Acaricide residues in honey, beeswax and propolis

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In Switzerland the acaricides Folbex VA (bromopropylate, BP), Perizin (coumaphos, CM), Apistan (fluvalinate, FV) Bayvarol (flumethrine, FM) are used for varroa control. We studied the contamination level of BP,CM and FV in brood and honey combs, sugar feed, honey after field trials. In samples of recycled pure beeswax and propolis, gathered by beekeepers we examined the level of all four acaricides. All samples were analysed by gas chromatography with ECD detection.

INTRODUCTION

The acaricides Folbex VA (bromopropylate), Perizin (coumaphos), Apistan (fluvalinate) and Bayvarol (flumethrine) are used world-wide for varroa control. In Switzerland in 1982 bromopropylate was the first homologated acaricide and by 1991 all acaricides were used for varroa control (see table 1). The active ingredients of the acaricides are non-polar and contaminate mostly the beeswax, while the residues in honey are very small (see Discussion). Only few publications examined the residue levels in bee products after a known number of acaricide treatments, mostly after one or two normal treatments (Lodesani et al. 1992; Hansen and Petersen, 1988 Thrasyvoulou and Pappas, 1988). Similar residue levels in honey and wax to the ones found in this study under comparable conditions were measured for bromopropylate (Hansen and Petersen, 1988, Lodesani et al., 1992), fluvalinate (Lodesani et al. 1992) and coumaphos (Thrasyvoulou and Pappas, 1988). In other studies the acaricide levels in wax and honey of market samples with unknown history were examined (Klein and Weber, 1986; Wallner, 1995; Effler, 1993; De Greef et al., 1993). In all studies quoted above the acaricide levels in wax and in combs were much higher than in honey. Most investigators have not examined the long-term contamination effects of these acaricides on honey and beeswax. Presently long term varroa control is necessary, so that the acaricide level in honey and beeswax should be examined after repeated or longer varroacide use.

Some preliminary results on the residues in wax and honey after trials with Folbex VA and Apistan have been published (Bogdanov, 1990 a and b). In the present study we report on the contamination level on all main acaricides used in Switzerland in the sugar feed, honey, brood and honey combs and propolis after longer or repeated use of the acaricides. Also, we report on the regular follow-up of the residues in recycled beeswax after a long-term use of the acaricides.

The results, presented here are reported in full detail elsewhere (Bogdanov et al. 1998).

MATERIALS AND METHODS

The materials and methods used in this study are described in detail elsewhere (Bogdanov et al. 1998).

RESULTS

Field studies

Folbex VA: In table 3 the Folbex residues in brood comb wax, feed and honey after repeated Folbex treatments are summarized. The ratio between bromopropylate (BP) and its disintegration product dibromobenzophenone (BBP) in feed and honey was 2:1 and did not change with increasing number of treatments. The BP and the BBP residues in both honey and feed remained

well below the Swiss MRL (0.1 mg/kg for each substance) and there was no significant increase of the residues with increasing number of Folbex treatments. The Folbex residues (BP and BBP together) were not significantly different from those in the feed.

The BP residues in the combs were 1900 to 3650 greater than in the corresponding feed. There was a significant correlation ($p=0.025$, $r = 0.96$) between the residue level in the brood comb wax and the number of Folbex treatments. After one normal treatment the residues in honey combs where on the average 12 times smaller than in the brood combs.

Fluvalinate In table 4 the residues of fluvalinate in combs, bee feed and honey after one treatment (control) and after a permanent Apistan treatment are shown. The residue values in the brood comb of the control varied between 0.2 and 7.3 mg/kg during the 15 months following the treatment with an average of 1.9 mg/kg. If Apistan strips were permanently present in the colony the fluvalinate residues increase with the duration of the strip exposition and reach a plateau of about 40 to 60 mg/kg after 6 months. The residue level in the honey combs of the same colony was on the average 5 times lower than in the brood combs. Two propolis samples were taken at the end of the treatment period from the colonies with permanent Apistan treatment. They contained 13.6 and 38.0 mg/kg fluvalinate.

The fluvalinate levels in the feed and in honey were mostly below the detection limit of 0.003 mg/kg. Fluvalinate is the most lipophilic of the tested acaricides, the ratio between brood comb wax and feed varying between 1825 and 10000. The ratio is probably even higher, because the residues in half of the feed samples were below the detection limit.

Perizin The coumaphos residues in brood comb wax, feed and honey do not show an increasing tendency with increasing number of Perizin treatments (table 5). The residues in brood combs were by a factor of 300-2000 higher than in feed and honey of the corresponding colonies. The residues, measured in honey combs were slightly above the detection limit and were ten times lower than in the brood comb wax of the same colonies.

The residue level in the feed was not significantly different from that of the corresponding honey samples and remained below the MRL.

Comparison of contamination levels of the different acaricides

The acaricide levels, found in the different products after treatment with the different acaricides, decrease in the following order:

brood combs > honey combs >> sugar feed ≥ honey.

These results indicate that all acaricides are very lipophilic. The lipophilicity of each acaricide can be determined by calculating the ratio between its concentration in the brood comb and in the feed (tables 3, 4 and 5). The higher the ratio, the more lipophilic the substance. The lipophilic character of the acaricide decreased in the following order:

fluvalinate > bromopropylate > coumaphos

The contamination level of the brood combs, found in our study, is:

bromopropylate > coumaphos, fluvalinate

The contamination level found in the brood combs is thus directly proportional to the amount of the active ingredient, released during the treatment (see table 1).

The contamination of honey and feed after the various acaricide treatments decrease in the following order:

bromopropylate ≥ coumaphos > fluvalinate

Although much more bromopropylate than coumaphos is released into the colony after one treatment (see table 1), the residues in feed and honey after treatments with the two acaricides are similar, because coumaphos is much less lipophilic.

The acaricide levels in feed and honey lied well below the Swiss MRL for honey.

Residues after melting of combs under laboratory conditions

The results are summarized in table 6. The acaricide concentration in the new recycled wax was on the average 1.7 times higher than in the old combs. Boiling for a longer period of time and at higher temperatures (autoclave) had no effect on the acaricide concentration.

Acaricide level in commercial beeswax

In fig.1 the residue level in Swiss commercial wax since 1991 is given. After the registration of Apistran and Bayvarol in 1991 Folbex was practically no more used for varroa control. As a result the residue level of bromopropylate is slowly declining from levels between 4.3 and 5.3 between 1991 and 1993 to reach a level of 2.4 mg/kg in 1996. The coumaphos residues remained fairly constant at a level between 1 and 1.3 until 1995 and diminished to 0.7 mg/kg in 1996. After the registration of Apistan in 1991 the fluvalinate residues rose rapidly during the following two years of use , to reach a plateau of 2.6-2.9 between 1994 and 1996. No flumethrine above the detection limit of 0.25 mg/kg was detected.

Propolis

The residues found in the propolis, sent to us by 27 beekeepers are summarized in table 7. All samples contained residues of at least one of the acaricides and all but one sample contained fluvalinate. The fluvalinate residues are 3.4 times higher, the bromopropylate ones two times lower than the corresponding residues found in the commercial wax of the same year (1996, see fig.1). Only two samples contained flumethrine (3.7 and 1.3 mg/kg), while the other ones did not contain residues higher than the detection limit of 0.4 mg/kg.

DISCUSSION

Our results with Folbex VA and Apistan suggest that the longer and the more frequent the treatment, the greater the residues in the combs. The lack of correlation between the number of Perizin treatments and the residue level in brood comb wax might be explained by the uneven distribution of the active ingredient than by possible coumaphos degradation in wax. Coumaphos, as well as the other acaricides are stable in wax (see Methods and Gajduskova et al, 1990). On the other hand the Perizin solution used for treatment is poured between the combs and is very probably not well distributed. By sampling small pieces of each comb we do not get samples, where the residues are evenly distributed. Indeed, the residues after each treatment are highly variable (table 5).

The honey acaricide amounts, found in our studies, as well as in all studies quoted above were below the Swiss acaricide MRL values (table 1). In Germany and Italy the MRL for coumaphos is 0.01 mg/kg. In that case some of the honey samples, examined in our study will not conform to

these quality standards. In an examination of German honeys 20 % of the examined samples had coumaphos residues, which were higher than this MRL value (Effler,1993).

The relatively low contamination of honey is due to the highly lipophilic character of the acaricides. Wallner (1995) found in laboratory model experiments the following lipophilicity order of the acaricides, tested in this study:

flumethrine > fluvalinate > bromopropylate > coumaphos

Our „in vivo“ results confirm this order, with the exception of flumethrine, where we did not conduct a study. According to Wallner (1995) flumethrine is 5 to 10 times more lipophilic than fluvalinate. The expected residue level of flumethrine in honey would therefore be about 50 to 100 times lower than of fluvalinate, i.e. far below the present detection limit of 0.003 mg/kg. The very low fluvalinate residues found in honey might be explained by its extremely lipophilic character. However, another explanation for the very low, mostly undetectable fluvalinate residues might be a possible fluvalinate degradation in honey during the Apistan treatment or during the honey storage. Indeed, such a degradation has been reported (Balayanis and Santis, 1992, Jimenez and Atienza, 1995) but since then in other reports (Sandoz report 1993, Tsigouri et al., 1997) no

degradation after storage was found in fluvalinate-spiked honey. The stability of coumaphos in honey is also controversial. While Thrasyvoulou and Pappas (1988) found a dissipation of coumaphos during the honey storage, other workers reported, that it is stable in honey (Gajduskova et al, 1990; Taccheo Barbina et. al., 1989).

The results of the model recycling experiment show that the active ingredients of the acaricides are stable in wax and do not change during the process of comb recycling. The comb and wax residues of the bromopropylate decomposition product dibromobenzophenone originate from the burning of Folbex VA during the treatment (Formica, 1984) are by a factor of 5 smaller than those of bromopropylate (Hansen and Petersen, 1988, Bogdanov et al., 1990 b). The results of our model wax-recycling experiment indicate that the acaricides concentration does not decrease in the process of producing new wax from old combs and that it is trapped by the new wax. The finding that the acaricide concentration in new beeswax is higher than in old combs can be explained by the enrichment of acaricides in new beeswax. It is probable, that the lipophilic acaricides have a better solubility in wax than in the debris, left after the melting of the combs. However, the results of this model recycling cannot be quite compared to the production of beeswax by beeswax manufacturers. Under practical conditions wax is recycled from different types of comb material: brood- and honey combs and cappings. There the recovery of new wax is generally higher - about 30-50%. A major part of this new wax is derived from honey combs and cappings, containing wax that is less contaminated that the brood combs. This explains, why the residues, that we found in the brood combs are generally higher, than those found in the commercial beeswax.

Our long term studies in Swiss beeswax show, that contamination of recycled beeswax after a certain acaricide starts to be intensively used (e.g. Apistan, fig.1) is rather fast. On the other hand, if an acaricide is no longer used it takes a long time for the residues to disappear. Folbex was practically stopped being used after 1991. If the disappearance rate on fig.1 of the bromopropylate residues is extrapolated, BP will theoretically sink below the limit of detection 12 years after.

The acaricide levels in beeswax, found in German and Austrian beeswax are similar (Wallner, 1995). In Germany, where Folbex has been used for a longer period of time, the bromopropylate residue level in commercial beeswax is higher, reaching in some cases 50 to 100 mg/kg (Wallner, 1995). In our new wax samples we did not find flumethrine above the detection limit of 0.20 mg/kg. In Austria, where Bayvarol has been used for a longer period the residues were higher: between 1 and 10 mg/kg (Wallner, 1995). The flumethrine residues in brood combs after two Bayvarol normal treatments were: average 0.051, minimum 0.026 (= analytical detection limit) and maximum 0.176 (Krieger, personal communication, 1994). This very low level is explained by the small amount of active ingredient, which is released during the acaricide treatment (table 2).

Our results show that propolis samples, gathered during 1996 in apiaries treated with different acaricides in different years, has an average fluvalinate content of 10.5 mg/kg, which is 6.2 times higher than the fluvalinate level of the commercial wax of the same production year. Wallner (1995, 1997) found, that propolis contained 3 to 16 mg/kg fluvalinate, which was by an order of magnitude greater than the residues in wax from the same bee hive. With the present method we could not examine the acaricide level of alcoholic propolis extracts. However, it was reported, that fluvalinate residues in propolis are fully extractable by alcohol and aqueous alcohol (Wallner, 1997). We do not have an explanation for the high contamination level of propolis, but its sticky nature might be one of the causes. Also, propolis was more severely contaminated by heavy metals than beeswax (Altmann, 1983).

All acaricides have been tested for bee toxicity prior to their registration and have been found as non-toxic even at higher residue levels, than those found in wax. However, the possibility exists, that synergetic effects between the different acaricides might lead to toxic effect on bees. Also, the persistence of the acaricide in the beehive wax favours the appearance of acaricide resistant mites. Indeed, resistance to the pyretroids fluvalinate and flumethrine in Italy has been reported for Italy (Milani 1995) and reports about pyrothroid resistant mites from other European countries are also spreading.

Significant acaricide levels in wax and propolis damage their quality in view of their use in pharmacy and medicine. MRL for acaricides should be introduced as a measure to control

contamination. Moreover, varroa can also be effectively controlled with organic acids, which do not contaminate wax and honey (Imdorf et al. 1996). A full version of this report has been published recently (Bogdanov et al. 1998).

After: Bogdanov S., Kilchenmann V., Imdorf A. (1998) Acaricide residues in some bee products, J.Apic.Res. 37: 57-67

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Table 1 Data of acaricides used in the present study

Acaricide manufacturer	in use since	Active ingredient (ai)	mg ai per treatment	Treatment after honey harvest in	MRL** mg/kg
Folbex VA CIBA-GEIGY	1982	bromopropylate	1600	autumn	0.1
Perizin BAYER	1987	coumaphos	32	autumn, winter	0.05
Apistan* SANDOZ	1991	fluvalinate	1600*	August, September	0.05
Bayvarol* BAYER	1991	flumethrine	14.4*	August, September	0.005

* - only a small part (about 5-10 %) of the total active ingredient can diffuse out of the ribbon.

** - MRL - maximal residue limit for Switzerland

Table 2 Validation data of acaricides

Determination of different concentration of acaricides in honey, wax and propolis.

	n	mg/kg	BBP	BP	CM	FV	FM
Honey		0.01, 0.05					
Recovery (mean)	12		94	105	99	84	nd
% RSD			6	6	11	13	nd
Wax		0.4,1,2,5,10,20,50					
Recovery (mean)	35		nd	101	95	80	88
% RSD				10	10	7	12
Propolis		1,2,5,10,20					
Recovery (mean)	20		Nd	85	nd	80	56
% RSD				5		5	7

RSD: relative standard deviation

Nd - not determined

Table 3. Residues (in mg/kg) of bromopropylate

In comb wax, feed and honey after repeated Folbex VA treatments

Folbex treatment	n*		Brood comb	Feed	Ratio*	Honey comb	Honey
1	3	BP BBP	47.9 ± 21.1	0.025 ± 0.005 0.017 ± 0.005	1916	3.8 ± 1.2	0.010 0.005
2	3	BP BBP	92.0 ± 33.9	0.090 ± 0.020 0.030 ± 0.010	1022		
4	3	BP BBP	116.7 ± 43.0	0.043 ± 0.005 0.020	2713		0.020 0.010
5	3	BP BBP	135.0 ± 59.4	0.037 ± 0.004 0.017 ± 0.005	3648		0.050 0.020

* - number of values, honey samples: single measurements

values: averages and standard deviations for comb and feed values. No deviation means that all values were equal.

BP: bromopropylate, BBP: dibromobenzophenone

*- ratio between the concentration in the brood comb and the feed

Table 4 Residues (in mg/kg) of fluvalinate

in combs, feed and honey after normal and continuous Apistan treatment

Months Apistan	n	Brood comb	Feed	Ratio *	Honey comb	Honey
1	9	1.8 ± 1.8	≤ 0.003			≤ 0.003
5	2	7.3 ± 1.7	0.004	1825		
6	2	14.7 ± 6.6	0.004	3675		
7	2	30.2 ± 5.1	≤ 0.003	10067		
8	2	20.6 ± 13.4				
9	2	20.0 ± 12.0	≤ 0.003	6667	2.7 ± 1.8	0.005 ± 0.001
10	2	17.6 ± 7.2	≤ 0.003	5867	8.4 ± 5.6	≤ 0.003
11	2	31.8 ± 4.6	0.007± 0.004	4543	5.9 ± 4.5	≤ 0.003
12.5	2	43.4 ± 23.4	0.011± 0.005	3945		

averages and ranges of a duplicate, no deviation was given when both values are equal

*: ratio between the concentrations in the brood combs and the feed

Table 5. Residues (in mg/kg) of coumaphos

in comb wax, feed and honey after repeated Perizin treatments

Perizin treatments	n	Brood comb	Feed	Ratio*	honey comb	Honey
1	4	4.3 ± 4.2	0.015 ± 0.015	287	0.7 ± 0.3	0.013 ± 0.003
2	4	7.4 ± 6.1	0.004 ± 0.001	1850	0.5 ± 0.1	0.010 ± 0.006
5	4	5.8 ± 5.4	0.006 ± 0.006	967	0.5 ± 0.1	0.005 ± 0.002

the values are averages and standard deviations

* ratio between the concentrations in the brood combs and the feed

Table 6. Melting of acaricide-contaminated old combs

to produce new beeswax under laboratory conditions

	% Wax recovery	BP	CM	FV	FM
Combs before melting		19.6 ± 0.3	14.8 ± 0.3	17.0 ± 0.5	20.3 ± 1.3
1 hour boiling	18	36.0 ± 1.7	28.9 ± 0.6	26.9 ± 0.7	34.8 ± 0.1
3 hours boiling	24	34.6 ± 1.2	27.8 ± 0.0	26.5 ± 0.8	33.4 ± 1.2
1 hour autoclave (140 °C)	25	34.8 ± 0.5	27.5 ± 0.6	27.1 0.7	34.4 ± 0.5
2 hours autoclave (140 °C)	22	34.0 ± 1.5	27.9 ± 2.0	24.3 ± 1.0	31.2 ± 1.7
Enrichment factor wax/comb		1.8	1.9	1.6	1.6

Results in mg/kg: averages of a duplicate ± range.

BP: bromopropylate; CM: coumaphos; FV: fluvalinate; FM: flumethrine

Table 7. Acaricide residues in propolis

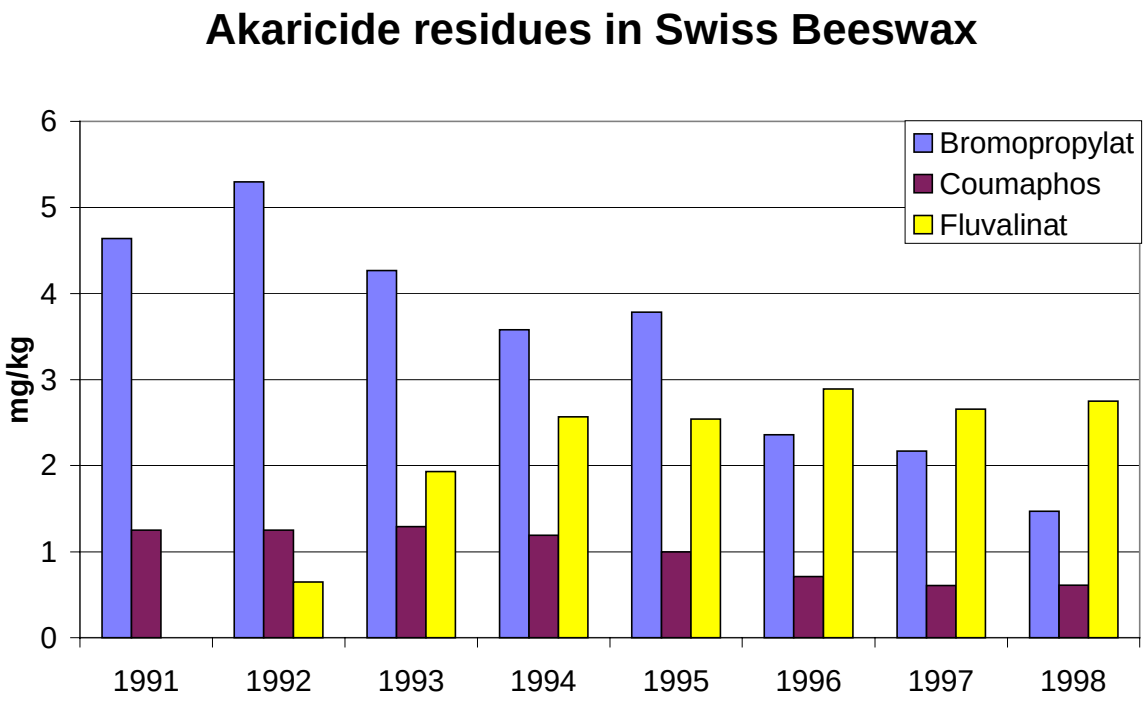
	BP	FV	FM
average	1.17	9.80	2.54
minimum	0.6	0.5	1.3
maximum	3.8	38.7	3.7
n total = 27			
n positive	10	26	2

only values (mg/kg) above the detection limit were considered

BP: bromopropylate; FV: fluvalinate;

FM: flumethrine

FIG.1. Acaricide residues in Swiss commercial beeswax



Apistan was homologated in 1991 Measurements were done in representative commercial samples, see Bogdanov et al. 1998**.**