*Apidologie*, 1985, **16** (1), 89-94

# MALATHION RESIDUES IN GREEK HONEY

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## SUMMARY

Sixty-one samples of honey collected from different parts of Greece during 1981-1982 were analyzed for malathion residues. Residues were extracted with acetonitrile/water solution, partitioned with n-hexane, cleaned up by florisil column, evaporated to dryness, redissolved in isooctane and then analysed by GLC with an electron capture detector. Recoveries of malathion residues varied from 80.0-98.0 %. Fifty-four samples contained no detectable malathion residue while the rest contained residues at 1-5 ppb level. This level is not considered hazardous for humans.

## INTRODUCTION

The possibility of contamination of food products by various types of pesticides was emphasized by different authors (KILIKIDIS, 1976 ; CREMLYN, 1978). Honey as a pure and natural product should be innocent of any taint due to chemical sprays. Residue analyses so far either failed to detect pesticides in honey (MORTON *et al.*, 1974 ; DENNIS *et al.*, 1981) or revealed very low concentrations (OGATA and BEVENUE, 1973 ; ESTER *et al.*, 1977). This suggested a natural provision against a contamination of the honey based on the physiology and behavior of honey bees (ATKINS, 1975).

Pesticides might be introduced into honey by bees that fed on nectar or pollen from contaminated blossoms. Furthermore, contamination of honey can arise by chemicals that are used to prevent infections of bees such as sulphonamides (BELLIARDO, 1981) ethylene oxide (VESELY *et al.*, 1981) and others (LYON, 1975). In recent years another source of honey contamination became predominant in several countries of the world. Beekeepers use a variety of pesticides within the colonies to control *Varroa* mite (*Varroa jacobsoni*). Since these

pesticides are put directly into the hives one could suspects contamination of the honey. Malathion has been used extensively against *V. jacobsoni* in Greece since 1978. This study reports the results of an investigation conducted to identify malathion residues existing in the honey produced and marketed in Greece.

## MATERIALS AND METHODS

### *Sample collection*

Twenty-three samples of honey were collected from the market of Greece and represented 12 different commercial firms. Another 38 samples were collected directly from beekeepers.

Thirteen of these 61 samples were produced in 1981, the rest in 1982. They represented different geographical areas that covered the north, the south, and some Greek islands as well.

### *Reagents*

| | |
|---|---|
| Acetonitrile | — Nanograde (Mallinckrodt, Inc.). |
| Ethyl acetate | — Nanograde (Mallinckrodt, Inc.). |
| Florisil | — 60/100 mesh (Fisher Scientific Co.), activated in a 130 °C oven overnight. |
| Glass wool | — Filtering fibre (Corning Glass Works) washed with hexane. |
| Hexane | — Nanograde (Mallinckrodt, Inc.). |
| Isooctane | — Resi-analyzed (J.T. Baker Co.). |
| Malathion | — Standard (Supelco, Inc. 4-9050). |
| Sodium sulfate | — Baker Analyzed (J.T. Baker Co.), heated at 600 °C overnight. Stored in 130 °C. |

### *Procedure*

The procedure that was used in this work, was a modification of the method employed by ESTER *et al.* (1977).

Ten grams of honey were weighed into a 250 ml separatory funnel. Fifty ml of a 10/90 water/acetonitrile solution was added and the funnel shaken vigorously until the honey was dissolved. Thirty ml of a 4 % aqueous sodium chloride solution was added and the funnel shaken for 2 min.

The acetonitrile/water solution was partitioned three times with 20 ml hexane by shaking for 2 min. The hexane layers were collected in 100-ml pear-shaped flasks. An emulsion usually formed between the hexane and acetonitrile/water layers, but it was easily broken by drawing off the acetonitrile layer and vigorously shaking the remaining hexane layer. Any samples with emulsion persisting were centrifuged for 5 min at 2,000 rpm.

The combined hexane layers were evaporated at 40 °C in a rotary evaporator to approximately 1 ml.

A 11-mm × 300-mm glass column was dry-packed with 1.5 g florisil and topped with 3 cm sodium sulfate. The column was eluted with 10 ml hexane, and without allowing the top of the column to run dry, the 1 ml sample was applied. The sample was eluted with 25 ml of 5/95 ethyl acetate/hexane. The first 10 ml eluant was discarded. The next 15 ml eluant was collected in a 50-ml

pear-shaped flask and evaporated to dryness with rotary evaporation at 40 °C. Exactly 1.0 ml isooctane was added and the sample transferred to a vial for GLC injection.

A « Varian 3700 » chromatograph equipped with a linearized Ni electron capture detector was used in the instrumental analysis. A 5 µl injection was made through a 4 mm × 3 ft 1.5 % SP-2250 / 1.95 % SP-2401 on 100/120 Supelcoport column (Supelco, INC) with a flow of 85 ml/min nitrogen. The injection port was kept at 220 °C, the column oven at 192 °C and the dectector at 250 °C. Chromatograms were recorded and peaks intergrated with a Vista CDS-401 (Varian). The presence of residue was confirmed only if it emerged on a second column was 10 % OV-101/chrom W HP 80/100, 200 cm × 10.35 mm × 2 mm glass.

## RESULTS AND DISCUSSION

The recovery from various levels of fortification indicate good extraction efficiency and cleanup recovery for malathion residue (table 1). Malathion was stable in isooctane, and chromatographed well. It was not necessary to make repeated injections.

TABL. 1. — *Recovery of malathion for fortified honey* (*)

| ppb added | ppb recovered | % recovery |
|---|---|---|
| 0.100 | 0.096 | 96.0 |
| 0.100 | 0.090 | 90.0 |
| 0.100 | 0.086 | 86.0 |
| 0.100 | 0.084 | 84.0 |
| 0.200 | 0.173 | 81.5 |
| 0.200 | 0.182 | 91.0 |
| 0.200 | 0.174 | 87.0 |
| 0.200 | 0.184 | 92.0 |
| 0.300 | 0.240 | 80.0 |
| 0.300 | 0.272 | 90.5 |
| 0.300 | 0.294 | 98.0 |
| 0.300 | 0.255 | 85.0 |

(*) Standard deviation of recovery percentage 5,51. Coefficient of Variation of recovery percentage 6.23.

Fifty-four samples (88.5 %) contained no detectable malathion residues while the rest contained residues less than 5.00 ppb (table 2) which is far from being hazardous for humans. The acceptable daily intake levels for malathion in food by FAO/WHO is 0.02 mg/kg/day of body weight (human) (MATSUMURA, 1976). Five of the seven samples containing malathion residues were produced

in 1982 and were either honey-dew honey (three samples) or blossom honey (two samples). The sample with 5.00 ppb malathion was collected from a hive where the beekeeper mistakenly used excess malathion and killed the bees. Malathion has attracted considerable, interest by Greek beekeepers because is very effective against *V. jacobsoni* (IFANTIDIS, 1980 ; PELEKASIS *et al.*, 1980) and because it is one of the safest insecticides (KRUEGER and O'BRIEN, 1959). It has been used almost exclusively by the majority of Greek beekeepers for the last 5 years. This research indicates that both the method and the time of application of malathion to control the mites does not cause contamination of honey. This also suggests that a mechanism similar to those that are described by ATKINS (1975) may exist and excludes malathion contaminants from the honey. Futhermore malathion may possibly degrades rapidly in honey.

TABL. 2. — *Malathion residues in Greek Commercial honeys* (*)

| Number of samples | Malathion residues ppb |
|---|---|
| 54 | none |
| 2 | 1.00 - 2.00 |
| 2 | 2.01 - 3.00 |
| 2 | 3.01 - 4.00 |
| 1 | 4.01 - 5.00 |

(*) Limit of detection 0.5 ppb.

Malathion was used against the fowl mite *(Ornithonyssus sylviarum)* (VINCENT *et al.*, 1954) but a marked tolerance was developed by that mite in 1961 (RODRIGUEZ and RIEHL, 1963, NELSON and BERTUM, 1965). It was suggested that insects and mites can develop a similar mechanism of resistance to malathion (NELSON and BERTUN, 1965). The information we have so far indicates that malathion is still effective against *Varroa* but a study should be conducted to investigate whether the mite can develop tolerance toward malathion. In such a case an alternative solution to the problem of controlling the mite must be developed.

*Received for publication in February 1984.*

*Accepted for publication in August 1984.*

## ACKNOWLEDGEMENTS

We are thankful to the Environmental Protection Service of the Greek Ministry of Agriculture for providing the Gas Chromatotraph. We also thank Dr R.O. MUMMA and Dr A.W. BENTON of the Pennsylvania State University for providing some of the reagents and their laboratory to conduct part of this research.

## RÉSUMÉ

### LES RÉSIDUS DE MALATHION DANS LES MIELS GRECS

Durant les 5 dernières années la majorité des apiculteurs grecs a presque uniquement utilisé le malathion pour lutter contre l'acarien *Varroa*. Ce produit a soulevé un très grand intérêt parmi les apiculteurs en raison de sa forte efficacité vis-à-vis de *Varroa jacobsoni* et de sa grande sécurité en tant qu'insecticide. On donne ici les résultats d'une étude destinée à identifier les résidus de malathion dans les miels produits et commercialisés en Grèce.

On a analysé 61 échantillons de miels récoltés dans diverses régions de Grèce. Vingt-trois d'entre eux provenaient du commerce et représentaient 12 firmes différentes, les autres ayant été prélevés directement chez les apiculteurs. Les résidus ont été extraits par une solution aqueuse d'acétonitrite, séparés à l'n-hexane, purifiés avec une colonne florisil (60/100 mesh), évaporés jusqu'à dessication, puis redissouts dans de l'isooctane et analysés en chromatographie gas-liquide avec un détecteur capteur d'électrons. On a récupéré 80,0 à 98,0 % des résidus de malathion présents dans les échantillons enrichis (Tabl. 1).

Cinquante-quatre échantillons ne renfermaient pas de résidus détectables de malathion, tandis que les 7 autres en contenaient 1-5 ppb (Tabl. 2).

## ZUSAMMENFASSUNG

### MALATHION-RÜCKSTÄNDE IM GRIECHISCHEN HONIG

In den letzten 5 Jahren wurde von der Mehrheit der griechischen Imker fast aussschließlich Malathion gegen die *Varroa*-Milbe benutzt. Es hat erhebliches Interesse bei den Imkern hervorgerufen, weil es sehr effektiv gegen die *Varroa*-Milbe wirkt und eines der sichersten Insektizide ist. Diese Studie berichtet über die Ergebnisse einer Untersuchung, die die Malathion-Rückstände des in Griechenland produzierten und vermarkteten Honigs aufzeigt.

Es wurden einundsechzig Honigproben aus verschiedenen Gebieten Griechenlands untersucht. Dreiundzwanzig davon waren aus dem Handel und repräsentierten 12 verschiedene Firmen, während die übrigen direkt von Imkern gesammelt wurden.

Die Rückstände wurden mit einer Acetonitril/Wasser-Lösung extrahiert, mit n-Hexan ausgeschüttelt, mit einer Florisil-Säule (60/100 mesh) gereinigt, zur Trockenheit einrotiert, wieder gelöst in Isooctan und dann mit einem GLC-Chromatographen mit einem Elektronenfang-Detektor identifiziert. Die Ausbeute der Malathion-Rückstände variierte zwischen 80 und 98 % (Tab. 1).

Vierundfünfzig der Proben enthielten keine nachweisbaren Malathion-Rückstände, während die übrigen Rückstände in Höhe von 1-5 ppb enthielten (Tab. 2).

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