Danish Research Service for Plant and Soil Science **Report no. 1921**
Administration
Department of Bee Diseases
Kongevejen 83
DK-2800 Lyngby

National Food Agency
Central Laboratory, Division 3
Chemical Contaminants in Food
Mørkhøj Bygade 19
DK-2860 Søborg

Residues in honey and wax after treatment of bee colonies with bromopropylate

Rester i honning og voks efter behandling af bifamilier med brompropylat

HENRIK HANSEN and JENS HØJSLEV PETERSEN

Summary

Bee colonies without the mite *Varroa jacobsoni* were treated, in the autumn, with bromopropylate in order to investigate the concentrations in residual feed, honey and wax. Samples for the investigation were taken eight to nine months after the treatment.

Treatment of bee colonies using four fumigant strips containing bromopropylate (Folbex VA) in accordance with the manufacturer's instructions left residues of 0.005–0.07 mg bromopropylate/kg in the honey. Residues of the breakdown product 4,4'-dibromobenzophenone were also discovered in the honey (0.003–0.05 mg/kg).

The concentration in the wax of the pesticide and its breakdown product was 500–1000 times higher (19–99 mg bromopropylate and 9–48 mg 4,4'-dibromobenzophenone/kg). The concentration present in the residual feed was similar to that of the honey.

That the pesticide and its metabolite accumulate in the bee wax through repeated treatment of the combs (over a two year period) cannot be confirmed or disproved due to the limited data of the analysis.

When using an alternative method of treatment, the residual concentration of the two substances in both the wax and the honey was approximately 20 times less than when using the normal method of treatment.

Key words: *Varroa jacobsoni, Apis mellifera,* honey bees, bromopropylate, 4,4'-dibromobenzophenone, Folbex VA, pesticide residues, honey, wax.

Resumé

Bifamilier uden miden *Varroa jacobsoni* blev behandlet med brompropylat om efteråret for at få kendskab til restkoncentrationer i foderrester, honning og voks. Prøver blev udtaget efter otte til ni måneder til undersøgelse for restkoncentrationer.

Ved behandling af bifamilier med fire brompropylatholdige rygestrimler (Folbex VA) efter producentens anvisninger blev der i honningen målt restindhold af brompropylat på 0,005–0,07 mg/kg. Der blev herudover målt restindhold af nedbrydningsproduktet 4,4'-dibromobenzophenon på 0,003–0,05 mg/kg i honningen.

I bivokset blev der målt mellem 500 og 1000 gange højere indhold af pesticidet og dets nedbrydningsprodukt (19–99 mg brompropylat og 9–48 mg 4,4'-dibromobenzophenon/kg). I foderresten var indholdene i samme størrelsesorden som i honningen.

En formodning om, at pesticidet og dets nedbrydningsprodukt akkumuleres i bivokset ved gentagne behandlinger af samme tavler over to år, kunne ikke be- eller afkræftes på grund af det begrænsede antal analysedata.

Ved en alternativ behandling blev restindholdene af de to stoffer i både voks og honning ca. 20 gange mindre end ved normal behandling.

Nøgleord: *Varroa jacobsoni, Apis mellifera,* honningbier, brompropylat, 4,4'-dibromobenzophenon, Folbex VA, restkoncentrationer, honning, voks.

Introduction

Honey bees *(Apis mellifera)* can be attacked by the mite *Varroa jacobsoni.* This mite has spread to honey bees in many parts of the world. In Central Europe, as in other places, experience has shown that bee colonies die after a few years unless treated (5). In a few years, the mite is expected to have spread to bee colonies in many parts of Denmark.

At present, treatment is being carried out abroad by means of a number of chemical substances. Many of the substances have to be used in the autumn, when there is no brood in the bee colonies (4). As there is no actual nectar flow at that time of the year, the risk of residues of the substances involved occurring in the honey is thereby reduced.

Bromopropylate is one of the substances that is effective in treating for *Varroa* (3). It is probable that this substance will be used in Denmark.

In order to investigate the level of chemical concentrations in honey, wax and remaining feed after treatment using fumigant strips containing bromopropylate, bee colonies without *Varroa* mites were treated with this substance. In this paper, an account is given of the experiments conducted.

Method

Treatment using bromopropylate

In autumn 1983, bee colonies were treated with bromopropylate (Folbex VA) at two separate locations on Zealand (St. Dyrehave and Pagterold).

In the apiary in St. Dyrehave, six bee colonies were treated, while four were treated, in the apiary at Pagterold. The treatment was carried out in the latter half of September.

The treatment was carried out four times as prescribed by Ciba-Geigy (1), which markets Folbex VA. Each time the treatment consisted of fumigating the bees using one fumigant strip per colony. Each bee colony had two boxes each containing nine waxcombs. During fumigation, the hives were closed. The fumigant strips each contained 370 mg bromopropylate.

In the middle of May 1984, samples were taken of remaining winter feed (sugar) and of comb wax from two bee colonies in St. Dyrehave and two at Pagterold. At the beginning of July 1984, after the first extraction, honey samples were taken from four colonies in each of the two apiaries. The honey from each box was kept separate. Thus each sample was a blend of honey from an individual box.

As a control in the treatment with bromopropylate six untreated bee colonies were wintered in St. Dyrehave and four at Pagterold.

It is usual apicultural practice to leave some of the combs in the bee hives for several years. If such a practice is followed, it is important to know whether an accumulation of bromopropylate and its breakdown product 4,4'-dibromobenzophenone occurs after treatment over two consecutive years.

To obtain further information four bee colonies were treated again in September 1984. As in the previous year, fumigation was carried out four times. Each colony had two boxes each containing nine combs. Combs in one box from each colony had not been treated in September 1983. The experiment was carried out only in St. Dyrehave. Two of the bee colonies had been moved to St. Dyrehave from Pagterold.

One of the bee colonies died during winter 1984–85. In early summer 1985 two of the remain-

ing colonies were given two boxes containing combs, while the last colony was given one box containing combs. These combs had not previously been treated.

Bromopropylate treatment combined with transfer

It has been shown *(W. Ritter,* pers. comm. 1984) that treatment of bee colonies with bromopropylate is much more effective in colonies with a few combs than in those with many. When treating a small number of combs two fumigant strips are sufficient. To investigate whether it is possible to reduce the chemical residue in honey and wax, treatment was undertaken involving the use of two fumigant strips followed by a transfer of the bee colonies concerned to artificial combs.

In the middle of August 1984 the brood combs were removed from eight bee colonies in St. Dyrehave, and the bees of each colony were shaken into three feed combs. Immediately after, one fumigant strip was used in each colony. Two days later, another fumigant strip was used and a further two days later, the bees were shaken into artificial combs and the feed combs removed. All the bee colonies were then fed normally with sugar water. They were wintered in one box per colony.

As a control for bromopropylate fumigant strip treatment and transfer, eight colonies were wintered in the normal way.

Two of the bee colonies which had been treated died during the winter. In the course of the spring, each of the remaining colonies were given two additional boxes so that, by the beginning of July 1985 they each had three boxes. At that time, samples of honey from the first extraction and of wax were taken from each colony. The honey was again extracted from each box separately. The samples were taken from the box in which the bees had wintered, and from one of the two new boxes they had been given that year.

Method of analysis for bromopropylate and 4,4'-dibromobenzophenone

Extraction and cleaning

Honey and remaining feed: 10 g or remaining feed is shaken with 20 ml water for 30 minutes. 15 ml n-pentane is added and the mixture is again shaken. It is then centrifuged and the pentane is removed with a pipette. The procedure is repeated with another 15 ml n-pentane, and the two pentane extracts are combined. The extract is then dried by means of Na_2SO_4, evaporated and transferred to isooctane.

Wax: 2 g rinsed wax is extracted by means of shaking with n-pentane. Impurities are removed by centrifuging. The extract is dried with Na_2SO_4, evaporated and transferred to isooctane.

Gas chromatography

A Packard-Becker 427 gas chromatograph equipped with capillary columns and an EC-detector is used. Isothermal analysis (200°C) is employed with split injection on a 25 m CP SIL 5-column (i.d. 0.22 mm, 0.12 μm film thickness) or alternatively temperature programmed operation (90°C for 1 minute, 10°C/min, final temperature 220°C) on-column injection with 10 m CP SIL 18-column (i.d. 0.20 mm, 0.18 μm film thickness).

Limit of detection

Honey and remaining feed: bromopropylate 0.001 mg/kg, 4,4'-dibromobenzophenone 0.002 mg/kg.

Wax: bromopropylate 0.3 mg/kg, 4,4'-dibromobenzophenone 0.6 mg/kg.

Results

Bromopropylate treatment

In Table 1, the results following treatment with bromopropylate in autumn 1983 are shown. The treatment was carried out with four fumigant strips as prescribed by Ciba-Geigy.

From Table 1 it can be seen that in the remaining feed there was 0.01–0.09 mg bromopropylate/kg and 0.003-0.04 mg 4,4'-dibromobenzophenone/kg. In the wax there was 25–86 mg/kg and 13–48 mg/kg respectively. In the honey there was 0.005–0.07 mg/kg and 0.002–0.03 mg/kg respectively. The residual concentrations were thus substantially higher in the wax than in the honey.

None of the ten colonies treated died during the winter, whereas one out of the ten untreated colonies died.

Table 2 shows the results from boxes/colonies treated over two consecutive years, those treated only once and those not treated at all (the last comb was added in the spring of the year the samples were taken). Each treatment was carried out with four bromopropylate fumigant strips as prescribed by the manufacturer.

Table 1. Residual concentrations in remaining feed and wax in May 1984 and in honey extracted in July 1984 following bromopropylate treatment in autumn 1983
Restkoncentration i foderrest og voks i maj 1984 samt i honning i juli 1984 efter behandling med brompropylat i efteråret 1983.

Apiary	Bee colony and box	Detected presence of 1) bromopropylate, and 2) 4,4'-dibromobenzophenone in mg/kg					
		Remaining feed		Wax		Honey	
		1)	2)	1)	2)	1)	2)
St. Dyrehave	10 b	0.09	0.04	85.5	47.7	0.04	0.02
	10 a	0.04	0.02	48.3	28.5	n.a.	n.a.
	11 b	0.01	0.01	33.7	16.0	0.05	0.02
	11 a	0.02	0.01	49.2	32.3	n.a.	n.a.
	11+18+9 b	n.a.	n.a.	n.a.	n.a.	0.07	0.03
Pagterold	323 b	0.01	0.003	25.0	12.9	0.01	0.006
	323 a	0.01	0.006	65.8	25.9	n.a.	n.a.
	334 b	0.02	0.01	47.3	24.2	0.02	0.01
	420+224 b	n.a.	n.a.	n.a.	n.a.	0.005	0.002

b = bottom box, a = top box, n.a. = not analysed

Limit of detection. Honey and remaining feed: bromopropylate 0.001 mg/kg, 4,4'-dibromobenzophenone 0.002 mg/kg. Wax: bromopropylate 0.3 mg/kg, 4,4'-dibromobenzophenone 0.6 mg/kg.

Table 2. Residual concentrations in honey and wax in July 1985 following bromopropylate treatment carried out in autumn 1983 and 1984 (using four fumigant strips on each occasion).
Restkoncentrationer i honning og voks i juli 1985 efter behandling med brompropylat i efteråret 1983 og 1984 (hver gang med fire rygestrimler).

Bee colony	No. of times box treated	Residuals of 1)bromopropylate, 2) 4,4'-dibromobenzophenone in mg/kg				Description used in Tab. 1.
		Honey		Wax		
		1)	2)	1)	2)	
10	2	0.03	0.02	34.4	9.1	10 a
	1	0.05	0.05	47.8	16.5	
	0	0.005	0.007	n.d.	n.d.	
11	2	0.05	0.04	98.0	27.5	11 a
	1	n.a.	n.a.	56.5	21.6	
323	2	0.03	0.03	38.9	20.8	323 a
	1	0.03	0.03	39.9	15.5	
	0	0.007	0.005	n.a.	n.a.	

n.d. = not detected, n.a. = not analysed

Limit of detection: Honey: bromopropylate 0.001 mg/kg, 4,4'-dibromobenzophenone 0.002 mg/kg. Wax: bromopropylate 0.3 mg/kg, 4,4'-dibromobenzophenone 0.6 mg/kg.

In this experiment, 35–99 mg bromopropylate/kg and 9–27 mg 4,4'-dibromobenzophenone were detected in the wax. Less was detected in the honey than in the wax, 0.03–0.05 mg bromopropylate/kg and 0.02–0.05 4,4'-dibromobenzophenone/kg. Furthermore, there were smaller concentrations of both substances in both the wax and the honey present in the combs that had been added in the spring following the treatment.

One of the four colonies involved in the experiment died during the winter.

Bromopropylate treatment combined with transfer

In Table 3, the results are shown of experiments with bromopropylate fumigation of three combs which, following treatment, were replaced by artificial combs.

As can be seen from Table 3 0.001–0.002 mg bromopropylate/kg was detected in the honey. The wax was analysed using two samples from combs to which the bees had been transferred after bromopropylate fumigation. In one of the samples the concentration of bromopropylate was below the limit of detection (0.3 mg/kg). In the other sample, there was 3.8 mg bromopropylate/kg and 1.1 mg 4,4'-dibromobenzophenone/kg. This is a considerably smaller residue in both the honey and wax than is found when using the traditional method of treatment.

Out of the eight colonies treated, two died during the winter. Three of the eight control colonies died.

Discussion and conclusion

In honey from the treated bee colonies, residual concentrations of maximally 0.07 mg bromopropylate/kg and 0.05 mg 4,4'-dibromobenzophenone/kg were detected. The maximum permitted limit in Denmark for residual bromopropylate in fruit and vegetables is between 1–3 mg/kg, therefore the residue found in the honey does not pose a health hazard.

The National Food Agency recently carried out an investigation of pesticide residues in honey. The samples used in this investigation were taken at random and included honey produced in Denmark as well as imported honey (6). Bromopropylate treatment of bee colonies is permitted in many countries but in none of the imported samples could a residue of bromopropylate above 0.01 mg/kg be detected. Nevertheless a German investigation of residues in 50 honeys after treatment against Varroa disease has shown that 45

Table 3. Residual concentrations in honey and wax in July 1985 following treatment with bromopropylate (using two fumigant strips) and subsequent transfer in August 1984.
Restkoncentrationer i honning og voks i juli 1985 efter behandling med brompropylat (to rygestrimler) og efterfølgende omsætning i august 1984.

Bee colony	Date of combs added	Residuals of 1) bromopropylate and 2) 4,4'-dibromobenzophenone in mg/kg.			
		Honey		Wax	
		1)	2)	1)	2)
9	1984/85	0.002	n.d.	n.a.	n.a.
12	1984/85	0.002	0.003	n.a.	n.a.
14	1984	0.002	n.d.	n.a.	n.a.
18	1984	0.002	0.004	3.8	1.1
101	1984/85	0.001	n.d.	n.a.	n.a.
177	1984/85	0.002	n.d.	n.a.	n.a.
Control	1984/85	n.d.	n.d.	n.d.	n.d.

n.d. = not detected, n.a. = not analysed

Limit of detection. Honey: bromopropylate 0.001 mg/kg, 4,4'-dibromobenzophenone 0.002 mg/kg. Wax: bromopropylate 0.3 mg/kg, 4,4'-dibromobenzophenone 0.6 mg/kg.

p.c. of the samples contained bromopropylate and 4,4'-dibromobenzophenone (2). The average content was 0.027 mg/kg and 0.012 mg/kg respectively. The honey originated from Rheinland-Pfalz where Folbex VA has been used since 1982.

The level of residues og bromopropylate and 4,4'-dibromobenzophenone in wax nine months after treatment is disturbingly high – as much as 99 mg bromopropylate/kg and half that figure for 4,4'-dibromobenzophenone. There is a risk that these chemicals might be preserved in the wax and increased as a result of repeated treatment of the same combs over a number of years. Investigations were started to establish whether this is the case. However, as can be seen from Table 2, the results provide no clear evidence of this.

The large variations in the residual contents may be due to various factors:

a) The method of treatment whereby fumigant strips impregnated with bromopropylate are burned in closed bee hives may be significant. There is reason to believe that the fumigation process itself gives rise to an unpredictable ratio of bromopropylate to 4,4'-dibromobenzophenone and to other possible products of combustion.
b) The bees themselves cause the smoke to be distributed in the bee hive by ventilating with their wings. This cannot result in an even distribution to all boxes (normally two in a hive when treatment is carried out) and combs (nine in each box).
c) It is normal for the bees to move wax, honey and sugar feed during the course of the season. With the present resources, it has not been possible to carry out detailed studies of the distribution of bromopropylate amongst all combs.

However, it appears to have been confirmed that the relative proportion og bromopropylate and 4,4'-dibromobenzophenone in wax and honey is fairly constant. If the wax contains a certain residue, the concentration in the honey will be 500–1000 times smaller. If the bromopropylate treatment becomes normal practice in apiculture, there will be a need to investigate further the accumulation of the chemical over several years. In addition, an investigation needs to be carried out to ascertain whether the bromopropylate can survive the melting down of the wax, as bees wax is reused year after year to produce new combs.

With treatment involving two fumigant strips followed by transfer to artificial combs, the residue in both wax and honey is considerably smaller (approximately 20 times smaller) than with the treatment of combs that remain in the bee hive. However, in all the honey samples a bromopropylate content of 0.001–0.002 mg/kg was found. In only one of the two wax samples analysed could a residue be detected. This residue was small (3.8 mg bromopropylate/kg and 1.1 mg 4,4'-dibromobenzophenone/kg) compared with the residue found after the ordinary recommended treatment.

The method of treatment by which fumigation is followed by transfer can be recommended if the apiarist wishes to reduce the residue in wax and honey. However, this method is somewhat more laborious than the method normally used.

Significant loss of bees has not been observed in using either of the methods of treatment. The loss of colonies amongst both the treated and the untreated groups can be ascribed to normal winter loss.

Translation into English by *Bodil Sampson.*

References

1. *Ciba-Geigy.* The control of honeybee mites. 14pp.
2. *Laub, E., Metzler, B., Pütz, A. & Roth, M.* 1987. Zur Rückstandssituation zugelassener Varroatosebekämpfungsmittel im Honig. Lebensmittelchem. Gerichtl. Chem. 41, 107–109.
3. *Ritter, W. & Rutner, F.* 1980. Chemotherapie. Allg. dt. Imkerztg. 14.
4. *Ritter, W.* 1981. Varroa Disease of the honey bee *Apis mellifera.* Bee World 62, 141–153.
5. *Ritter, W.* 1986. Die Varroatose der Honigbiene, *Apis mellifera,* und ihre Bekämpfung mit Perizin. Veterinär-Medizinische Nachrichten 1, 3–16.
6. *Wienberg, A. & Guldborg, M.* 1987. Honning, varestandard og uønskede stoffer (Honey, food standard and undesireable constituents). Publication 142, National Food Agency.

Manuscript received 4 February, 1988.