

Long-term monitoring of lipophilic acaricide residues in commercial Swiss beeswax

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Abstract

BACKGROUND: A national survey on pesticides in recycled beeswax originating from beekeeping has been conducted in Switzerland for almost three decades. It allowed obtaining a good overview of the lipophilic products used for beekeeping within the last 30 years.

RESULTS: The use of the veterinary drugs containing bromopropylate or *tau*-fluvalinate two decades ago led to substantial residues in commercial beeswax. These contaminants are still detectable although in Switzerland the corresponding products have been out of use for many years. The level of coumaphos substantially increased in 2015 up to an annual value of 3.25 mg·kg⁻¹, suggesting that at least a few beekeepers used coumaphos-containing products. Consequently, an information campaign was launched, and the annual value decreased again. Maximal levels of thymol up to an annual value of 87.5 mg·kg⁻¹ were measured in 2009. Since that time, a steady decrease of thymol residues suggests that beekeepers less frequently use thymol-containing products. Twenty-five years ago, 1,4-dichlorobenzene (PDCB) was widely used for the control of the wax moth, resulting in residues in beeswax up to an annual value of 10.9 mg·kg⁻¹ whereas nowadays, PDCB residues are rarely detected in Swiss beeswax.

CONCLUSIONS: Our survey illustrates that several beekeeping-associated pesticides persist in recycled beeswax for many years. Most recent analyses show lower residue levels in Swiss beeswax as compared to previous years. Nowadays Swiss beekeepers mostly use hydrophilic substances for treatment against the *Varroa destructor* that do not accumulate in beeswax, thus reducing exposure of the honey bees to lipophilic contaminants.

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Keywords: *Apis mellifera*; *Varroa destructor*; acaricide; bromopropylate; *tau*-fluvalinate; coumaphos; thymol; 1,4-dichlorobenzene; beeswax

1 INTRODUCTION

Losses of honey bee colonies are associated with many different causes. *Varroa destructor* in particular is a major threat to honey bees. To fight the *Varroa* mite, beekeepers use veterinary drugs. However, these substances can accumulate in the beehive compartments. The octanol–water partition coefficient ($\log P_{o/w}$) gives an indication about the lipophilicity of a substance and hence about the preferential distribution within the various hive compartments. Lipophilic substances have a tendency to accumulate in wax and much less in honey. Hence, veterinary drugs with a higher $\log P_{o/w}$, such as *tau*-fluvalinate ($\log P_{o/w}$ 7.0),¹ bromopropylate ($\log P_{o/w}$ 4.9),² coumaphos ($\log P_{o/w}$ 3.9)¹ or thymol ($\log P_{o/w}$ 3.3)³ contaminate mainly beeswax. There is increasing evidence that such contaminants have multiple negative effects on the growing honey bees, underlining the importance of minimizing the level of contaminants in beeswax.

In beekeeping, beeswax is recycled to produce foundation sheets. Honey bees secrete wax as small wax scales from special wax glands located in the abdomen. Beekeepers usually place frames containing foundation sheets as a template for the bees to build new combs. A good beekeeping practice is to exchange old wax combs at an interval of about 3 years. Hence, the

beekeepers melt the old frames and produce wax blocks, which they take to manufacturers for the production of new foundation sheets. In Switzerland, the beeswax used for the fabrication of new foundations originates mostly from recycled old combs and capping wax. Lipophilic substances, such as veterinary drugs used to treat bee diseases, insecticides, or plant protection products, accumulate in beeswax. The quantitatively most important contaminants in beeswax are the lipophilic acaricides that beekeepers use for the control of *Varroa destructor*. Beekeepers apply these drugs directly on the beehives and on a regular basis. Hence, it is not surprising that many studies show that acaricides are the most frequently detected residues in beeswax of European^{1,4–17} or North American^{18–20} origin. Furthermore, when wax is recycled, bromopropylate, *tau*-fluvalinate, and coumaphos remain in the wax and hence are still present in the newly produced wax foundation sheets.^{21,22} The exposure of the

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developing honey bee to contaminants in the brood comb can delay larval development and reduce the longevity of the adult bee.²⁰ As an alternative to lipophilic acaricides, organic acids, such as formic, lactic and oxalic acid, are recommended for mite control. They do not contaminate beeswax, as they are hydrophilic, but rather accumulate in honey. If applied according to the recommendation, the residues of organic acids in honey are within their natural concentrations.

Residues can migrate from wax into the stored honey. Thus, high residue levels in wax may lead to residue levels in honey that exceed the maximal limits and thus can pose a health risk for consumers. An example of a substance that migrates from wax into honey is 1,4-dichlorobenzene (PDCB). PDCB is an insecticide that was used against different types of moths. However, PDCB-containing products were never authorized for use in apiculture in many countries, including Switzerland. Nevertheless, beekeepers used PDCB to protect stored combs from the greater wax moth *Galleria mellonella* and the lesser wax moth *Achroia grisella*. Although PDCB was applied on combs stored outside of the hive, this substance was found in approximately 30% of Swiss honeys during the years 1997 to 2002, as reported by the cantonal food control authorities.²³ Hence, PDCB migrated from the contaminated wax into honey.

In a long-term project, we are following the acaricide and PDCB levels in Swiss beeswax by analyzing representative annual samples from all major manufacturers of foundations in Switzerland. The earlier results from 1991 until 2002 were published by Bogdanov and colleagues.^{21,23–25} These results are included in this study for a more comprehensive picture. We now present results for an additional 16 years (years 2004–2019). As a result, we obtained an indication of the long-term behavior of acaricides in beeswax. Furthermore, our study gives an overview of the use of lipophilic acaricides in Switzerland during the last three decades and its implications for beekeeping.

2 MATERIALS AND METHODS

2.1 Material

Bromopropylate, coumaphos, and *tau*-fluvalinate were purchased as a mix (each 0.1 mg·mL⁻¹ in cyclohexan) from Neochema (Bodenheim, Germany). 1,4-dichlorobenzene Pestanal (Art. 35775) and thymol (99.5%; Art. 89330) were obtained from Sigma-Aldrich (Seelze, Germany).

Florisil (60 to 100 mesh; Art. 1.12518) and Na₂SO₄ sicc. were obtained from Merck, Darmstadt, Germany, and the SPE columns (Bakerbond spe, C18, 6 mL, 500 mg; Art. 7020-06) from J.T. Baker (Deventer, Holland). The solvents, acetone, isooctane, hexane, and ethanol (min. 995 g·kg⁻¹) were of analytical grade for residue analysis and obtained from Merck. Chromafil AO-45/15 MS (0.45 µm; Art. 729049) nylon membrane filters were obtained from Macherey-Nagel, Düren, Germany.

2.2 Wax samples from the manufacturers of beeswax foundations

All major commercial manufacturers of foundations in Switzerland participated in our long-term study. This means between eight and 11 participants depending on the year of investigation. Most manufacturers produced between 400 and 8000 kg of new foundations yearly. However, the largest manufacturer produced between 28 000, and 45 000 kg per year. During the years of the investigations, which was usually every alternate production year, the manufacturers collected wax samples from each production

lot. The samples were stored in the dark at -20 °C. At the beginning of the subsequent year, the manufacturer sent all samples to the Swiss Bee Research Centre together with information on the size of each production lot.

2.3 The annual residue value per manufacturer and the annual value for all Switzerland

In our laboratory, we prepared representative annual samples from each manufacturer in proportion to the weight of each production lot. The wax of each manufacturer was melted for homogenization before the extraction procedure. After analysis by gas chromatography, we obtained the annual residue values per manufacturer. A theoretical average annual value for the total amount of beeswax produced in Switzerland, the annual value of the residues for all Switzerland, was calculated, taking into consideration the different amounts of the foundations (measured in weights) produced by each manufacturer during the year.²³

2.4 Sample preparation

Extraction of bromopropylate, coumaphos, and *tau*-fluvalinate was performed as described by Bogdanov and colleagues,²¹ with minor modifications. One gram of wax was extracted for 45 min with 10 mL hexane in an ultrasonic bath. Subsequently, the sample was placed at -20 °C for at least 1½ h, followed by centrifugation (Sigma 4-16KS) at -5 °C at 10010 × *g* for 15 min. The supernatant was decanted, and the freezing procedure was repeated before purification of the supernatant on a column (5 mm id and 10 cm length; filled with 0.1 g Na₂SO₄ sicc. and 1.5 g florisil containing 5% water). Five milliliters of the hexane supernatant was poured on the column, washed with 20 mL hexane, and the substances subsequently eluted with 20 mL of an acetone-hexane mixture (1:1). The solvent was evaporated, and the substances were dissolved in 2 mL isooctane. The sample was placed at -20 °C for at least 2 h before filtration through a 0.45 µm membrane.

Extraction of thymol and PDCB was performed as described by Bogdanov and colleagues,²⁶ with minor modifications. One gram of wax was extracted for 1 h with 10 mL ethanol in an ultrasonic bath, followed by centrifugation (Sigma 4-16KS) for 20 min at room temperature at 27800 × *g*. The supernatant was transferred into a new centrifugation tube, and the sample was placed at -20 °C for at least 2 h before centrifugation at -5 °C for 20 min 27 800 × *g*. The freezing and centrifugation step was repeated. Five milliliters of the clear supernatant were diluted with 20 mL Milli-Q-water. An SPE column (Bakerbond spe, C18) was activated with ethanol and subsequently with water. The diluted wax extract was passed through the SPE column and the column rinsed twice with 10 mL 20% ethanol. The substances were subsequently eluted twice with 1 mL of acetone. A spatula of Na₂SO₄ was added to absorb the water, and the sample was placed at -20 °C at least for 2 h before filtration through a 0.45 µm membrane.

2.5 GC-MS/MS analysis of bromopropylate, coumaphos, and *tau*-fluvalinate

Gas chromatography analysis of the wax samples from 1991 to 2007 was performed on a Hewlett Packard 5890 gas chromatograph equipped with an electron capture detector (ECD) and a flame ionization detector (FID) detector, as described earlier by Bogdanov and colleagues.^{21,24} The limit of detection (LOD) for bromopropylate, coumaphos, and *tau*-fluvalinate was 0.25 mg·kg⁻¹.²⁴

Analysis of the samples from 2009 to 2019 was performed on a Thermo Trace Ultra 2000 gas chromatograph equipped with a Deans Switch system coupled with a MS/MS triple quadrupole mass spectrometer (Thermo Quantum) and an FID detector with a 15-m transfer column. An autosampler (CTC Combi PAL Systems) was used for automatic injection. The carrier gas was helium. A retention capillary column deactivated with OV-1701-OH (0.53 mm i.d.) of 50 cm and a DB-1 analytical capillary column (J + W, 0.25 mm i.d., 0.25 μm film thickness) of 30 m, as well as a transfer column Rxi[®]-5 Sil MS (0.25 mm i.d., 0.25 μm film thickness), were used. One microliter of the final beeswax extract was injected on the column. The gas chromatograph was operated using the constant pressure mode (225 kPa). The gas chromatograph temperature program was 2.0 min at 90 °C, 90 to 250 °C at 5 °C·min⁻¹, 250° to 300 °C at 3 °C·min⁻¹, where it was held for 50 min. The source temperature (TSQ Quantum) and the temperature of the transfer column were 250 °C. The electrospray ionization was operated in the positive mode and the ionization energy I was 70 eV. The Deans Switch allowed directing the window 34.5 to 37 min as well as the window 39 to 47 min to the MS/MS triple quadrupole mass spectrometer for the detection of the bromopropylate, coumaphos, and *tau*-fluvalinate peaks, while the rest of the chromatogram was directed to an FID detector to prevent pollution of the MS system. Bromopropylate, coumaphos, and *tau*-fluvalinate were identified using their mass fragmentation. For bromopropylate, the transitions of m/z 341 to 155 (collision energy (CE) 41) and m/z 341 to 185 (CE20) were used for identification, and the transition m/z 341 to 183 (CE 20) was used for quantification. For coumaphos, the transitions of m/z 362 to 334 (CE10), m/z 362 to 109 (CE25), and m/z 362 to 226 (CE25) were used for identification, and the transition m/z 226 to 163 (CE18) was used for quantification. For *tau*-fluvalinate, the transitions of m/z 181 to 152 (CE20), m/z 250 to 208 (CE20), and m/z 252 to 200 (CE20) were used for identification, and the transition m/z 250 to 200 (CE 20) was used for quantification.

Quantification was achieved through external calibration. The standard solutions containing bromopropylate, coumaphos, and *tau*-fluvalinate were prepared in blank matrix extract to compensate for matrix effects. The limit of detection (LOD) was experimentally determined using spiked blank wax extracts. The LODs (signal to noise [s/n] 100) were 0.01 mg·L⁻¹ or 0.04 mg·kg⁻¹ in wax. The recovery was tested at seven spiking levels (0.05, 0.1, 0.2, 0.5, 2.0, 10, 20 mg·kg⁻¹) with five repetitions each. The recoveries for bromopropylate ranged between 78% and 93%, for coumaphos between 82% and 89%, and for *tau*-fluvalinate between 83% and 91%. The detector had linear responses for all spiking levels from 0.05 to 20 mg·kg⁻¹ ($R^2 = 0.9997$, $R^2 = 0.9999$, $R^2 = 0.9991$, respectively). The limits of quantitation (LOQ) for coumaphos, *tau*-fluvalinate, and bromopropylate were 0.05 mg·kg⁻¹. They were defined as the lowest validated spiked level where the recovery was above 75%.

2.6 GC-MS/MS analysis of thymol and PDCB

Gas chromatography analysis of the wax samples from 1991 to 2009 was performed on a Hewlett Packard 5890 gas chromatograph as described earlier by Bogdanov and colleagues.^{23,26} The LOD for thymol was 0.4 and 0.7 mg·kg⁻¹ for PDCB.^{23,26}

Analysis of the samples from 2011 to 2019 was performed on the Thermo Trace Ultra 2000 gas chromatograph. A DB-5 retention capillary column (J + W; 0.32 mm i.d., 1 μm film thickness) of 50 cm and a DB-5 MS analytical capillary column (J + W,

0.32 mm i.d., 1 μm film thickness) of 30 m, as well as a transfer column Rxi[®]-5 Sil MS (0.25 mm i.d., 0.25 μm film thickness), were used. One microliter of the final beeswax extract was injected in splitless mode (1.5 min) at a temperature of 240 °C. The gas chromatograph was operated using the constant pressure mode (225 kPa). The gas chromatograph temperature program was 2.0 min at 50 °C, to 200 °C at 4 °C·min⁻¹, to 280° at 30 °C·min⁻¹, where it was held for 60 min. The source temperature (TSQ Quantum) and the temperature of the transfer column were 250 °C. The ionization was operated in the positive mode and the ionization energy I was 70 eV. The Deans Switch system allowed directing the window of 16 to 33 min of the chromatogram to the MS/MS triple quadrupole for the detection of the PDCB and the thymol peak, while the rest of the chromatogram was directed to an FID detector to prevent pollution of the MS system. PDCB and thymol were identified using mass fragmentation. For PDCB, the transition of m/z 111 to 75 (CE12) was used for identification, and the transition m/z 146 to 111 (CE 22) was used for quantification. For thymol, the transitions of m/z 135 to 115 (CE12) and m/z 150 to 135 (CE7) were used for identification, and the transition m/z 135 to 91 (CE 14) was used for quantification.

Quantification was achieved through external calibration. The standard solutions containing PDCB and thymol were prepared in blank matrix extract to compensate for matrix effects. The limit of detection (LOD) was experimentally determined using spiked blank wax extracts. The LOD (signal to noise [s/n] 100) was 0.05 mg·L⁻¹ corresponding to 0.2 mg·kg⁻¹ in wax. The recovery for PDCB was tested at seven spiking levels (0.2, 0.4, 1.0, 2.5, 5.0, 25, 50 mg·kg⁻¹) with at least six repetitions each. The recoveries for PDCB ranged between 49% and 57% and were, on average, 53%. The detector had linear responses for all spiking levels from 0.2 to 50 mg·kg⁻¹ ($R^2 = 0.9904$). The results for PDCB were multiplied by a factor of 1.85 to compensate for the incomplete recoveries. The LOQ for PDCB was 0.37 mg·kg⁻¹ defined as the lowest validated spiked level multiplied by the factor 1.85. The recovery for thymol was tested at nine spiking levels (0.4, 0.8, 2.0, 5.0, 10, 50, 100, 250, 500 mg·kg⁻¹) with six repetitions each. The recoveries for thymol ranged between 80% and 95%. The detector had linear responses for all spiking levels from 0.4 to 500 mg·kg⁻¹ ($R^2 = 0.9904$). The LOQ for thymol was 0.40 mg·kg⁻¹, defined as the lowest validated spiking level with a recovery above 80%.

3 RESULTS

The first product for *Varroa* control was approved in 1984 in Switzerland containing bromopropylate as the active substance (Table 1).²⁷ Consequently, bromopropylate residues in beeswax were highest in the early 1990s, reaching a maximal annual value of 5.3 mg·kg⁻¹ in 1992 (Fig. 1). The residue levels have decreased constantly since 1992, and 25 years later, its annual value dropped below 0.1 mg·kg⁻¹ (Fig. 1).

Products containing coumaphos or *tau*-fluvalinate as the active substances were approved in Switzerland in 1987 and 1991, respectively (Table 1).²⁴ Residue levels of *tau*-fluvalinate were highest in 1996 with an annual value of 2.89 mg·kg⁻¹ (Fig. 1). Since then, *tau*-fluvalinate residues have decreased steadily until 2011. In the last few years, we observed slightly higher annual values of *tau*-fluvalinate residues as compared to the value in 2011 (Fig. 1, Table 2), although approval of the corresponding product expired in 2006 (Table 1). In the early years of the monitoring program, the annual values of coumaphos were around

Table 1. Lipophilic acaricides used for apiculture in Switzerland

Active substance	Commercial product	Period of authorization in Switzerland
Bromopropylate	Folbex VA®	1984–1999
<i>Tau</i> -Fluvalinate	Apistan®	1991–December 2006
Coumaphos	Perizin®	1987–November 2017
	CheckMite+®	2006 –still approved
Flumethrin	Bayvarol®	1991–still approved
Thymol	Api Life Var®	1996–still approved
	Thymovar®	1998–still approved
	Apiguard®	2003–May 2016

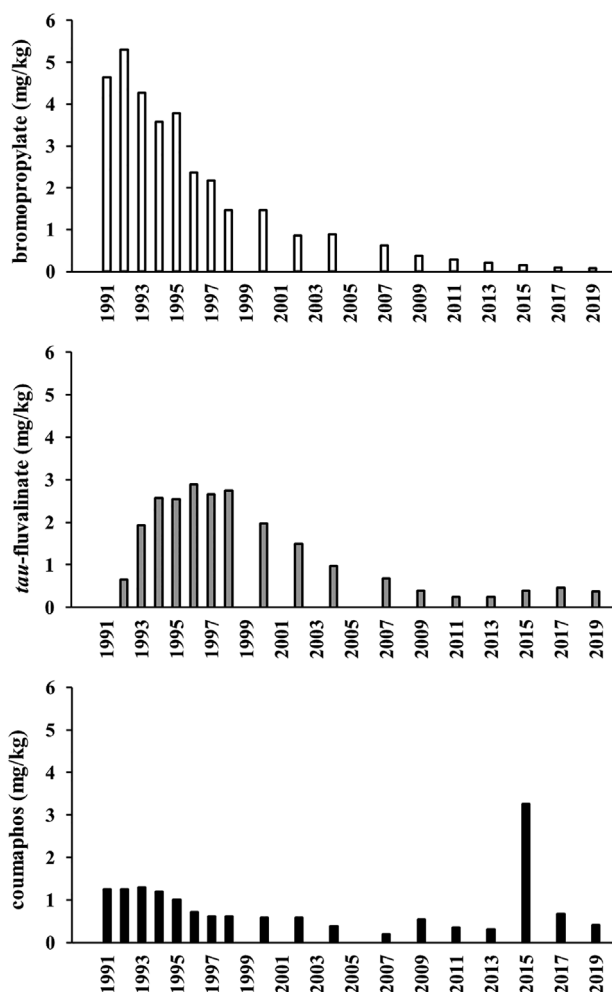


Figure 1. Monitoring of acaricide concentrations in commercial Swiss beeswax. The annual value of bromopropylate, *tau*-fluvalinate, and coumaphos for each year was calculated using the individual values obtained from the manufacturers' samples in proportion to the amounts of foundations produced per manufacturer during the year of investigation.

1 mg·kg⁻¹. During the years 1996 through 2004, values below 1 mg·kg⁻¹ were measured (Fig. 1). In 2006, an additional product containing coumaphos was approved in Switzerland for use in beekeeping (Table 1). Annual values, however, remained low until 2013, but they increased suddenly to an annual value of 3.25 mg·kg⁻¹ in 2015 (Fig. 1, Table 3). Although annual values

dropped in subsequent years, the annual samples of a few manufacturers remained high (up to 6.24 mg·kg⁻¹ in 2017 or up to 3.23 mg·kg⁻¹ in 2019; Table 3), suggesting that a few beekeepers were still using coumaphos-containing products during the last years.

Several thymol-containing products have been approved during the years 1996 to 2003 (Table 1).²⁶ Our survey shows that thymol residues in beeswax increased progressively starting from 1996 and reached a maximal annual value of 87.5 mg·kg⁻¹ in 2009 (Fig. 2), with individual annual samples of the different manufacturers varying between 39.4 and 119.0 mg·kg⁻¹ (Table 4). Since then, thymol residues have decreased continuously to an annual value of 17.4 mg·kg⁻¹ in 2019, suggesting that nowadays fewer beekeepers use thymol-containing products as compared to 2009.

Although not authorized, beekeepers used PDCB to protect beeswax combs from wax moth. Between 1992 and 2002, we found substantial PDCB residues in Swiss bees wax (Fig. 3).²³ The highest annual value of 10.9 mg·kg⁻¹ was found in 1994.²³ Since 2002, the values have decreased substantially and dropped to an annual value of 0.007 mg·kg⁻¹ in 2019 (Fig. 3). The individual annual samples of eight out of nine different manufacturers were below our LOQ of 0.37 mg·kg⁻¹ in 2019. As residue levels of PDCB are nowadays very low, Swiss beeswax is of good quality with respect to residues of PDCB.

In a repeated survey on voluntary participation, Swiss beekeepers were asked about the type of *Varroa* treatment that they used for summer and winter treatments. This question was part of a survey on winter mortality.^{28,29} The first survey was performed in 2002, and from 2012 onwards, surveys were completed annually by administering the questionnaire developed by the COLOSS group. For summer treatment, the majority of Swiss beekeepers used formic acid: 56% of the beekeepers in 2002 and 86% of the beekeepers in 2019 (Table 5). Furthermore, the survey shows that the use of thymol-containing products has decreased over the last 17 years. In 2002, 30% of the participating beekeepers were using thymol-containing products for summer treatments, whereas in 2019, fewer beekeepers (3%) used these products (Table 5). For winter treatment, the big majority of Swiss beekeepers used oxalic acid: 65% of the beekeepers in 2002/03 and 93% of the beekeepers in 2019/20 (Table 6).

4 DISCUSSION

Our survey allowed the study of the long-term behavior of pesticides in beeswax under real beekeeping conditions for nearly three decades. An identical sample collection and preparation guaranteed the consistency required for an ideal long-term study. Furthermore, this national survey on contaminants originating from beekeeping gives a general idea of the acaricides used in Switzerland in the past 30 years.

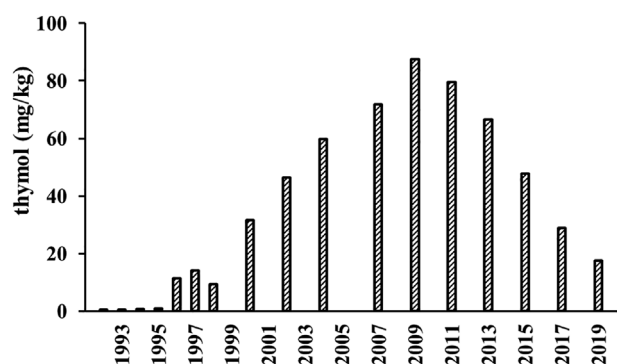
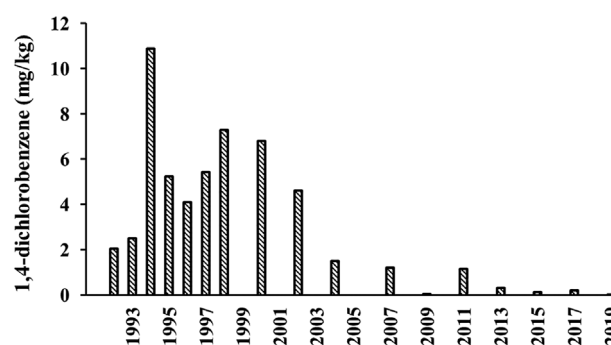
Bromopropylate is a good model substance for demonstrating the persistence of lipophilic acaricides in beeswax. Folbex VA® was the first product registered for mite control in beekeeping in Switzerland (Table 1).²⁷ After 1991, Folbex VA® was no longer used in Switzerland, as new, easy-to-use, and more efficient products like Apistan® (*tau*-fluvalinate) and Perizin® (coumaphos) became available on the market.²⁵ As a consequence, residues of bromopropylate have been steadily decreasing since 1992. Bogdanov predicted that it would take approximately 20 years for this acaricide to disappear from beeswax.⁴ In fact, 13 years later (2019), levels of bromopropylate below 0.1 mg·kg⁻¹ were

Table 2. *tau*-Fluvalinate residues in Swiss beeswax in the last 10 years

		2009	2011	2013	2015	2017	2019
Annual value	mg/kg	0.39	0.24	0.24	0.39	0.46	0.38
Proportion of positive annual samples of the manufacturers	%	100	100	100	100	100	100
Minimal value	mg/kg	0.22	0.12	0.10	0.09	0.13	0.12
Maximal value	mg/kg	0.72	0.35	0.45	0.94	0.96	0.55

Table 3. Coumaphos residues in Swiss beeswax in the last 10 years

		2009	2011	2013	2015	2017	2019
Annual value	mg/kg	0.53	0.36	0.31	3.25	0.68	0.41
Proportion of positive annual samples of the manufacturers	%	100	100	87.5	100	100	100
Minimal value	mg/kg	0.21	0.07	<0.05	0.08	0.08	0.08
Maximal value	mg/kg	0.59	1.04	0.74	4.46	6.24	3.23

**Figure 2.** Monitoring of thymol concentrations in commercial Swiss beeswax. The annual value of thymol for each year was calculated using the individual values obtained from the manufacturers' samples in proportion to the amounts of foundations produced per manufacturer during the year of investigation.**Figure 3.** Monitoring of PDCB concentrations in commercial Swiss beeswax. The annual value of PDCB for each year was calculated using the individual values obtained from the manufacturers' samples in proportion to the amounts of foundations produced per manufacturer during the year of investigation.**Table 4.** Thymol residues in Swiss beeswax in the last 10 years

		2009	2011	2013	2015	2017	2019
Annual value	mg/kg	87.5	79.5	66.7	47.8	28.8	17.4
Proportion of positive annual samples of manufacturers	%	100	100	100	100	100	100
Minimal value	mg/kg	39.4	49.6	42.5	29.2	22.0	13.0
Maximal value	mg/kg	119.0	124.8	108.6	125.6	83.2	58.9

still measurable in beeswax, as shown in this study. If a lipophilic acaricide is no longer used, residues are gradually diluted through newly produced wax, which has not been exposed to this specific acaricide. As shown in the case of bromopropylate, this process is very slow, taking more than two decades. Hence, it is important to prevent contamination of beeswax from the beginning, ideally by using organic acids for mite control, which are not lipophilic and do not accumulate in beeswax.

Tau-fluvalinate residues in beeswax were highest during the years when Apistan® was authorized in Switzerland. However, residues persisted at lower levels until now. This could be due to the import of wax from countries where *tau*-fluvalinate is authorized

in beekeeping³⁰ or as a plant protection product. Indeed, *tau*-fluvalinate is one of the most frequently detected pesticides at higher levels in beeswax of European or North American origin.^{7,9,18,31–33}

In 2002, 11% of the beekeepers used Perizin®, a coumaphos-containing product (64 mg active ingredient per application) authorized for winter treatment until 2017, while few beekeepers used this product after 2012/13. Coumaphos residue levels were low for two decades but increased suddenly in 2015, suggesting an increased use of coumaphos-containing products and/or the use of products containing higher coumaphos dosage. On the other hand, the survey shows that only a few beekeepers used

Table 5. Summer treatments according to the yearly survey in Switzerland

Year of the survey		2002	2012	2013	2014	2015	2016	2017	2018	2019
Beekeepers (%)	Thymol	29.7	15.4	13.3	10.0	8.5	5.9	4.1	4.2	3.4
	Formic acid	56.1	76.9	77.3	82.1	84.4	85.1	84.2	84.6	86.1
	Oxalic acid*	3.5	3.1	4.4	3.4	3.0	5.6	5.8	6.4	5.8
	Pyrethroids [†]	2.4	1.2	1.4	1.3	0.9	1.3	1.4	0.8	0.5
	CheckMite+ [‡]	0.0	0.0	0.0	0.2	0.5	0.6	0.2	0.0	0.1
	Other products	8.0	3.0	2.6	2.0	1.7	0.8	1.2	0.3	0.6
	Other methods [§]	0.0	0.0	0.5	0.5	0.7	0.4	3.1	3.4	2.9
	No treatment	0.4	0.3	0.6	0.5	0.3	0.3	0.1	0.2	0.6
Number of apiaries (n) included in the survey [¶]		549	1323	1237	1282	1254	1369	1379	1461	1666

*Oxalic acid with or without brood interruption.

[†] Pyrethroids (flumethrin, tau-fluvalinate in year 2002).[‡] Coumaphos-containing product authorized for summer treatment in Switzerland since 2006.[§] Other methods include hyperthermia, total removal of brood, brood interruption with a trapping comb.[¶] The number of apiaries included in 2019 (n = 1666) corresponds to apiaries of approximately 8% of the Swiss beekeepers.**Table 6.** Winter treatments according to the yearly survey in Switzerland

Year of the survey		2002/03	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19	2019/20
Beekeepers (%)	Oxalic acid	65.1	87.7	89.0	89.9	91.1	91.8	94.2	94.0	93.1
	Lactic acid	1.4	0.0	0.6	0.4	0.5	0.0	0.0	0.0	0.0
	Pyrethroids*	0.2	0.1	0.1	0.2	0.2	0.0	0.0	0.0	0.0
	Perizin [†]	10.8	0.7	0.6	0.5	0.2	0.0	0.0	0.0	0.0
	Other products or methods [‡]	3.9	5.3	3.9	4.2	3.7	1.7	0.8	1.0	1.0
	No treatment	18.7	6.2	5.7	4.8	4.3	6.5	5.0	5.1	5.9
Number of apiaries (n) included in the survey [§]		518	1322	1238	1282	1254	1369	1379	1462	1665

*Pyrethroids (flumethrin, tau-fluvalinate in year 2002).

[†] Coumaphos-containing product authorized for winter treatment in Switzerland until 2017.[‡] Other products and methods include formic acid, thymol and hyperthermia.[§] The number of apiaries included in the survey for the winter treatments can differ from the numbers in the survey of the summer treatment due to colony losses in the fall or due to missing responses from the beekeepers in the second part of the survey.

coumaphos-containing products during this period (CheckMite+[®]: 0.2% in 2014, 0.5% in 2015; Perizin[®]: 0.5% in 2014/15). As the survey was conducted on a voluntary basis, it is possible that it does not entirely reflect the average of the treatments used in Switzerland. However, we have previously shown that a single application for CheckMite+[®] (2.72 g of coumaphos in two strips) can lead to levels ranging from 36 to 159 mg·kg⁻¹ in the wax of the brood frames next to the strips 7 months after the end of the treatment.^{34,35} Hence, residue levels in the common beeswax may substantially increase if only a few beekeepers use products with a high dosage.

On account of the rise in coumaphos residues in 2015, we started an intensive information campaign for beekeepers to advise against using products containing lipophilic acaricides (except thymol). Furthermore, the manufacturers of beeswax signed an agreement to no longer sell these products. Additional measures included that beekeepers who returned wax to the manufacturers for the production of foundation sheets were required to sign that they did not use lipophilic acaricides, and at times, samples were taken for further analysis. As an alternative, beekeepers did not receive money for their wax

blocks, but instead obtained a voucher for buying new foundations later on in the season. This should encourage beekeepers to recycle solely wax that has not been exposed to lipophilic acaricides. All of these measures contributed to reduce residue levels in beeswax so that annual coumaphos residue values dropped in subsequent years, almost back to the levels before 2015. The example of coumaphos underlines the value of the Swiss monitoring program. Our collaboration with the main manufacturers of foundations allows regular exchange of information and demonstrates how both beekeepers and manufacturers can work together to maintain the good quality of bee products.

Flumethrin has been authorised in Switzerland since 1991, but very few beekeepers have used Bayvarol[®].^{24,36} Furthermore, the product contains a low dosage of flumethrin (14 mg) and only a small part of it diffuses out of the strips.³⁶ Hence, the contamination level of this acaricide in the comb wax is low²⁴ and no residue levels above the detection limit of 0.25 mg·kg⁻¹ were observed in the annual samples until 2009 (Bogdanov *et al.*³⁶; our own analysis after 2001). The changes we made to the analytical procedure during the year 2009 did not allow the detection of flumethrin,

and thus, this acaricide was not included in the monitoring from 2011 to 2019. We are currently establishing more sensitive methods for several pesticides, including flumethrin.

Thymol-containing products are part of the treatment scheme for mite control recommended by the Swiss Bee Research Centre. These products are effective against the *Varroa* mites at thymol concentrations that are tolerated by the bees.³⁷ Furthermore, thymol evaporates easily out of the foundations if they are stored in frames exposed to air.²⁶ Although our monitoring study on beeswax suggests that beekeepers gradually used thymol-containing products more frequently until 2009, the study also suggests that these products have been used less often since 2009. Indeed, yearly surveys on winter mortality in which we also ask for the type of *Varroa* treatment suggest that in 2019, fewer beekeepers in Switzerland used thymol-containing products such as Api Life Var® or Thymovar® for summer treatment as compared to the previous years (Table 5).^{28,29} Instead, the majority of beekeepers nowadays use products containing formic acid (Table 5).^{28,29} While thymol acts solely on the phoretic mites,³⁸ formic acid acts also on the mites in the capped cells.³⁹ Hence, the effect on mites sets in more quickly using formic acid as compared to thymol and thus beekeepers may prefer products based on formic acid.

Thymol can diffuse from wax into honey and thus poses a risk for honey quality, especially if the thymol concentration in honey exceeds the taste threshold of 1.1 mg·kg⁻¹.²⁶ In a laboratory assay, honey was exposed to wax containing thymol at various concentrations. Thymol levels in wax up to 500 mg·kg⁻¹ resulted in thymol levels of 0.44 ± 0.08 mg·kg⁻¹ in honey.²⁶ To verify the laboratory assay findings, we performed an additional small-scale, more field realistic experiment by pouring wax sheets containing thymol at a concentration of 500 mg·kg⁻¹. The sheets were placed in the honey supper of three colonies. Subsequently, the bees were fed with sugar to provoke the construction of the combs. Two months later, the stored honeys were collected and analyzed. The thymol concentrations in these honeys were all between 0.01 and 0.05 mg·kg⁻¹, thus well below the taste threshold of 1.1 mg·kg⁻¹. This confirms that thymol levels in wax up to 500 mg·kg⁻¹ are not problematic for honey. Given that the annual wax samples of our monitoring program never exceeded the level of 500 mg·kg⁻¹, we consider thymol-containing products used for summer treatment as safe. In fact, thymol concentrations in Swiss honeys are usually well below the taste threshold of 1.1 mg·kg⁻¹.⁴⁰

In Switzerland, the use of amitraz-containing products was never authorized for beekeeping. Therefore, amitraz as well as the metabolites of amitraz are not included in our monitoring program.

As the wax moths *Galleria mellonella* and *Achroia grisella* are a threat to stored beeswax, beekeepers have been fighting these lepidopteran pests using PDCB. PDCB levels in beeswax were highest in the 1990s. In 1994, the annual values reached a maximal level of 10.9 mg PDCB per kg wax and annual samples up to maximal levels of 60.5 mg·kg⁻¹ per manufacturer.²³ During the years 1997 to 2002, food authorities found PDCB residues in up to 46% of Swiss honey samples,²³ which led to broad information campaigns for beekeepers as well as efforts from the manufacturers of foundations to prevent contaminated wax from entering the recycling process. Furthermore, stores for beekeeping equipment stopped selling PDCB-containing products. Nowadays, Swiss beeswax is almost free of PDCB, as shown in this study. The same is true for honey as reported in 2012,⁴⁰ which indicates

a tremendous improvement in comparison to the situation of 20 years ago.

We studied annual samples that represented average values of the entire production produced during a whole year. Therefore, individual production lots may contain substantially higher residue levels. Our values probably compare best with the mean values of many individual wax samples analyzed in so many other studies. In the early years of the Swiss wax monitoring program, levels of the three acaricides bromopropylate, coumaphos, and *tau*-fluvalinate have been mostly comparable to the levels found in wax from the neighboring countries Germany and Italy.⁴ The same was true for the years 2013 and 2014.⁵ Many years later, in 2019, coumaphos and *tau*-fluvalinate levels in Swiss wax were both, on average, approximately 0.4 mg·kg⁻¹ and thymol levels were 17 mg·kg⁻¹. These acaricides are among the most frequently detected pesticides in German beeswax,^{32,33,41} reported at levels comparable to that of our study (means of 0.7 mg·kg⁻¹ for coumaphos and 0.2 mg·kg⁻¹ for *tau*-fluvalinate).⁴² High variations were observed from one sample to the other^{32,42} and *tau*-fluvalinate values were measured up to 8.5 mg·kg⁻¹ in individual samples,³² despite the fact that *tau*-fluvalinate was never authorized in Germany for beekeeping. This might be explained by the import of wax from other countries or by contamination resulting from agricultural use. Indeed, higher residue levels were found in wax on the international market,^{18–20,31,43} suggesting that the use of lipophilic acaricides might be more prominent in some non-Central European countries.

In conclusion, our study makes it evident that during the last three decades, the quality of beeswax with respect to residue levels of lipophilic synthetic acaricides has clearly improved in Switzerland. For example, when a beekeeper bought wax during the year 1997, the wax contained on average 5.3 mg of lipophilic acaricides (sum of bromopropylate, *tau*-fluvalinate, and coumaphos) per kg. More than 20 years later, in 2019, wax contained substantially lower levels of the same acaricides (0.4 mg·kg⁻¹ *tau*-fluvalinate, 0.4 mg·kg⁻¹ coumaphos and 0.08 mg·kg⁻¹ bromopropylate corresponding to a total of 0.9 mg·kg⁻¹). As a very positive development, the majority of beekeepers now mostly use hydrophilic organic acids that do not accumulate in beeswax.

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