Bull. Environ. Contam. Toxicol. (1997) 58:341–347


Bulletin of Environmental Contamination and Toxicology

# Evaporated Extracts of Samples for Pesticide Residue Analysis Simplifies Transport from Remote Places

**O. Terenius, M. Åkerblom**

**Department of Environmental Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, Uppsala S-75007, Sweden**

Received: 23 September 1996/Accepted: 2 December 1996

The increasing use of pesticides demands a world-wide control of residue levels in food and the environment. However, pesticide residue analysis is challenging and needs both experience and instrumentation. Although much can be done with fairly simple means (Åkerblom and Cox 1996), many countries with limited resources can only perform certain analyses. Also in technologically more advanced counties, different laboratories may specialise in certain kinds of analyses. There is also a need to confirm results with an exchange of samples between laboratories. There are usually several technical difficulties connected with the transport of pesticide samples, in addition to the risk of transmission of pests and diseases and related customs control. Currently, frozen samples are sent in insulated containers with dry ice to keep the samples in intact condition. When possible, there are large costs for the transport of heavy packages, and delay in customs may lead to destruction of the samples. In many areas of the world this procedure is not feasible at all.

A way to circumvent these difficulties is presented here. Even if a laboratory lacks the basic requirements to purchase, run and maintain instrumentation to analyse pesticide residues, there are often possibilities to extract the pesticide samples. The extracts are taken to dryness in the presence of a 'keeper' substance, and the residues can then be sent in apparently empty glass vials. The use of keepers is well known in residue analysis: a small amount of the keeper, a high boiling compound which dissolves the pesticides, is added to an extract before evaporation of the solvent to avoid loss of volatile compounds. Thus, Thornburg (1963) suggests the use of ethylene glycol, stearic acid, or white oil as keeper, Baumgarten and Pfrang (1989) have investigated the use of dodecane to reduce loss of organochlorine compounds on evaporation, and Albro et al. (1984) used propylene glycol to reduce losses of dibenzo-*p*-dioxines and dibenzofurans. The natural waxes of pine needles on growing pine trees retain organochlorine compounds sufficiently to be used as sampling medium (Kylin et al. 1994).

We have investigated the use of polyethylene glycol as a keeper at harsh conditions, for up to three weeks at 44°C. This was done to find out whether the residues remain in the vials at simulated transport conditions. In all 64 pesticides were tested.

*Correspondence to:* M. Åkerblom

## MATERIALS AND METHODS

Gas chromatographic analyses were performed according to Andersson and Ohlin (1986), on Hewlett-Packard 5890 gas chromatographs, one equipped with two $^{63}$Ni electron capture detectors (ECD), one with two nitrogen-phosphorus detectors (NPD). Typical columns used were SE-30 (Quadrex) and OV-1701 (J&W Scientific) with dimensions of 25 m x 0.25 mm id (0.25 µm film thickness). The following conditions were used: splitless injection, 60 sec; carrier gas, nitrogen, injector temperature 25°C, detector temperature 300°C. The oven temperature was held at 90°C for 1 min, increased by 30°C/min to 180°C, increased by 4°C/min to 260°C, and held there for 25 min.

Chemicals were of analytical or pesticide grade. Pesticides had a purity of 98-100% (Dr. Ehrenstorfer, GmbH). Standard solutions of the pesticides were prepared in cyclohexane:acetone 9:1. The keeper, the polyethylene glycol Carbowax 20 M®, was used as a 10% solution in dichloromethane.

In a preliminary study four substances were tested, c.f. Table 1. A set of test tubes was filled with 1 mL each of a standard solution of a mixture of these substances. Keeper solution (100 µL) was added to half of the tubes. The solvent was evaporated with a gentle stream of nitrogen. Half of the samples of each type were evaporated just to dryness, and the others received nitrogen flow for another 15 min. To retrieve the pesticide the residue was dissolved in hexane (where Carbowax is insoluble) in an ultrasonic bath till the wax had disappeared from the walls of the tubes. The tubes were centrifuged, and the supernatant was analysed by gas chromatography.

Next, two similar studies were made, both with the same basic set-up. All experiments were run in duplicate. Four pesticide solutions, each containing 10 to 15 different pesticides, 0.2-0.4 µg/mL in cyclohexane:acetone 9:1, were prepared (Table 2). An aliquot of 500 µL of these solutions was drawn into 1.5 mL glass vials (Varian, for GC auto sampler). Keeper solution (100 µL) was added. The content of the vials was evaporated with a gentle stream of nitrogen; when the solvent had disappeared, gas flow was allowed to run for 5 more minutes.

In the first of these studies, the glass vials were left open during storage. They were kept in the dark at 22°C for 1 hour, 1 week and 3 weeks, respectively, and at 44°C for 1 week and 3 weeks, respectively. In all 52 substances were tested. The residue was dissolved in acetone (where Carbowax is barely soluble) and analysed as in the preliminary study.

In the second study the vials were closed with Teflon® lined screw caps during storage. Another set-up of 52 substances was studied under the following conditions: First vials were kept in the dark at 44°C for 3 weeks. Then half of the vials were opened at room temperature, and the residue dissolved as above. Half of the vials were stored in a freezer at -18°C for 24 hr before they were opened and the residue was dissolved.

Every sample was chromatographed twice on each of the two GC columns, and the mean of the four results was calculated. Recoveries are expressed as percentage of

the original amount added, and are rounded off to the closest 5%. When the difference between the duplicates was >30%, both recoveries are shown (Table 2). In the second study the standard error of recovery for each substance was calculated.

## RESULTS AND DISCUSSION

In the preliminary study the more volatile of the four substances showed larger recovery with than without keeper (Table 1). This encouraged us to continue with a larger set of substances.

**Table 1.** Recovery of pesticides (%) with and without keeper.

| Pesticide | RT SE-30 min | With keeper | | Without keeper | |
|---|---|---|---|---|---|
| | | 0 min* | 15 min* | 0 min* | 15 min* |
| Fenitrothion | 9.8 | f | f | 85 | 75 |
| Malathion | 10.2 | f | f | 65 | 40 |
| Endosulfan-α | 12.8 | f | f | f | f |
| DDT-*p,p'* | 16.6 | f | f | f | f |

f= fully recovered; the percentage level is ≥90. *= extra flow of nitrogen after dryness.

In the following two studies, each comprising 52 substances, a total of 64 substances were examined (Table 2). The retention times of the pesticides on the non-polar GC column SE-30 have been given as an indication of their relative volatility. This gives a practical way of judging whether a given pesticide with known retention time would be a candidate for this type of transport.

**Table 2.** Recovery of pesticides (%) with keeper at different storage temperature and time.

| Pesticide | RT SE-30 min | Uncapped vials | | | | | Capped vials | |
|---|---|---|---|---|---|---|---|---|
| | | 22°C 1h | 22°C 1w | 22°C 3w | 44°C 1 w | 44°C 3 w | non-frozen 44°C 3w | frozen 44°C 3w |
| Azinphos-ethyl | 20.7 | f | f | f | f | f | 85 ± 7.6 | 80 ± 2.5 |
| Azinphos-methyl | 19.1 | f | f | 70 | 75 | 60 | 70 ± 21 | 75 ± 3.3 |
| Binapacryl | 14.5 | f | f | f | 70 | 65;30 | - | - |
| Bromophos | 11.0 | - | - | - | - | - | 65 ± 9.3 | 85 ± 6.5 |
| Bromopropylate | 14.8 | f | f | f | f | f | - | - |
| Captan | 11.5 | 80 | 65 | 70;15 | 5 | 1 | 40 ± 34 | 0 |
| Carbaryl | 9.1 | 50 | 45 | - | 40 | 60;5 | 45 ± 52 | 80 ± 2.9 |
| Carbofuran | 7.4 | f | 80 | f | 70 | 65 | 55 ± 42 | 85 ± 3.4 |
| Carbophenothion | 15.9 | f | f | f | f | f | - | - |
| Chlorfenson | 12.7 | f | f | f | f | 85 | - | - |
| Chlorfenvinfos | 11.7 | f | f | f | f | 80 | 90 ± 2.7 | 85 ± 1.5 |
| Chlorpropham | 6.7 | f | f | f | 60 | 25 | 95 ± 1.4 | 80 ± 2.1 |
| Chlorpyrifos | 10.6 | f | 85 | 80 | 55 | 30 | 75 ± 16 | 80 ± 1.4 |
| Cyanophos | 7.8 | - | - | - | - | - | 60 ± 25 | 75 ± 4.8 |
| Cypermethrin | 25.2 | f | f | f | f | f | - | - |
| DDD-*p,p'* | 15.0 | f | f | f | 80 | 80 | 95 ± 4.3 | 95 ± 1.7 |
| DDE-*p,p'* | 13.6 | f | f | f | f | f | 95 ± 3.0 | 130 ± 3.9 |
| DDT-*o,p'* | 15.3 | f | f | f | 80 | 80 | 90 ± 2.6 | 95 ± 5.3 |
| DDT-*p,p'* | 16.6 | f | 85 | f | 65 | 50 | 100 ± 7.6 | 90 ± 11 |
| Deltamethrin | 29.8 | f | f | f | f | f | - | - |
| Diazinon | 8.2 | f | 85 | 80 | 30 | 5 | 85 ± 3.2 | 80 ± 2.1 |

**Table 2.** (cont.)

| Pesticide | RT SE-30 min | Uncapped vials 22°C 1h | 22°C 1w | 22°C 3w | 44°C 1 w | 44°C 3 w | Capped vials non-frozen 44°C 3w | frozen 44°C 3w |
|---|---|---|---|---|---|---|---|---|
| Dichlobenil | 4.4 | 65 | 0 | 0 | 0 | 0 | 75 ± 9.7 | 75 ± 5.1 |
| Dichlorvos | 3.7 | 45 | 0 | 0 | 0 | 0 | 15 ± 13 | 25 ± 6.4 |
| Dicofol | 18.7 | f | f | f | 80 | 45 | - | - |
| Dimethoate | 7.3 | 85 | 80 | 75 | 55 | 30 | 50 ± 37 | 90 ± 1.7 |
| Ditalimfos | 12.5 | f | 85 | 70 | 80 | 70 | - | - |
| Endrin | 14.5 | f | f | f | 75 | 55 | 95 ± 0.96 | 100 ± 2.9 |
| Ethion | 15.0 | f | f | f | f | 85 | 100 ± 14 | 105 ± 3.3 |
| Fenamifos | 12.8 | - | - | - | - | - | 115 ± 2.7 | 130 ± 4.4 |
| Fenchlorphos | 9.7 | f | 60 | 45 | 15 | 1 | 50 ± 33 | 65 ± 5.9 |
| Fenpropimorf | 10.9 | - | - | - | - | - | 85 ± 12 | 80 ± 0.0 |
| Fenvalerate | 27.3 | f | f | f | f | f | - | - |
| Folpet | 11.8 | 80 | 55 | 60;5 | 0 | 0 | 35 ± 35 | 0 |
| HCH-alpha | 7.1 | - | - | - | - | - | 85 ± 2.8 | 15 ± 7.6 |
| Hexazinon | 16.1 | - | - | - | - | - | 90 ± 7.8 | 85 ± 4.1 |
| Lindane | 7.8 | 80 | 65 | 70 | 10 | 2 | 95 ± 2.6 | 30 ± 14 |
| Malathion | 10.2 | f | 75 | 60 | 40 | 50;20 | - | - |
| Mecarbam | 11.8 | f | f | f | f | 85 | - | - |
| Mephosfolan | 11.0 | - | - | - | - | - | 95 ± 11 | 90 ± 4.1 |
| Metamitron | 13.2 | - | - | - | - | - | 105 ± 12 | 115 ± 20 |
| Methidathion | 12.0 | f | 85 | 70 | 60 | 25 | 85 ± 3.3 | 95 ± 2.6 |
| Methoxychlor | 18.8 | f | f | f | 85 | f | 95 ± 3.8 | 105 ± 9.6 |
| Metoxuron | 4.8 | - | - | - | - | - | 40 ± 5.6 | 80 ± 6.4 |
| Mevinphos | 5.1 | 85 | 35 | - | 0 | 0 | 45 ± 30 | 75 ± 4.1 |
| Omethoate | 6.1 | 45 | 40 | 50 | 10 | 3 | 40 ± 48 | 60 ± 3.7 |
| Parathion | 10.5 | f | f | f | 85 | 70 | 100 ± 8.6 | 100 ± 5.4 |
| Parathion-methyl | 9.2 | f | 75 | 50 | 30 | 35;5 | 70 ± 5.0 | 80 ± 3.5 |
| Permethrin | 22.7 | f | f | f | f | f | - | - |
| Phosalone | 19.5 | f | f | f | 80 | 80 | 95 ± 12 | 100 ± 7.2 |
| Phosmet | 17.6 | f | 85 | 70 | 80 | 40 | 85 ± 3.4 | 95 ± 3.2 |
| Pirimicarb | 8.6 | f | f | f | 65 | 20 | 95 ± 7.0 | 100 ± 2.7 |
| Pirimiphos-methyl | 10.0 | f | f | f | 70 | 25 | 95 ± 12 | 100 ± 4.1 |
| Prochloraz | 23.3 | - | - | - | - | - | 100 ± 24 | 105 ± 20 |
| Procymidone | 12.0 | f | f | f | f | 85 | - | - |
| Profenophos | 13.4 | f | f | 80 | 75 | 60 | 65 ± 31 | 75 ± 1.8 |
| Promecarb | 7.0 | - | - | - | - | - | 60 ± 35 | 85 ± 3.0 |
| Propham | 5.3 | f | 70 | 40 | 0 | 0 | 85 ± 2.2 | 75 ± 3.1 |
| Prothiophos | 13.4 | f | f | f | 80 | 60 | 100 ± 15 | 110 ± 6.4 |
| Pyrazophos | 21.1 | f | f | f | 85 | 85 | 100 ± 9.5 | 105 ± 6.4 |
| Quinalphos | 11.8 | f | f | f | 85 | 70 | 100 ± 14 | 105 ± 5.1 |
| Tecnazene | 6.3 | 85 | 20 | 10 | 0 | 0 | 90 ± 6.4 | 75 ± 3.6 |
| Tetrachloraniline | 6.5 | 85 | 75 | 60 | 10 | 1 | 100 ± 7.6 | 100 ± 8.8 |
| Tionazin | 6.3 | - | - | - | - | - | 85 ± 2.9 | 75 ± 2.7 |
| Triazophos | 15.1 | f | f | 85 | f | f | 90 ± 7.7 | 85 ± 4.1 |
| **Total ≥90%** | | 41 | 30 | 29 | 14 | 11 | 25 | 23 |
| **Total 70-<90%** | | 7 | 12 | 10 | 17 | 12 | 11 | 21 |
| **Total <70%** | | 4 | 10 | 13 | 21 | 29 | 16 | 8 |

f= fully recovered; the percentage level is ≥90.

In the first study 52 pesticides were tested with uncapped vials. The recovery of the substances increased with increasing retention time and decreased with longer duration of storage and higher temperatures. This is indicated in Figure 1, representing 33 substances that were also run in the second study. Temperature was

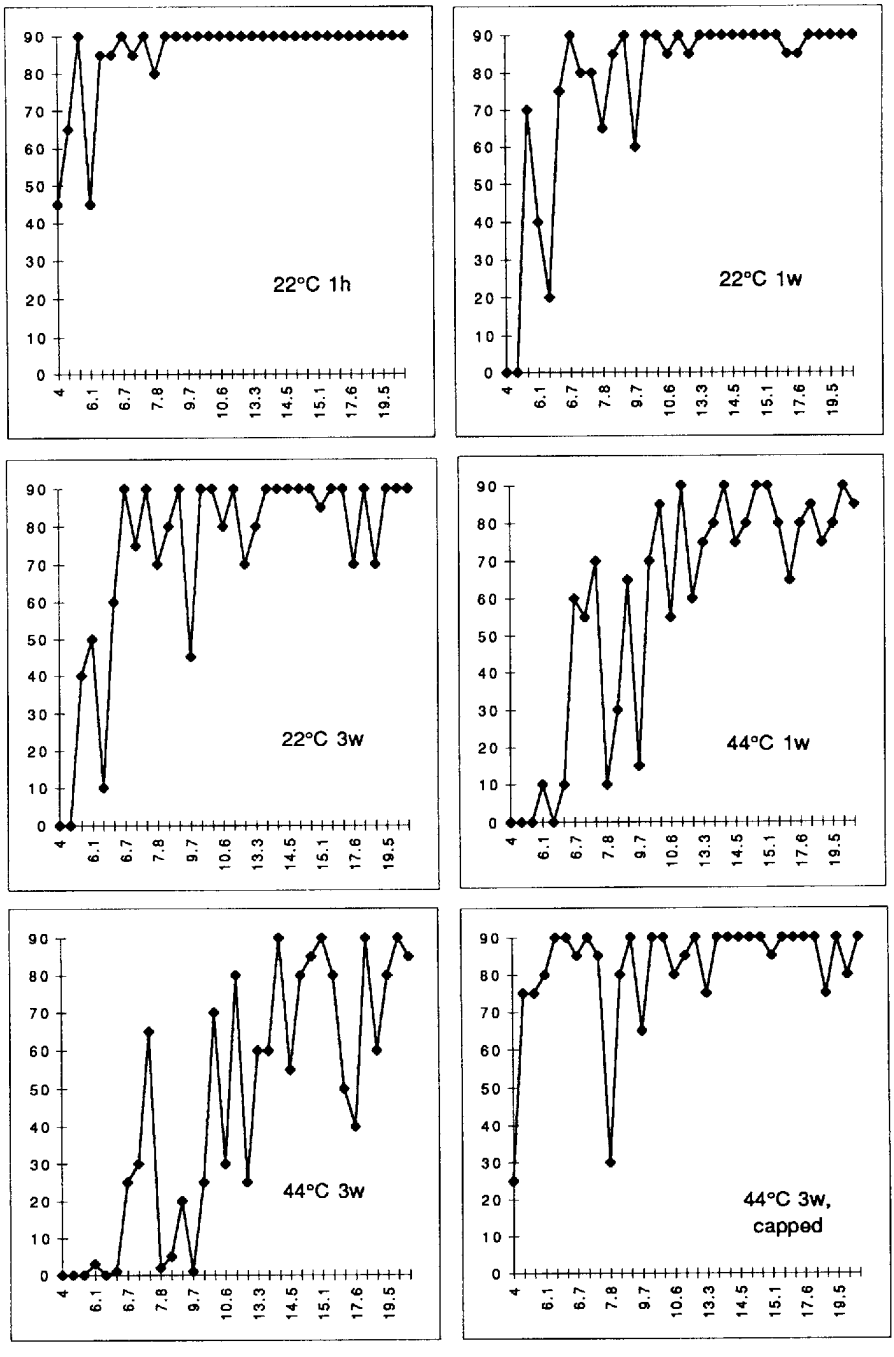


Figure 1. Recovery (%, ordinate) of selected pesticides in relation to retention time (min, abscissa) after given time at temperature indicated. Five trials with uncapped vials, one trial with capped, frozen vials.

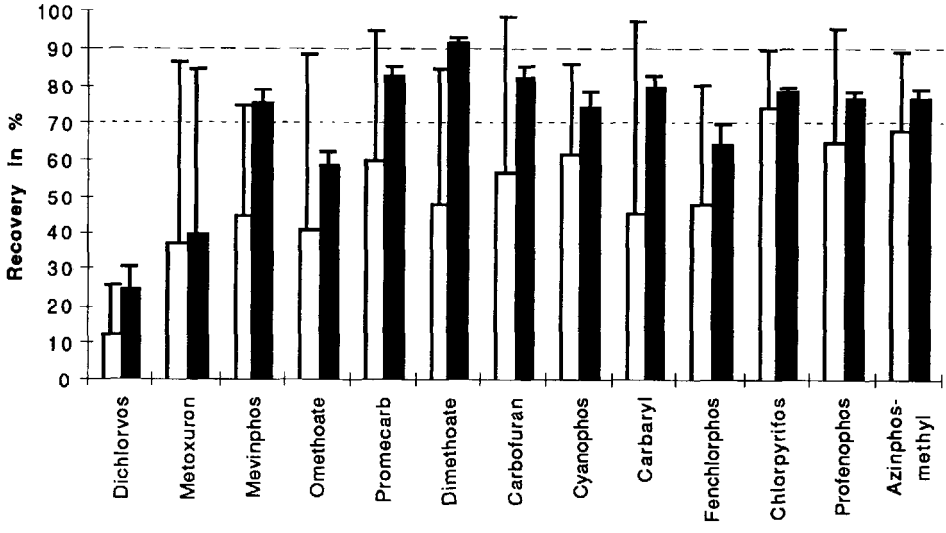


**Figure 2.** Recoveries of the more volatile substances after 3w at 44°C, in non-frozen (open bars) and frozen (solid bars) vials, respectively. The horizontal lines indicate 'full' recovery level and 'acceptable' level, respectively. Error bars represent standard deviation.

a more important factor than time. Out of the 52 substances, 42 were acceptably (>=70%) recovered and 30 of these were well (>=90%) recovered after 1 week at 22°C, but only 23 were acceptably recovered after 3 weeks at 44°C.

Capped vials were used in the second study with additional substances. Included were also 27 substances that did not work acceptably in the first study for 3 weeks at 44°C. The recovery of 22 of these 27 substances rose to >=70%. For comparison these results are presented in Figure 1. In order to condense substances that might be volatilized in the vial, half of the vials were frozen before uncapping to add solvent. The recoveries rose for the most volatile compounds and the results were generally more reproducible when the vials were frozen before opening. This is demonstrated in Figure 2 by selected substances giving large differences between duplicates when not frozen. However, HCH- $\alpha$ and HCH- $\gamma$ (lindane) were lost to a great extent by freezing. No explanation to this phenomenon has yet been found.

According to the results it would be, in many cases, possible to send pesticide samples as evaporated extracts even in a hot climate. In the second study we noticed that the recovery is much better when the vials are capped. In total we find that 56 out of 64 substances give recoveries of at least 70% after storage at 44°C for 3w.

These studies were made with pure standard solutions. Sample extracts may contain compounds that will catalyse degradation of pesticides, although this is unlikely when dissolved in a keeper. It is suggested that any extract - pesticide - keeper combination is tested beforehand, and that with each set of samples sent there are control samples fortified at different levels with the pesticide(s) sought.

The results presented here clearly show that a much simplified method of transfer of samples between laboratories is adequate, which enables monitoring of pesticide residues in any part of the world.

## REFERENCES


Åkerblom M, Cox J (1996) Appropriate technology for pesticide analysis in developing countries. In: Ekström G (ed) World directory of pesticide control organisations, 3rd edition. Royal Society of Chemistry, UK, pp 19-40

Albro PW, Schroeder JS, Harvan DJ, Corbett BJ (1984) Characteristics of an extraction and purification procedure for chlorinated dibenzo-p-dioxins and dibenzofurans in soil and liver. J Chromatog 312:165-182

Andersson A, Ohlin B (1986) A capillary gas chromatographic multiresidue method for determination of pesticides in fruits and vegetables. Vår Föda 38, Suppl 2:79

Baumgarten D, Pfrang I (1989) Schwerflüchtige halogenierte Kohlenwasserstoffe in Wasserproben Teil 2. GIT Fachz Lab 9:815-821

Kylin H, Grimvall E, Östman C (1994) Environmental monitoring of polychlorinated biphenyls using pine needles as passive samplers. Environ Sci Technol 28:1320-1324

Thornburg WW (1963) Extraction and cleanup procedures. In: Zweig G (ed) Analytical methods for pesticides and plant growth regulators, vol 1, Academic Press, New York, pp 87-108