both to establish the 'safety period' (time which must elapse between pesticide spraying and consumption of the fruit) and the 'activity period' (time during which the fruit is protected) of pesticides. Likewise, it is necessary to perform these studies individually for each pesticide, and not to extrapolate the results obtained with one pesticide to all the members of its family.

(2) Overall, the differences between the in-vivo and in-vitro studies can be attributed to the fact that the decay agents are different in each case. In the laboratory, mechanical agents are not involved, nor is photodecomposition, since the fruit is not directly exposed to sunlight. In this case, the main decay agent is atmospheric oxygen.

(3) It is difficult to quantify the contribution of each agent to the overall decay rate, since the curves do not all respond to the same mathematical model, and the initial amount of pesticide is different in each fraction. Nevertheless, in general terms, physical agents and photodecomposition are more important decay agents than chemical oxidation.

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REFERENCES

Bargnoux, H., Pepin, D., Chabard, J-L., Vedrin, F., Petit, J. & Berger, J-A. (1978). Application of freeze-drying to analytical pre-concentration of organothio-

phosphorus pesticides in waters. 1. Influence of the inorganic composition of the water. *Analysis*, **6**(3), 107–12.

Belashova, I. G. (1984). Systematic identification of trace pesticides by thin-layer chromatography. *Zh. Anal. Khim.*, **39**(12), 2238–41.

Belashova, I. G., Klisenko, M. A. & Khokhlova, G. A. (1983). Determination of the levels in air of pesticides used for beet crops by thin-layer chromatography. *Gig. Sanit.*, **9**, 44–6.

Brown, R. L., Farmer, C. N. & Millar, R. G. (1987). Optimization of sweep co-distillation apparatus for determination of coumaphos and other organophosphorus pesticide residues in animal fat. *J. Assoc. Off. Anal. Chem.*, **70**(3), 442–5.

Cainus, T., Siegmund, E. G. & Froberg, J. E. (1985). Identification of diazinon and its metabolite in spinach by chemical-ionization mass spectrometry. *Bull. Environ. Contam. Toxicol.*, **35**(3), 291–5.

Chapman, R. A. & Harris, C. R. (1980). Persistence of chloropyrifos in a mineral and organic soil. *J. Environ. Sci. Health, Part B*, **15**(1), 39–46.

Cochrane, W. P., Maybury, R. G. & Greenhalgh, R. G. (1979). Comparative study of the linearity and sensitivity of electron-capture and flame-photometric detectors using a pesticide standard. *J. Environ. Sci. Health*, **14**(2), 197–212.

Dishburger, H. J., Mckellar, R. L., Pennington, J. V. & Rice, J. R. (1977). Determination of residues of chlorpyrifos, its oxygen analogue and 3,5,6-trichloropyridin-2-ol in tissues of cattle fed chlorpyrifos. *Agric. Food Chem.*, **25**(6), 1325–9.

Farran, A. & De Pablo, J. (1987). Evaluation of combined flow injection-high-performance liquid chromatography for the determination of three organophosphorus pesticides in liquid wastes. *Int. J. Environ. Anal. Chem.*, **30**(1–2), 59–68.

Federici, J. A. & Paul, J. (1986). Thin-layer chromatographic separation of dimethoate, dimethoate (oxygen analogue), disulfoton, dioxiathion, fonofos, fonofos (oxygen analogue) and oxydemeton-methyl from each other. *Microchem. J.*, **34**(2), 211–18.

Guinivan, R. A., Thompson, N. P. & Bardalaye, P. C. (1981). Simultaneous electron-capture detection of chlorpyrifos and its major metabolite 3,5,6-trichloropyridin-2-ol, after gel-permeation chromatography. *J. Assoc. Off. Anal. Chem.*, **64**(5), 1201–4.

Inman, R. D., Kiigemagi, U. & Deinzer, M. L. (1981). Determination of chlorpyrifos and 3,5,6-trichloro-pyridin-2-ol residues in peppermint hay and peppermint oil. *J. Agric. Food Chem.*, **29**(2), 321–33.

Irudayasamy, A. & Kamala, T. R. (1975). Micro sublimation step in ultra-violet spectrophotometric determination of diazinon. *Curr. Sci.*, **44**(8), 265.

James, J. P., Spence, J. H. & Ford, J. H. (1984). Gas-chromatographic determination of chlorpyrifos residues in greenhouse vegetables after treatment of potting media with Dursban [chlorpyrifos] for imported fire ant. *J. Assoc. Off. Anal. Chem.*, **67**(6), 1091–4.

Karr, J. J. (1985). Analysis of diazinon for impurities and degradation materials. *J. Assoc. Off. Anal. Chem.*, **68**(5), 929–34.

Kirkbride, K. P. (1987). Estimation of diazinon in omental tissues. *J. Anal. Toxicol.*, **11**(1), 6–7.

Lawrence, J. F. & Iverson, F. (1975). Analysis for the diazinon metabolites, G 27550

[6-hydroxy-2-isopropyl-4-methylpyrimidine] and GS 31144 [6-hydroxy-2-(1-hydroxy-1-methyl-ethyl)-4-methylpyrimidine] by gas–liquid chromatography with nitrogen-specific detection after derivatisation. *J. Chromat.*, **103**(2), 341–7.

Lhuguenot, J. C., Pochat-Pochatoux, N., Witkowski, M. & Baron, C. (1980). Detection of trace levels of organophosphorus pesticides by g.l.c.; comparison between electron capture and flame photometry. *Ann. Falsif. Expert. Chim. Toxicol.*, **73**(784), 139–52.

López-Avila, V., Hirata, P., Kraska, S., Flanagan, M., Taylor, J. M. & Hern, S. C. (1985). Determination of atrazine, lindane (gamma-HCH), pentachlorophenol and diazinon in water and soil by isotope-dilution gas chromatography-mass spectrometry. *Anal. Chem.*, **57**(14), 2797–2801.

Osterloh, J., Lotti, M. & Pond, S. M. (1983). Toxicological studies in a fatal overdose of 2,4-D, MCPP [mecoprop] and chlorpyrifos. *J. Anal. Toxicol.*, **7**(3), 125–9.

Petrova, T. M. & Andreev, Y. B. (1980). Determination of Basudin [diazinon] and Dursban [chlorpyrifos] in soil and water. *Khim. Sel'sk. Khoz.*, **18**(10), 52–4.

Potti, E. E., Kaimal, P. M. & Nair, P. G. (1975). Detection and identification of mixed 'benzoic dialkyl phosphorodithioic anhydride' pesticides by thin-layer chromatography. *J. Forens. Sci. Soc.*, **15**(4), 309–11.

Poziomek, E. J., Crabtree, E. V. & Mullin, J. W. (1981). Fluorescence detection of specific insecticides, rodenticides, herbicides and fungicides. *Anal. Lett., Part A*, **14**(11), 825–31.

Simonaitis, R. A., Cail, R. & Zehner, J. M. (1981). Rapid clean-up procedure for gas–liquid chromatographic determination of chlorpyrifos-methyl residues in cat food. *J. Assoc. Off. Anal. Chem.*, **64**(5), 1227–31.

Skelly, N. E., Jackson, D. J. & Anderson, P. K. (1981). High-pressure liquid-chromatographic analysis of chlorpyrifos-containing insecticidal formulations—Collaborative study. *J. Assoc. Off. Anal. Chem.*, **64**(3), 628–34.

Sovocool, G. W., Harless, R. L. & Bradway, D. E. (1981). Recognition of diazinon, an organophosphorus pesticide, when found in samples in the form of decomposition products. *J. Anal. Toxicol.*, **5**(2), 73–80.

Sultatos, L. G., Costa, L. G. & Murphy, S. D. (1982). Determination of organophosphorus insecticides, their oxygen analogues and metabolites by high-pressure liquid chromatography. *Chromatographia*, **15**(10), 669–71.

Trujillo, A., Gnanasambandan, T. & Freiser, H. (1984). Determination of organophosphorus compounds by dye-assisted chromatography. *Anal. Chim. Acta*, **162**, 333–8.