ELSEVIER

Food Chemistry 52 (1995) 305–309

0308-8146/95/$9.50
Analytical, Nutritional and Chemical Methods Section

Evaluation of the decay of malathion, dichlofluanid and fenitrothion pesticides in apple samples, using gas chromatography

C. Sáenz Barrio, J. Sanz Asensio,* M. Plaza Medina, M. Pérez Clavijo

Departamento de Química (Area de Química Analítica), Universidad de La Rioja, Logroño-26001, La Rioja, Spain

&

J. Galbán Bernal

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Zaragoza, Zaragoza-50009, Spain

(Received 12 March 1994; revised version received and accepted 8 June 1994)

In this paper an analytical study of the decay, both in the laboratory and on the trees of malathion, fenitrothion and dichlofluanid pesticides in apple samples is reported. 'Safety periods' (time which must elapse between pesticide spraying and consumption of the fruit), their penetration inside the fruit in each situation and the main reasons of the decay are evaluated. Studies were independently performed on each pesticide. To undertake the study in the laboratory, the apple samples were previously sprayed with an aqueous suspension of commercial pesticides and apples were periodically taken. Afterwards, apples were subjected to extraction by Soxhlet using ethyl acetate as solvent and the pesticides were determined by gas chromatography (using a capillary column and NPD detector). The results showed a poor decay of fenitrothion after 2 months of treatment and a logarithmic decay for the other two pesticides. The study on the trees was performed following a similar analytical methodology. In this case, the three compounds showed logarithmic decay curves, indicating more rapid decay than in the laboratory. In no case was any pesticide observed to penetrate inside the apple (under the peeling).

INTRODUCTION

One of the most important problems with the use of pesticides is their possible persistence in the environment and therefore, their possible incorporation into the food chain, which affects all the ecosystem and all human beings (Pimentel *et al.*, 1991). Organophosphorus (OP) pesticides arose as an alternative to the inorganic, natural organic and organochloride pesticides because they are characterised by a lower persistence in the environment (they are easily oxidised or hydrolysed by natural agents) and a more efficient specificity to the target species. Nevertheless, the reality is that most OP pesticides present a considerable persistence and a poor selectivity. According to the World Health Organization data, in 1977 (Copplestone *et al.*, 1977) about 20 000 deaths caused by OP pesticide food poisonings and 500 000 poisonings, more or less serious, were registered. Thus, the investigations about OP pesticides are nowadays concentrated on

(1) the study of the persistence and toxicity from the resulting metabolites after their decay, and
(2) the application of real studies of the persistence of OP pesticides in growing grounds and in horticultural products intended for human consumption.

Our OP pesticide decay studies on chlorpyrifos and diazinon (Sanz *et al.*, 1991) showed that the persistence of these compounds on fruits (*on the trees*) can be of 40 days, but if the decay is studied in the laboratory (with comparable conditions to the study in a greenhouse) the decay time can even be doubled; these results are qualitatively similar to those obtained from other types of pesticides (Mónico-Pifarre & Xirau-Vayreda, 1990). The OP pesticides inhibit the catalytic action of acetylcholinesterase on the hydrolysis of the acetylcholine, which produces considerable nervous disorders. Since

*To whom correspondence should be addressed.

the inhibition is irreversible, the effect provoked by the OP pesticides is accumulative, therefore the presence of small quantities of these compounds in food over a long period can provoke serious poisonings. It is therefore necessary to know the rate of transformation and decay of these compounds in real situations.

In spite of the great increase of the high-performance liquid chromatography (HPLC) (Guiberteau & Bushway, 1991; Yao *et al.*, 1991) and the incorporation of supercritical-fluid chromatography (Kalinoski & Smith, 1988; Mol *et al.*, 1991), from an analytical point of view, the most usual technique to perform the determination of thermically stable OP pesticides, in solid samples, is capillary gas chromatography (CGC) with columns of medium or low polarity. However, some authors advise the use of packed columns for samples with a large quantity of organic matter (Allender, 1991). In general, detectors such as flame ionisation detectors (FID) (Grob & Li, 1989; Liu *et al.*, 1989) or capture electron detectors (CED) (Alawi *et al.*, 1990; Neicheva *et al.*, 1988), are often used when the OP pesticides contain halogens or nitrogen, although more selective detectors such as the flame photometric detector (FPD) (Holstege *et al.*, 1991; Leoni *et al.*, 1992), the nitrogen phosphorus detector (NPD) (Mourer *et al.*, 1990; Sanz *et al.*, 1991), or the mass spectrometer (Mattern *et al.*, 1990; Durand & Barcelo, 1991), are used more frequently. Nowadays, new detectors such as ozone-induced chemiluminescence, when the OP pesticides contain nitrogen (Courthaudon & Fujinari, 1992) are used. The CGC–NPD system is the most recommended for OP pesticides determination (Sharp *et al.*, 1988).

One of the most important aspects of the methodology is the sample treatment. Different schemes of treatment were suggested (Wells, 1988), and the most recommended consists of performing a first step involving maceration with an organic solvent of medium–high polarity (acetone (Miyahara *et al.*, 1991), ethyl acetate (Durand *et al.*, 1989)), followed by a clean-up of the organic extracts by solid-phase extraction with Florisil cartridges (Brayan *et al.*, 1988) or, more common C_{18} cartridges (Brayan *et al.*, 1992), from which the pesticides are later eluted with a solvent of similar polarity to that used in the maceration step. Nevertheless, depending on the characteristics of the sample, the type of the study to realise and the selectivity of the chromatographic detector, a maceration and a later dehydration of the organic phase (Cabras *et al.*, 1989; Sanz *et al.*, 1991) only is necessary. In the future, the treatment of solid samples for OP pesticides will tend to use automated systems of solid–liquid extraction (Marvin *et al.*, 1990) and extraction with supercritical fluids (Majors, 1991).

In this paper, the study of the decay of malathion, fenitrothion and dichlofluanid (not an OP pesticide but it exhibits a similar behaviour) on apple samples, is reported. Objectives of this study are (i) to find the decay time of these pesticides; (ii) to determine the penetration of the pesticides inside the apple; (iii) to compare the decay on the trees and in the laboratory.

MATERIALS AND METHODS

Apparatus

All measurements were made with a Hewlett-Packard HP 5890 gas chromatograph, with a HP Ultra 2 fused silica capillary column (crosslinked 5% phenyl–methyl silicone gum phase of 0·33 μ film thickness, 0·2 mm internal diameter and 25 m length) a NPD detector and HP 3390 A integrator. The optimum working conditions are shown in Table 1.

A battery of 6 Soxhlet (Selecta) (250 ml flasks and 125 ml condensers) with individual temperature regulation in each one (from 5 to 110°C) and evaporator Buchi RE-1 10-B (Switzerland), were also used.

Reagents

Pesticide standards (malathion, fenitrothion and dichlofluanid) were 99·9% pure, and obtained from Riedel-de-Häen (Seelze, Germany). All solvents used were HPLC or PRS quality: ethyl acetate from Lab-Scan (Dublin, Republic of Ireland), isobutyl methyl ketone (IBMK), isooctane and xylene from Panreac (Barcelona, Spain); anhydrous calcium chloride from Merck (Darmstadt, Germany) and bidistilled water were used.

Solutions

Pesticide solutions (1000 mg/litre) were prepared by dissolving the standards in ethyl acetate. More dilute solutions were prepared by dilution with ethyl acetate.

The aqueous pesticide suspensions were prepared following commercial suggestions for each pesticide (fenitrothion 1 ml/litre, malathion 2 ml/litre, and dichlofluanid 1·5 g/litre).

Diazinon (5·05 μg/ml) was used as internal standard throughout the study.

RESULTS AND DISCUSSION

Gas chromatography

Internal standard (diazinon, 50 mg/litre) was used as the quantitative determination method. Peak height of the chromatographic peaks, which presented a better

Table 1. Instrument conditions used in gas chromatograph

Detector	NPD
Detector temperature (°C)	300
Column temperature (°C)	215
Injector temperature (°C)	275
Carrier gas flow rate (nitrogen, ml/min)	0·6
Split (nitrogen, ml/min)	100
Purge flow rate (nitrogen, ml/min)	3
Flow rates for NPD detector	
Auxiliary gas (nitrogen, ml/min)	30
Air (ml/min)	70
Hydrogen (ml/min)	4
Sample volume injected (μl)	2

repeatability than the area, was chosen as the quantification parameter.

The chromatographic response was calibrated separately for each pesticide. Under the experimental conditions, collected in Table 1, other signals different from pesticides (coextractives from the apples or pesticide metabolites) were not observed.

Two linear response (concentration) ranges were observed for fenitrothion (one between 0·09 and 3·3, and the other from 3·3 to 60 mg/litre), and dichlofluanid (one between 0·45 and 6·0, and the other from 6·0 to 60 mg/litre), while malathion present a single linear response range from 0·09 to 60 mg/litre.

In all cases, repeatability values of the signals, expressed as relative standard deviation (RSD) of 10 determinations were 4·8% for fenitrothion in 0·09 mg/litre, 4·4% for malathion in 0·09 mg/litre and 2·8% for dichlofluanid in 0·45 mg/litre.

Solid–liquid extraction (SLE) study

Several solvents were tested for SLE in order to obtain the better recoveries with the three pesticides.

Firstly, isooctane was considered good since extraction recoveries (almost 100%) had been obtained with diazinon and chlorpyrifos (Sanz *et al.*, 1991). Similar recoveries were obtained with malathion and fenitrothion, but only 60% average recovery was obtained for dichlofluanid.

Subsequently, solvents of higher polarity were tested: (i) a mixture of ethyl acetate:xylene (1:1); (ii) ethyl acetate, and (iii) a mixture of ethyl acetate: IBMK (1:1) using magnetic stirring and Soxhlet extraction. The obtained results indicated the following:

- By using magnetic stirring, all solvents produced recoveries of the three pesticides almost 100% in 2 h.
- With Soxhlet recoveries of about 100% were obtained in a smaller extraction time than 90 min using ethyl acetate as solvent.
- The chosen system of SLE consisted of the use of 100 ml of ethyl acetate in a Soxhlet system and with an extraction time of 90 min.

Decay study

To realise both decay studies (in the laboratory and on the trees), the apple samples were sprayed with the aqueous suspension of the commercial products:

For the study in the laboratory, three groups of 20 apples were considered, one group for each pesticide, and each apple was sprayed with 50 ml aqueous suspension of either pesticide. This volume is enough to wet all apple surface. Then, the apples were maintained free of contact with any surface to avoid losses. The residual pesticide on the apple was periodically determined by taking samples in duplicate.

For the study on the trees, six apple trees, two for each pesticide, which had not been sprayed with these or any pesticides, were sprayed with the aqueous suspension using a spraying machine, in excess of real suspensions volume to wet all the surface of each apple (8 litres per tree), but at the doses recommended by the manufacturer. At the same time intervals, the corresponding aliquots were taken in duplicate.

In order to perform the pesticide penetration study at the same time, four fractions from each apple at different depths, where the pesticides had been tested, were obtained by the following procedure:

- The apple (weight 90 ± 10 g) is washed twice with (2 × 10) ml ethyl acetate. The washings are collected and labelled 'fraction 1'.
- The apple is left to dry and peeled. The weight of the peelings is about 10% of the total weight and the thickness is about 1 mm. The peeling is extracted with 100 ml ethyl acetate, using the procedure ESL (Soxhlet) described before. The extract is 'fraction 2'.
- The rest of the apple is washed twice with (2 × 10) ml solvent. The washings are collected and labelled 'fraction 3'.
- The sample is left to dry and a spherical shell, about 5 mm thickness and 35% weight of the apple, is cut from the apple, peeled and then extracted with 100 ml ethyl acetate using the procedure ESL (Soxhlet). This extract is 'fraction 4'.

Each one of these four fractions was evaporated in an evaporator to small volume (not dryness), the residues were dried with anhydrous calcium chloride, the internal standard was added, and final volume was adjusted to 10 ml. The solution (2 μl) was injected into the chromatograph.

Decay study in the laboratory

From the addition of the aqueous suspension of the commercial pesticides to the apples, (2 × 9) apples for each pesticide were analysed for 2 months. Using the established procedure, only in fraction 1 and 2 pesticides were found. Fraction 2 contains in all cases about 20% of the found pesticides in fraction 1, which indicates that the penetration into the fruit is low, probably due to the impermeable structure of the skin.

In general, the found values of concentration for the pesticides (expressed as pesticide μg/apple g) showed variations in sawtooth way, and thus, a statistical treatment of the experimental data was performed, using the 'Analysis of Time Series'. Applying a 'Movement of Order 3' (Spiegel, 1988) better and more reliable results were obtained. In the Fig. 1 the results obtained for the three pesticides are presented. So, the following can be deduced:

- For fenitrothion, after a little initial variation of the pesticide, there is no decay during the interval of tested time, that is to say, the pesticide does not decay during the 2 months in which the study was carried out.
- For dichlofluanid it can be concluded that the pesticide is going to decay in a linear way in time. In fact, the minimum square fitting gives the equation:

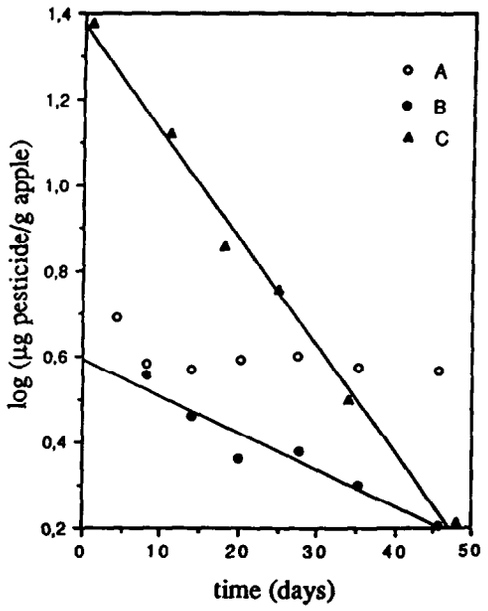

Fig. 1. Results obtained for the decay of the three pesticides in the laboratory. A, fenitrothion; B, dichlofluanid; C, malathion

$$\log [\text{dichlofluanid}] = 0{\cdot}591 - 0{\cdot}0085 \times t,\ r = 0{\cdot}96$$

where [dichlofluanid] comes in μg of pesticide per gram of apple, and t in days.

- For malathion, the observed results are very similar to that indicated for the dichlofluanid, although the decay rate is greater as deduced from slope of equation obtained by least squares:

$$\log [\text{malathion}] = 1{\cdot}370 - 0{\cdot}025 \times t,\ r = 0{\cdot}995$$

Decay study on the trees

This study was carried out in a similar form to that indicated in the laboratory study.

Penetration of the pesticides was not observed and variations of the concentration were also presented in a sawtooth way, being necessary to use the same mathematical treatment as in the laboratory study. The obtained results are shown in Fig. 2. The three pesticides show a behaviour which can be explained through the mathematical equations of logarithmic type:

$$\log [\text{fenitrothion}] = -0{\cdot}050 - 0{\cdot}026 \times t,\ r = 0{\cdot}96$$

$$\log [\text{dichlofluanid}] = 0{\cdot}273 - 0{\cdot}020 \times t,\ r = 0{\cdot}992$$

$$\log [\text{malathion}] = 0{\cdot}179 - 0{\cdot}055 \times t,\ r = 0{\cdot}994$$

To compare the obtained results on the trees and in the laboratory is very interesting. Malathion and dichlofluanid suffer the quickest decay on the trees; for both pesticides the equation slopes are practically duplicated, but if we take into account that they are logarithmic equations, the concentration decrease rate on the trees, is practically 10 times superior to that of the compound in the laboratory. In the case of fenitrothion its decay in the laboratory is negligible while, on the trees, exhibits a larger decay rate than the dichlofluanid.

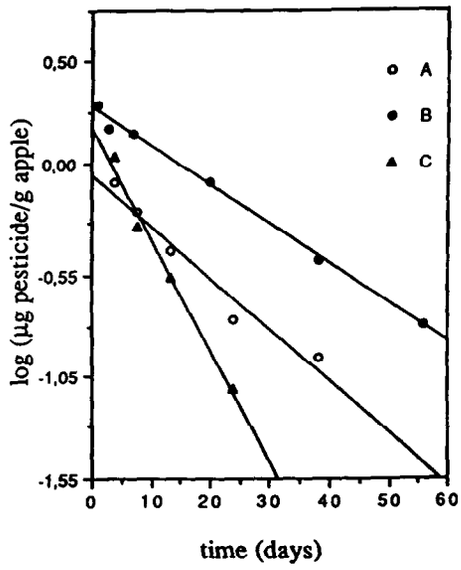

Fig. 2. Results obtained for the decay of the three pesticides on the trees. A, fenitrothion, B, dichlofluanid; C, malathion.

Considering persistence time, the results show a different behaviour, too. While, malathion practically disappears in 20 days after the addition, fenitrothion persists for approximately 50 days and at this time dichlofluanid has not decayed, showing a larger persistence compared with the nitrogen-containing pesticides.

CONCLUSIONS

The results obtained in the laboratory are never extrapolable to the results on the trees, and so, it is necessary to perform the decay studies in the conditions in which the growing is realised.

Taking into account that the pesticides do not penetrate inside the fruit and, thus, the decay occurs by biological ways, the main difference between the studies on the trees and in the laboratory can be due to the more drastic action that the environment agents (sunlight, rain, temperature) produce on the trees.

Again, to emphasise the enormous persistence of these compounds (except the malathion), they show longer decay times than 2–3 weeks, that is the common time considered as a safe interval.

ACKNOWLEDGEMENTS

This work has been supported by the Instituto de Estudios Riojanos de La Rioja (La Rioja, Spain), by Estación de Avisos Fitosanitarios de La Comunidad Autónoma de La Rioja (Spain), and by CAICYT (project. No. 541-A 783).

REFERENCES

Alawi, M. A., Gharaibeh, S. & Al-Shureiki, Y. (1990). Determination of fenitrothion and pyrethroid residues in water,

soil and plants after combating locusts in Jordan, 1989. *Chemosphere*, **20**(3–4), 443–7.

Allender, W. J. (1991). Column extraction of chlorpyrifos from contaminated fish. *J. Anal. Toxicol.*, **15**(3), 141–3.

Brayan, J. G., Haddad, P. R., Sharp, G. J., Dilli, S. & Desmarchelier, J. M. (1988). Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, **447**(1), 249–55.

Brayan, J. G., Haddad, P. R., Sharp, G. J., Dilli, S. & Desmarchelier, J. M. (1992). Extraction of carbaryl from stored rice, maize, peas and sunflower seeds prior to chromatographic analysis. *Pestic. Sci.*, **34**(3), 215–9.

Cabras, P., Meloni, M., Plumitallo, A. & Gennari, M. (1989). High-performance liquid-chromatographic determination of ethiofencarb and its metabolic products. *J. Chromatogr.*, **462**, 430–4.

Copplestone, J. F., Watson, D. L. & Brown, A. W. A. (ed) (1977). *Pesticide Management and Insecticide Resistance*. Academic Press, New York, p. 147.

Courthaudon, L.O. & Fujinari, E.W. (1992). Nitrogen specific gas chromatographic. Detection based on chemiluminescence. *LC–GC Int.*, **5**, 44–8.

Durand, G. & Barcelo, D. (1991). Confirmation of chlorotriazine pesticides, their degradation products and organophosphorus pesticides in soil samples using gas chromatography–mass spectrometry with electron impact and positive and negative-ion chemical ionization. *Anal. Chim. Acta*, **243**(2), 259–71.

Durand, G., Forteza, R. & Barcelo, D. (1989). Determination of chlorotriazine herbicides, their dealkylated degradation products and organophosphorus pesticides in soil samples by means of two different cleanup procedures. *Chromatographia*, **28**(11–12), 597–604.

Grob, K. & Li, Z. (1989). Coupled reversed-phase liquid chromatography-capillary gas chromatography for the determination of atrazine in water. *J. Chromatogr.*, **473**(2), 423–30.

Guiberteau, C. C. & Bushway, R. (1991). Analysis of phosmet and azinphos-methyl in apples by high-performance liquid chromatography. *J. Liq. Chromatogr.*, **14**(19), 3603–13.

Holstege, D. M., Scharberg, D. L., Richardson, E. R. & Moeller, G. (1991). Multi-residue screen for organophosphorus insecticides using gel-permeation chromatography–silica gel clean-up. *J. AOAC*, **74**(2), 394–9.

Kalinoski, H. T. & Smith, R. D. (1988). Pressure-programmed micro-bore-column supercritical-fluid chromatography–mass spectrometry for the determination of organophosphorus insecticides. *Anal. Chem.*, **60**(6), 529–35.

Leoni, V., Caricchia, A. M. & Chiavarini, S. (1992). Multi-residue method for quantitation of organophosphorus pesticides in vegetable and animal foods. *J. AOAC Int.*, **75**(3), 511–8.

Liu, J., Suzuki, O., Kumazawa, T. & Seno, H. (1989). Rapid isolation with Sep-Pak C_{18} cartridges and wide-bore capillary gas chromatography of organophosphate pesticides. *Forensic Sci. Int.*, **41**(1–2), 67–72.

Majors, R. E. (1991). Supercritical fluid extraction — an introduction. *LC–GC Int.*, **4**(3), 10–7.

Marvin, C. H., Brindle, I. D., Hall, C. D. & Chiba, M. (1990). Automated high-performance liquid chromatography for the determination of pesticides in water using solid phase extraction. *Anal. Chem.*, **62**, 1495–8.

Mattern, G. C., Singer, G. M., Louis, J., Robson, M. & Rosen, J. D. (1990). Determination of several pesticides with a chemical-ionization ion-trap detector. *J. Agric. Food Chem.*, **38**(2), 402–7.

Miyahara, M., Sasaki, K., Suzuki, T. & Saito, Y. (1991). Expanded coagulating cleanup procedures for simultaneous gas-chromatographic determination of organophosphorus pesticides in crops and fruits. *Chem. Pharm. Bull.*, **39**(4), 1055–8.

Mol, J. G., Zegers, B. N., Lingeman, H. & Brinkman, U. A. (1991). Packed-capillary supercritical-fluid chromatography of pesticides using phosphorus-selective detection. *Chromatographia*, **32**(5–6), 203–10.

Mónico-Pifarré, A. & Xirau-Vayreda, M. (1990). Study of carbendazin residue accumulation on greenhouse and field-grown strawberries, after successive treatments with benomyl. *J. AOAC*, **73**, 553–6.

Mourer, C. R., Hall, G. L., Whitehead, W. E. & Shibamoto, T. (1990). Gas-chromatographic method for determination of chlorpyrifos and its metabolite 3,5,6-trichloropyridin-2-ol (TCP) in dates. *J. AOAC*, **73**(2), 294–7.

Neicheva, A., Kovacheva, E. & Marudov, G. (1988). Determination of organophosphorus pesticides in apples and water by gas–liquid chromatography with electron capture detection. *J. Chomatrogr.*, **437**(1), 249–53.

Pimentel, D. (1991). Pesticides and world food supply. *Chem. Br.*, **29**(7), 646–7.

Sanz, J., Sáenz, C., Galarreta, M.T. & Galbán, J. (1991). Study of the decay of diazinon and chlorpyrifos in apple samples using gas chromatography. *Food Chem.*, **42**, 413–24.

Sharp, G. J, Brayan, J. G., Dilli, S., Haddad, P. R. & Desmarchelier J. M. (1988). Extraction, clean-up and chromatographic determination of organophosphate, pyrethroid and carbamate insecticides in grain and grain products. A review. *Analyst*, **113**(10), 1493–507.

Spiegel, M.R. (1988). *Teoría y Problemas de Estadística (serie Schaum)*. McGraw Hill, Bogotá, Colombia, pp. 283–313.

Wells, D.E. (1988). Extraction, clean-up and group separation techniques in organochlorine trace analysis. *Pure Appl. Chem.*, **60**(9), 1437–48.

Yao, S., Meyer, A. & Henze, G. (1991). Comparison of amperometric and UV-spectro-photometric monitoring in the HPLC analysis of pesticides. *Fresenius' J. Anal. Chem.*, **339**(4), 207–11.