Bull. Environ. Contam. Toxicol. (1996) 56:881-887

Bulletin of Environmental Contamination and Toxicology

Acaricide Residue Determination in Honey

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Received: 1 June 1995/Accepted: 1 March 1996

The Varroa mite appeared in Spain in 1985, though it was detected in Galicia (NW Spain) in 1987 for the first time (Asorey 1987). The control of this mite was carried out by the use of different acaricides: amitraz (active component of Taktic) and coumaphos (active component of Perizin) were first used. After 1989, many beekeepers used fluvalinate, the active component of three formulations: Mavrik, Klartan and Apistan (Asorey 1989).

In the past few years, several methods for the gas chromatographic determination of acaricide residues in honey with different extraction procedure and detection system have been repported (Klein et al. 1986; Hemmerling 1987; Thrasyvoulou and Pappas 1988; Lubinevski et al. 1988; Taccheo et al. 1988; Van Rillaer and Beernaert 1989; Stricker et al. 1989; Taccheo et al. 1990; Sancho et al. 1991; Fernández and Simal 1993; García et al. 1994).

The application of these methods to honey samples of different origins suggest that the treatment with the recommended dose during the adequate time, does not necessarily result in acaricide residues in honey. On the other hand, the inadequate treatment of hives with incorrect amount and/or exposure time can result in pesticide contamination in honey.

The objective of this work is to analyse the acaricide residues in honey samples in order to evaluate their level of contamination and so, to prevent toxicological risks that the treatment with the phytosanitary products described above might cause.

MATERIALS AND METHODS

The study was carried out in 221 honey samples of four different years (36 of these 221 samples were produced in 1988, 68 in 1989, 73 in 1990 and 44 in 1991) provided by the local association of beekeepers with guaranteed

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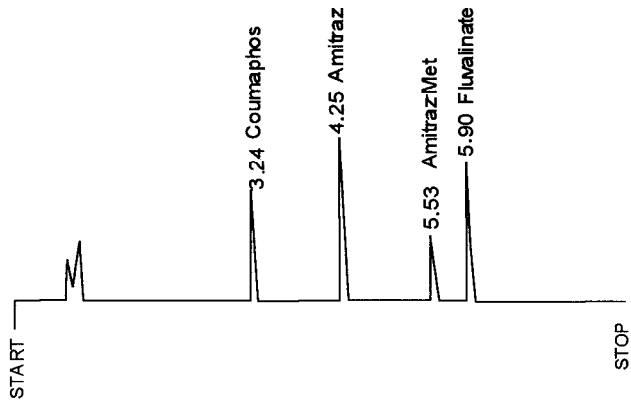

Figure 1. Gas chromatogram test solution of amitraz (6 µg mL^{-1}), coumaphos (150 µg mL^{-1}) and fluvalinate (40 µg mL^{-1}).

origin and made with traditional procedures in the region of production. All samples examined were unpasteurised honeys of random (mixed) floral type. Samples were collected in glass bottles and stored in the dark at 4 °C until analysis.

Pesticide standards of amitraz (Chem Service, 99% purity), coumaphos (Supelco Inc., 99% purity) and fluvalinate (Chem Service, 99% purity) were used. Solutions of amitraz, coumaphos and fluvalinate were prepared in methanol, containing concentrations which varied between 1-25 µg mL^{-1} for amitraz, 100-300 µg mL^{-1} for coumaphos and 10-60 µg mL^{-1} for fluvalinate.

Methanol, n-hexane (Aldrich, grade HPLC), acetonitrile (Panreac, P.S) were used as solvents. Florisil Sep-Pak (waters No 51960) was used as a cleanup procedure. The acaricide extraction was performed on 10 g of honey using a mixture of acetonitrile-water (2:1, v/v) heated at 75 °C. Organic phase was partitioned by adding 10 mL of n-hexane, and concentrated to 2 mL. Concentrated extract was purified through a Florisil Sep-Pak cartridge and eluted with n-hexane and finally with methanol. These extracts were analysed by Capillary Gas-Liquid Chromatography and nitrogen-phosphorus detection.

The apparatus was a Hewlett-Packard 5890A with nitrogen-phosphorus detector equipped with a capillary column HP-101 containing methylsilicone fluid as non polar stationary phase (12 m lenght, 0,2 mm i.d. and 0,2 mm film tickness) and splitless injection technique.

The operating conditions for chromatographic analysis of the considered compounds were as follows. Carrier gas: nitrogen (N-50,); split flow, 75 mL min^{-1}; purge flow, 1.05 mL min^{-1}; column flow, 1.4 mL min^{-1}; make-up flow, 36 mL min^{-1}; hidrogen flow, 3,5 mL min^{-1}; air flow, 100 mL min^{-1}; head column pressure, 9 psi; ratio split column, 53; initial temperature column, 60 °C (1

min); final temperature, 240 °C (1 min); ramp, 30 °C min^{-1}; injection port temperature, 200 °C; detector temperature, 250 °C; injection volume, 2 µL.

RESULTS AND DISCUSSION

Amitraz, coumaphos and fluvalinate were identified by comparison of their retention times to the standards. Figure 1 shows the chromatogram obtained when injecting 2 µL of the standard solutions of amitraz (6 µg mL^{-1}), coumaphos (150 µg mL^{-1}), and fluvalinate (40 µg mL^{-1}) in methanol. The presence of residues was confirmed by a column HP-5 (25 m lenght x 0.2 mm i.d. x 0.33 mm) of different polarity.

Recoveries were established by adding increasing amounts of considered acaricides. Table 1 shows the recovery data of products added to honey samples. These tests (repeated three times) were performed in two different concentration levels for each acaricide. Solid phase extraction recoveries have been 95%, 94% and 98% for honey samples additions of 10 µg g^{-1}, 325 µg g^{-1} and 40 µg g^{-1} of amitraz, coumaphos and fluvalinate respectively. Reproducibility of the GC response for five injections was 100 ± 5.6 for amitraz, 100 ± 5.5 for coumaphos and 100 ± 3.5 for fluvalinate.

Table 1. Recovery of amitraz, coumaphos and fluvalinate added to honey samples with florisil Sep-Pak

	µg amitraz in 2 µL injection	µg coumaphos in 2 µL injection	µg fluvalinate in 2 µL injection
Sample	Added Recovery	Added Recovery	Added Recovery
1	0.02 0.018 (90%)	0.13 0.12 (92%)	0.045 0.036 (81%)
2	0.02 0.017 (85%)	0.13 0.11 (85%)	0.045 0.037 (82%)
3	0.02 0.017 (85%)	0.13 0.11 (85%)	0.045 0.036 (81%)
	$\bar{X}$ = 87% ± 3.0	$\bar{X}$ = 87% ± 4.0	$\bar{X}$ = 81% ± 0.7
4	0.05 0.048 (96%)	0.65 0.61 (94%)	0.080 0.079 (99%)
5	0.05 0.047 (94%)	0.65 0.60 (93%)	0.080 0.078 (97%)
6	0.05 0.048 (96%)	0.65 0.61 (94%)	0.080 0.078 (97%)
	$\bar{X}$ = 95% ± 1.0	$\bar{X}$ = 94% ± 0.6	$\bar{X}$ = 98% ± 1.0

An external standard method was used to determinate the linearity of response of the NPD, to calculate the percentages of the acaricides recovered and to evaluate the level of the residues in honey samples.

The minimun detectable levels (MDL) (signal/noise=2) for the acaricides were

the following: amitraz, 20 pg, coumaphos, 1.5 ng and fluvalinate, 400 pg. The limit of quantification was 2.6 ng for amitraz, 200 ng for coumaphos and 18 ng for fluvalinate. The described method is rapid and accurate, being readily used in routine analysis.

The analysis of 221 honey samples corresponding to several years (1988-1991) were performed according to the described method. 138 (66%) of all honey samples considered do not present acaricide residues. As for the results per year, it was observed that 28 honey samples (77%) from 1988 were not contaminated. In the year 1989, 48 honey samples (83%) do not present any acaricide residue. While in 1990 and 1991, the uncontaminated samples were 39 (55%) and 16 (45%) respectively.

Table 2. Acaricide Residue Levels.

Acaricide	No.apppearances	Mean	Maximun	Minimum	SD
Amitraz (ng kg^{-1})	19	420	1820	33	413
Coumaphos (µg kg^{-1})	32	6	53	1	9
Fluvalinate (µg kg^{-1})	39	2	15	1	7

Table 3. Number of appearances of acaricides in 221 honey samples from 1988, 1989, 1990 and 1991.

Acaricide	No. appearances	1988	1989	1990	1991
Amitraz	19	8	6	4	1
Coumaphos	32	0	14	10	8
Fluvalinate	39	0	0	20	19

These values were confirmed with the data obtained in a public-opinion poll to 456 beekeepers. The poll showed that 317 of them (70%) utilized acaricides. Fluvalinate was the most used. 5% of the beekeepers utilized other acaricides (Asorey, 1989). This fact matched our results, in the contaminated samples, 60% of them corresponding to fluvalinate, 12% to coumaphos and 12% to amitraz.

The levels of acaricide residues were low, as show in Tables 2 and 3. In the case of amitraz, the mean value was 420 ppt (ng kg^{-1}), 6 ppb (µg kg^{-1}) for coumaphos and 2 ppb (µg kg^{-1}) for fluvalinate. There were two exceptions with a high concentration of fluvalinate (15 µg kg^{-1}) and coumaphos (53 µg kg^{-1}). The high concentration of fluvalinate was due to a long-time exposure of Apistan in the hives. In the case of coumaphos, the doses employed at the time of honey collecting were higher than those recommended.

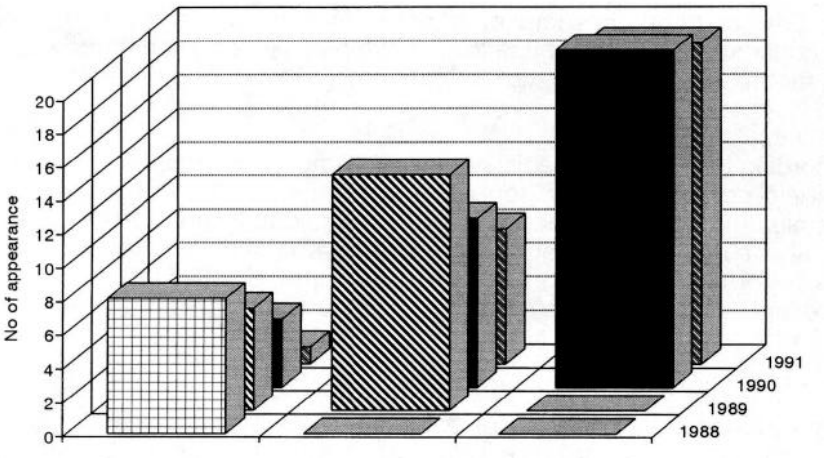

Figure 2. Frequency of the acaricides.

The amitraz concentrations detected were lower than other acaricides. These values were measured in ng kg^{-1} (ppt), as well the limit of quantification. These results are influenced by the instability of the amitraz at pH <7 (honey has a pH that varied between 3.9 and 4.2). Under these conditions amitraz produces metabolites with retention times of 4.24; 5.27 and 5.60 minutes. In this case, only the latter was detected.

The method of treatment had an influence in the acaricide levels found. Amitraz (Taktic), coumaphos (Perizin) and fluvalinate (Mavrik) are applied spraying bees with product solutions. Fluvalinate (Apistan), which is the most commonly used product today, is applied by contact, introducing three strips of the product into hives.

The values of the fluvalinate detected were very low if we compare them to the values obtained in other studies carried out in Spanish honeys (Sancho et al. 1991) or in German honeys (Van Rillaer and Bernaert 1989). In Figure 2 it can be seen that the frequency of amitraz is low and it decreases each year. The use of amitraz is being replaced by more active products (Ducos de Lahitte 1987) like coumaphos and lately fluvalinate, still the alternative use of two or three products to avoid the resistence of the mite to the acaricides es frequently recommended.

It must be pointed out that the maximum residue levels (MRL) in honey have not been established either by CEE (76/895/CEE; 86/362/CEE and 86/363/CEE) or by Spanish legislation (BOE 21/3/87 and BOE 9/5/90). The acceptable daily intake levels (IDA) for amitraz by FAO/WHO is 0.003 mg kg^{-1} of body weight (FAO/OMS 1986) being 0.0005 mg kg^{-1} in 1983. The acceptable daily intake levels (IDA) for coumaphos by FAO/OMS is 0.0005 mg kg^{-1} of body weight (FAO/OMS 1987). This level is being revised.

The daily average consumption of honey is rarely higher than 50 g and so, the concentration levels of acaricide residues detected in our honey samples are far too low to cause toxicity in humans (70 kg of body weight).

According to the present legislation, and taking into account the low levels of acaricide detected in the honey samples studied, it can be said that the samples did not overcome the maximum residue levels (MRL), except the two above mentioned samples. This and several of our studies (García et al. 1992, Fernández and García, 1993, García et al. 1994) demonstrate the excellent quality of honeys from Lugo and the appropriate treatments applied to control Varroa mite.

Acknowledgments. The authors thank the Asociación Lucense de Apicultura for providing honey samples. This work was partly financed by the fund for scientific research of the Instituto Lucense de Desarrollo (INLUDES), Lugo, Spain.

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