ACARICIDE RESIDUES IN BEESWAX AND HONEY

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Summary

We studied the contamination level of the acaricides Folbex VA (bromopropylate, BP), Perizin (coumaphos, CM) Apistan (fluvalinate, FV) and Bayvarol (flumethrine, FM) in brood combs, sugar feed, honey and in new beeswax. All samples were analysed by gas chromatography with ECD detection.

After one normal acaricide treatment, in autumn, the brood comb wax was contaminated by all these acaraicides with residues ranging from 0.03 to 48 mg/kg. The degree of contamination decreases in the following order: BP > FV ≈ CM >> FM.

The amount of these residues increases with the increase in the number or the duration of the treatments. The residues in the sugar feed were much smaller than in the corresponding brood comb wax and varied from 0.004 to 0.04 mg/kg. The residues in honey were all beyond the tolerance levels and varied from 0.003 to 0.015 mg/kg. The degree of contamination decreases in the following order: BP ≈ CM > FV. In a model study, we examined the behaviour of acaricide-contaminated old comb wax when melted into new beeswax.

The acaricide concentration in the new recycled was wax on average 1.7 times higher than in the old combs, regardless of the processing conditions (longer boiling times and higher temperatures). In 1995, the commercial samples in Switzerland contained 1 mg/kg CM, 2.5 mg/kg FV, 3.8 mg/kg BP and ≤ 0.25 mg/kg FM.

Keywords*: residue, acaricide, beeswax, comb, feed, honey, fluvalinate, bromproplylate, coumaphos, flumethrine.*

Introduction

The acaricides Folbex VA (active ingredient: bromopropylate), Perizin (coumaphos), Apistan (fluvalinate) and Bayvarol (flumethrine) are used in Switzerland for Varroa control in autumn, after the flow season. These acaricides are used world-wide and there are several reports dealing with their residues in honey and beeswax (KLEIN et al., 1986; THRASYVOULOU and PAPPAS 1988; LODESANI et al., 1992; HANSEN and PETERSEN 1988; WALLNER, 1995). These authors report that, due to their non-polar nature, acaricides mostly contaminate beeswax, while honey remains relatively free of contaminants. Most investigators have not examined the long-term effects of these acaricides in the contamination of honey and beeswax. As these acaricides are used for a long-term Varroa control, it is important to study the acaricide level in honey and beeswax after repeated use of the varroacides.

In earlier works, we published preliminary data on the residues after Folbex VA and Apistan treatments (BOGDANOV et al., 1990a, b). In the present study, we summarized all our studies on the contamination level of all four acaricides in brood and honey combs, in sugar feed, in honey and in new recycled beeswax. We tried to answer the following questions:

1. What is the acaricide level in honey and in beeswax after one normal treatment and after repeated or permanent varroacide treatments?
2. What is the behaviour of the acaricides when old combs are recycled into new beeswax?
3. What is the long-term level of the acaricides in Swiss beeswax?

Materials and Methods

Materials

All reagents were of analytical purity grade.

We used C-18 SPE (Solid Phase Extraction) Baker 7020 06 disposable columns, mounted on a Baker-10 SPE extraction manifold with vacuum.

Honey, sugar feed and comb samples

All the samples were extracted after specific acaricide treatments, as indicated below.

Sugar feed and combs: From each comb, also containing sugar feed, about 10 cm^2 were scratched with a spoon down to the foundations. The sugar feed was separated from the comb by sieving. The sugar feed was analysed by using the same method as in the case of honey.

Commercial Swiss beeswax

Representative samples from all the major Swiss beeswax producers were made in every production year (BOGDANOV and KILCHENMANN, 1993).

Methods

Since 1991, our laboratory has been certified with the EN 45001 European norm and works under quality assurance conditions. This guarantees the reproducibility of the residues measurements in bee products during our long-term studies.

Extraction of honey acaricides

10 g of honey were dissolved in 15 ml of ethanol-water 2:3.

The Baker C-18 columns were activated with one volume of ethanol, followed by one volume of water. The honey solution was then passed under constant vacuum. The column was dried for half an hour and then, it was eluted with 2 ml of ethyl acetate and 2 ml of hexane. The solvent is evaporated under vacuum and the residue is dissolved in 2 ml of isooctane, thus being prepared for analysis.

Extraction of wax acaricides

The extraction of bromopropylate, coumaphos and fluvalinate from wax was essentially as described below (ZIMMERMANN et al., 1993):

Extract 1 g of sample with 10 ml hexane, eliminate high molecular compounds by repeated freezing and centrifugation, purify the florisil columns, elute with petrolether-hexane (1:1); evaporate to dryness and redissolve in 2 ml of isooctane, then analyse.

Determination by gas chromatography

Inject 1 µl honey or wax extract by means of an autosampler on-column in 30 m DB 1 (wax analysis) or 30 m DB5 (honey analysis), both J+W, 0.25 mm id, 0.25 µm film thickness. The GC analysis was done either with a Carlo Erba MEGA Series or with a Hewlett Packard 5890 chromatograph, both equipped with an ECD detector.

Quantification was done by the external standard method.

The recoveries were between 80 and 100% and the detection limits were 0.003 mg/kg in honey and 0.25 mg/kg in wax for all the acaricides.

Flumethrin analysis by HPLC of selected wax comb samples was done by Bayer, Leverkusen, Germany.

Acaricide treatments

All treatments were carried out in autumn, after the sugar feeding of the bee colonies. Generally, beekeepers in Switzerland renew their combs every spring with 2-3 foundations per hive.

Folbex VA: One treatment included 4 strips per year, in “Swiss bee hives”. One strip contains 0.4 g bromopropylate. Samples were taken from apiaries to which 1 to 5 treatments were applied. Every year, one Folbex treatment per apiary was effected.

Apistan: The treatment was carried out in Dadant hives, with 3 Apistan strips, for 4 weeks. One Apistan strip contains 900 mg of fluvalinate. We made a special experiment with 2 bee colonies. The control colony was treated only once during the 4 weeks and then the strips were taken away. In the other hive, the strips remained for 13 months (permanent treatment).

Perizin: The treatments were carried out in “Swiss bee hives”. For one treatment, we used 50 ml Perizin, containing 32 mg coumaphos. Samples were taken from hives in which 1 to 5 treatments were effected. Every year, 1-2 treatments per apiary were effected.

Bayvarol: Treatments were carried out with 4 strips per hive, during 4 weeks. One strip contains 3.6 mg flumethrine. The samples were taken from apiaries to which two treatments were applied.

Table 1

Data on the Four Acaricides Used in This Study

Acaricide	In use since	Active ingredient (a.i.)	mg a.i. per treatment	Time of treatment
Folbex VA	1982	bromopropylate	1600	autumn
Perizin	1987	coumaphos	32	Autumn, winter
Apistan*	1991	fluvalinate	1600*	August, September
Bayvarol*	1991	flumethrine	14.4*	August, September

** only a small part pf the active ingredient is released during the treatment*

Results and Discussion

Residues in Brood Comb Wax

Folbex VA

Fig. 1 shows the accumulation of Folbex residues in the brood comb wax, depending on the number of Folbex treatments. The values in the diagram represent the combined residues of bromopropylate (BP) and of its metabolite, dibromobenzophenone (BBP). A part of the bromopropylate (about 20%), disintegrates into dibromobenzophenone during the burning of the Folbex strips for the treatment. The concentration ratio (in wax) between bromopropylate and its metabolite, was about 5:1 and remained constant for all the wax samples. There is a significant correlation (p=0.025) between the number of Folbex treatments and the amount of residues found. The residue level was 2 to 5 times lower if 3-4 foundations per colony and year were replaced (BOGDANOV et al., 1990b).

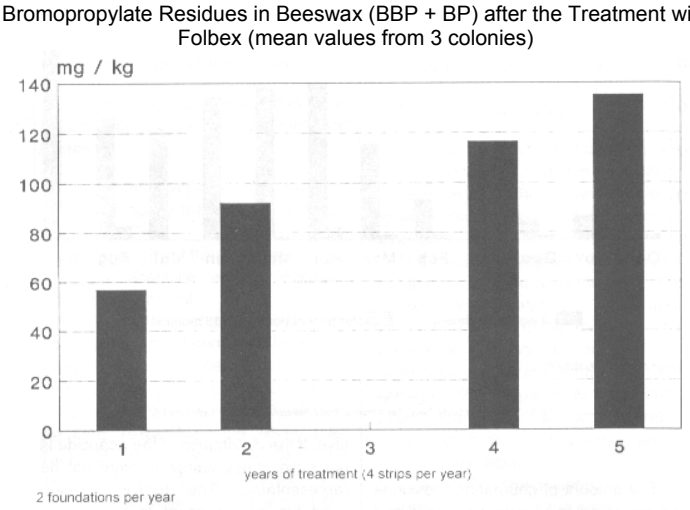

Fig. 1 – Folbex VA Residues in Brood Comb Wax after Repeated Treatments

Apistan

In Fig. 2, the fluvalinate residues in wax, after one treatment (control) and after a permanent Apistan treatment, are shown. The residue values in the comb of the control hive varied between 0.2 and 7.3 mg/kg, with an average of 1.9 mg/kg. When the same Apistan strips are kept in the colony all the time, the amount of fluvalinate residues increases with the duration of exposure, to reach a constant level of about 40-60 mg/kg at the end of the experiment. If the strips are left in the colony for one more year, the residues stay at the same level (results not shown). Propolis samples taken from the colony with the permanent Apistan treatment had the same fluvalinate level as the comb wax.

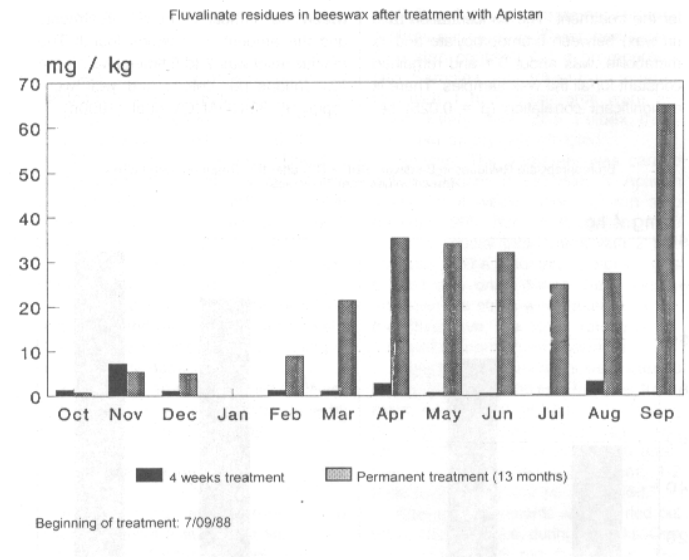

Fig. 2 – Residues in Comb Wax after a Normal and a Permanent Apistan Treatment

Perizin

The amount of coumaphos residues did not show any tendency to increase with the increasing number of Perizin treatments (Table 2). An explanation of this behaviour might be the sampling problem. We extract the comb wax randomly, as pieces from each comb of the hive. If the distribution of the acaricide is not even, these samples might not be representative. The Perizin solution used for the treatment is poured between the combs and, possibly, it cannot be evenly distributed among the brood combs. The residues resulted after the Folbex and Apistan treatments seem to be more evenly distributed.

Table 2

Coumaphos Residues (mg/kg) in Brood Comb Wax after repeated Perizin Treatments

Number of treatments	1	2	5
Average	3.8	7.4	5.8
Minimum-maximum	0.4-11.9	1.6-14.2	2.2-13.8
n	10	4	4

Bayvarol

We have the data of only one study. After two normal treatments, the amount of residues in the brood combs was on average 0.051, with a minimum of 0.026 (= analytical detection limit) and a maximum of 0.176. The residues level is lower by a factor of 40 than after the Apistan treatment (see above).

Distribution of Acaricides between Wax Combs, Sugar Feed and Honey

The contamination level in the wax and in the honey theoretically depends upon two factors:

a) lipophilicity of the acaricide;
b) the acaricide amount released during the treatment.

The lipophilic character of each acaricide can be determined by establishing the ratio between its concentration in the brood comb, respectively the honey comb, and its concentration in the feed, respectively the honey (see Table 3). The greater the ratio, the more lipophylic the substance. The lipophilic character of the acaricide decreases in the following order:

fluvalinate > bromopropylate > coumaphos

The more lipophilic the substance, the more the wax is contaminated and the less the sugar feed and the honey are.

Table 3 shows the residues in brood combs, honey combs, sugar feed and honey after acaricide treatments.

The acaricide levels found in these products decrease in the following order:

brood comb wax > honey comb wax >> sugar feed ≥ honey

Table 3

Acaricide Residue Levels (mg/kg) in Brood Comb Wax, Honey Comb Wax, Sugar Feed and Honey

Product	Bromopropylate		Fluvalinate		Coumaphos	
Number of treatments	1	3	1	*	1	2
Brood comb wax	47.8	116.7	2.9	24.8	3.8	7.4
Honey comb wax	2.4		≤0.01	14.0	0.7	0.4
Sugar feed	0.03	0.07	≤0.003	≤0.003	0.013	0.010
Honey	0.01	0.02	≤0.003	≤0.003	0.015	0.004
Honey tolerance limit	0.1		0.01		0.05	
Ratio brood comb wax/sugar feed	1600	1670		8300	290	740
Ratio honey comb wax/honey	240			4670	54	100

** the hive was treated for 11 months with Apistan. (for details see Methods and text)*

Wax

The amount of residues in the brood comb wax is larger than that in the honey comb wax in the case of all the acaricides used. The contamination level of both brood comb and honey comb wax after one normal treatment decreases in the following order:

bromopropylate > fluvalinate ≈ coumaphos >> flumethrin

Sugar feed and honey

The acaricide concentration in the sugar feed and in honey is a result of the equilibrium of the active ingredients between the sugar and the wax compartments.

The more hydrophilic the product and the more of the active ingredient is released, the greater the sugar feed and the honey contamination. The amount of acaricide residues in the sugar feed and in the honey is on the average 2,500, respectively 1,700 times smaller than that in the brood combs, respectively in the honey combs.

The contamination of honey and of sugar feed after one acaricide treatment decreases in the following order:

bromoprophylate ≈ coumaphos > fluvalinate

Although much more bromopropylate is released into the colony after one treatment than coumaphos (a factor of 50), the honey residues after treatments with either acaricides are similar, because coumaphos is much less lipophilic.

These results show that the acaricide level in honey did not reach values above the tolerance limits, even after several years of treatment (see Table 3).

The danger of honey contamination, and of the residues level exceeding the tolerance limit is greater after the treatment with Perizin, because this acaricide has the weakest lipophilic character.

We did not measure the amount of Bayvarol residues in honey, but according to the experience of Bayer, the flumethrine levels in honey, after the Bayvarol treatments, are below our detection limit of 0.003 mg/kg.

Behaviour of the Acaricides during the Production of New Beeswax

Brood comb wax was sprayed with 20 mg/kg of each acaricide and, then, was melted under different laboratory conditions (see table 4). The recovery of new wax, regardless of the processing conditions, was of 25%. The results are sumarised in Table 4. The acaricide concentration in the new recycled wax was, on the average, 1.7 times higher than that in the old combs. Boiling for a longer period of time and at higher temperatures (autoclave) had no effect on the acaricide concentration. The increase of the acaricide amount into the new beeswax might be explained by the better solubility of the substances in the wax matrix than in the comb debris.

Table 4

Acaricide Residues after Recycling Old Comb Wax.
(Combs were sprayed with 20 mg/kg acaricide and then melted under different conditions.
The results are averages of a duplicate.)

	Bromopropylate (mg/kg)	Coumaphos (mg/kg)	Fluvalinate (mg/kg)	Flumethrine (mg/kg)
Combs before recycling	19.6	14.8	17.0	20.3
1 hour boiling	36.0	28.9	26.9	34.8
3 hours boiling	34.6	27.8	26.5	33.4
1 hour autoclave (140 ^{O}C)	34.8	27.5	27.1	34.4
2 hours autoclave (140 ^{O}C)	34.0	27.9	24.3	31.2
Enrichment factor wax/comb	1.8	1.9	1.6	1.6

Acaricide Level in Commercial Beeswax

In Fig. 3, the residue level in Swiss beeswax for 5 consecutive years is given. The amount of bromopropylate residues is slowly decreasing, because Folbex VA no longer used. If one extrapolated the decline curve for bromopropylate from Figure 3, a half life of about 7 years will result. This means, that it will take approximately 16 years until the residues reach the detection limit of 0.25 mg/kg. On the other hand, the amount of fluvalinate residues rose rapidly after the year of registration 1991 and reached a maximum of about 2.5 mg/kg in 1994 and 1995. The coumaphos residues amount remained relatively constant, at a level of about 1.2 mg/kg, as Perizin is rarely used in Switzerland. No flumethrine above the detection limit of 0.25 mg/kg was detected. The contamination level of this acaricide in the combs is very low (see section *Residues in Brood Comb Wax*) and it will take some time for this substance to increase above the detection limit.

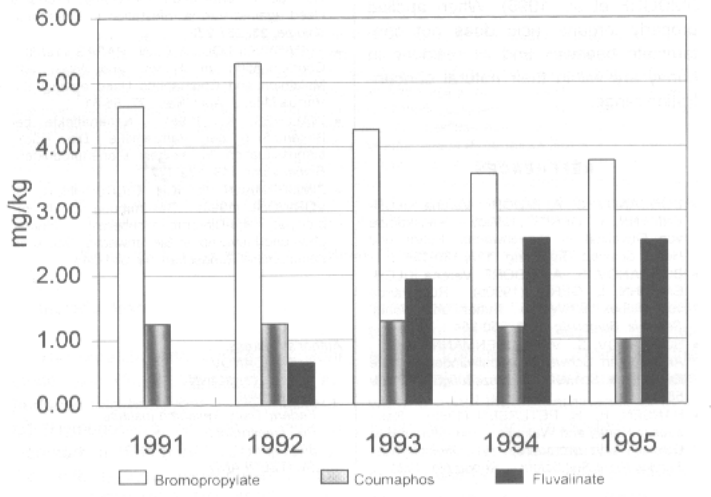

Fig. 3 – Acaricide Residues in Swiss Beeswax

Conclusions

If the residues levels did not exceed the tolerance limit during the time of our studies, they might do this if the acaricides are used for longer periods of time. All the acaricides examined contaminate beeswax to a much higher degree than they contaminate honey, in a dose- and time-dependent manner. Therefore, the disappearance of the acaricide residues, when the respective acaricides are no longer in use, is extremely slow. This means that significant acaricide residues amounts will remain for a long time in wax. As there are no tolerance limits for acaricides in wax, there are no legal means for the control of these residues. Therefore, it is imperative that such limits be established. While the individual acaricides do not have a toxic effect on bees, long-term and side effects cannot be excluded. Also, the constant presence of sublethal acaricide quantities favours the selection of acaricide-resistant mites. Because of the problems stated above, in Switzerland, we favour the use of organic acids for Varroa control (IMDORF et al., 1996). When applied properly, organic acid does not contaminate beeswax and its residues in honey are within their natural concentration range.

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