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Flumethrin

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Flumethrin

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Identity

Flumethrin is a pyrethroid acaracide composed of a mixture of two diastereomers (trans-Z1 and trans-Z2, with an approximate ratio 60:40).

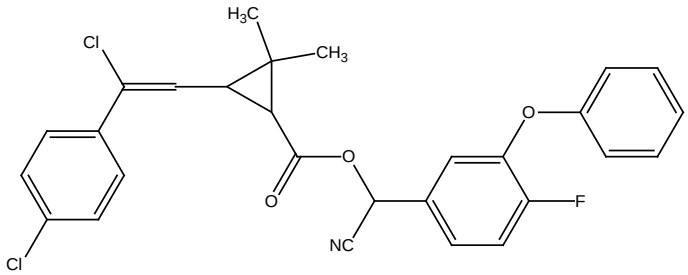
The laboratory studies to determine the physical properties of flumethrin were conducted to GLP.

International Non-proprietary Name (INN): Flumethrin

IUPAC name: (±)-α-cyano-4-fluoro-3-phenoxybenzyl-3-(β,4-dichlorostyryl) 2,2-dimethylcyclopropanecarboxylate or cyano(4-fluoro-3-phenoxyphenyl)methyl 3-[2-chloro-2-(4-chlorophenyl)vinyl]-2,2-dimethylcyclopropanecarboxylate.

Chemical Abstract Service (CAS) No.: 69770-45-2

Structural formula:



Molecular formula: $C_{28}H_{22}Cl_2FNO_3$

Molecular weight: 510.39 g/mol (pure substance)

Other information on identity and properties

Pure active ingredient: Flumethrin

Appearance: Highly viscous yellowish oil; no characteristic odour.

Solubility (at 20 °C):

In pure water: trans-Z1: 0.1 µg/l; trans-Z2: 0.1 µg/l; sum of trans-Z1 + Z2: 0.2 µg/l

In 1 % NaCl solution: < 0.03 µg/l (both diastereomers)

In water buffered at pH 4 or pH 7: < 0.03 µg/l (both diastereomers)

In water buffered at pH 9: hydrolysis

Table 1 . Solubility in organic solvents

	diastereomer		
	trans-Z1	**trans-Z2**	**trans-Z1 + Z2 (sum)**
n-Heptane	11 g/l	8 g/l	19 g/l
Xylene			> 250 g/l
1,2-Dichloroethane			> 250 g/l
2-Propanol	36 g/l	29 g/l	65 g/l
1-Octanol	69 g/l	56 g/l	130 g/l
Polyethyleneglycol			100 - 200 g/l
Acetone			> 250 g/l
Dimethylformamide			> 250 g/l
Acetonitrile			> 250 g/l
Ethylacetate			> 250 g/l
Dimethylsulfoxide			> 250 g/l

Log K_{ow} or Partition Coefficient: Log P_{ow} = 6.2

pH: The substance has no acidic or basic properties in aqueous solutions (the low water solubility makes the substance unsuitable for experimental methods to determine the pH).

Optical rotation: No data were provided.

UVmax: No data were provided.

Stability: On the basis of DTA/TGA measurements carried out in accordance with OECD Guideline No. 113 the substance is thermally stable at ambient temperature under air.

Vapour pressure: The substance has a very low vapour pressure.

Background

Flumethrin is registered in several countries worldwide for the diagnosis and control of varroatosis (aka varroosis) in honey bee colonies. Varroatosis is a disease of honey bees caused by the parasitic mite *Varroa destructor*. The substance can be formulated into a Low Density Polyethylene (LDPE) strip, which is impregnated with 3.6 mg flumethrin (0.5 mg/cm^3). This product is inserted between the combs in the brood chamber of the beehive. When used as recommended, no withdrawal period is required, although this is based on various restrictions to use.

The product is authorised for use in the following countries: Canada, Greece, Ireland, Mexico, New Zealand, Spain, Turkey and the United Kingdom.

Another type of beehive strip, where the flumethrin is impregnated into polyvinyl chloride (PVC), has also been authorised in some member states, which instead of being inserted between the honey combs, is used as a gate at the entrance of the beehive.

Residues in food and their evaluation

Conditions of use

Between-comb strips:

The product should be used after the honey is harvested, usually in late summer. It should not be used during the period of honey flow. For use as a diagnostic tool, or in cases of severe infestation, the product can be used at any time of the year (all regions).

In cases of treatment for high infestation during honey flow periods, the comb honey should not be sold (New Zealand only; this is not stated directly in other regions, but it is implied).

Beehive gates:

All colonies located on the same apiary should be treated simultaneously.

The product is intended to be used as part of an integrated varroa control programme.

As an effective method to reduce the risk of resistance selection, flumethrin – as for other acaricides – should not be used in consecutive years. Instead, strict rotation with products containing active substances from other chemical classes should be applied. Depending on the regional resistance situation, a longer treatment break than one year may be necessary. As flumethrin and tau-fluvalinate belong to the same class, they are not suitable for rotation with each other.

Inappropriate use of the product could result in an increased risk of resistance development and could ultimately result in ineffective therapy and colony losses.

Flight activity is necessary for exposure to the active substance. In case of prolonged periods of low flight activity, e.g., due to unfavourable weather conditions, efficacy may be reduced.

The beehive gates should not be reused.

Dosage

Between-comb strips:

The usual dose recommended in all regions where the between-comb product is authorised is 4 strips per chamber in developed colonies, or 2 strips per chamber in young colonies. The strips are reported to contain 3.6 mg flumethrin each (0.5 mg/cm^3). The strips are suspended in the spaces between the combs in the central brood rearing area. The strips should remain in the colony for 24 h (diagnosis) or for 4 to 8 weeks (treatment), although the most common recommendation is for 6 weeks.

Beehive gates:

The recommended dose for the beehive gate strips (275 mg flumethrin per strip) is 2 strips per beehive, fitted to the entrance of the hive, so that the bees are forced to use the holes that are in the strips (15 per strip) to enter or leave the hive. The recommended duration of application is between 9 weeks and 4 months, just after honey flow and extraction.

Pharmacokinetics and metabolism

Data on mammalian pharmacokinetic and metabolism are provided in the toxicology submission.

Pharmacokinetics in laboratory animals

When groups of rats were given 1 mg/kg bw ^{14}C[Cl]-flumethrin PO in 5 % aqueous Cremophor vehicle, 68 % of the radioactivity was eliminated in the faeces, and around 2 % in the urine, during the first 24 h.

Conversely, after PO administration of 1 mg/kg bw ^{14}C[F]-flumethrin, 45 % of the radioactivity was eliminated in urine and the rest in the faeces. Females appeared to absorb a considerably higher percentage of the dose than males after oral administration.

The plasma elimination half-lives of ^{14}C[Cl]- and ^{14}C[F]-flumethrin in PO-dosed rats were approximately 160 h and 8 h, respectively. No information on the nature of the metabolites derived from the acid moiety (which was radiolabelled in the chlorophenyl ring), which remained in the blood of treated rats for prolonged periods, are available.

Repeated daily PO dosing of 1 mg/kg bw ^{14}C[Cl]-flumethrin to 8 male rats resulted in an accumulation of radioactivity in the plasma.

After PO administration of 1 mg/kg bw ^{14}C-flumethrin to rats of both sexes, residues concentrations were highest in plasma (V_{ss} approximately 0.4 l/kg), and most of the radioactivity remained in the stomach for up to 312 h after administration.

Pharmacokinetics in Food-producing Animals (bees/honey)

Pharmacokinetic studies in beehives or honey are not applicable.

When bee colonies were exposed to the flumethrin-impregnated strips in the spring, pre-winter and during the nectar flow period, the flumethrin concentration in honey was found to be < LOD (1-2 µg/kg). The content in wax from the nearby combs was 30, 40 and 90 µg/kg, respectively. The highest concentration of flumethrin detected in beeswax was 130 µg/kg, found in a sample from a hive treated during the honey flow period.

Transfer of flumethrin from beeswax to honey was negligible, but residues in beeswax may accumulate if the wax is reused over several years. Residues of up to 61 µg/kg were found in wax from hives that had been treated annually for approximately 10 years.

In supervised residue trials for beehive gates used according to proposed label, with 2 strips (275 mg/strip) applied at the entrance of the hive, residues of flumethrin in honey were <LOQ

(<3 µg/kg). Residues of flumethrin in wax were in the range of <25 – 119 µg/kg. ***Metabolism in Laboratory Animals***

Rats

In rats, flumethrin is metabolised by hydrolysis of its central ester bond to form flumethrin acid and 3-phenol-4-fluorobenzyl alcohol. Flumethrin acid is then conjugated with glucuronic acid to form the glucuronide. 3-phenol-4-fluorobenzyl alcohol is not detected in the rat, but is oxidised to 4-fluoro-3-phenoxybenzoic acid and then to 4-fluoro-3-(4-hydroxyphenoxy)benzoic acid, both of which form glycine conjugates.

Metabolism in Food Producing Animals (bees/honey)

There is no known metabolism of the substance by the bees; the majority of the residues end up in the beeswax and honey, where no biotransformation can take place. Therefore, the only way of reducing the concentration of flumethrin in the honey is via a degradation process. However, investigations into the stability of residues of flumethrin in honey stored at room temperature have shown that no significant decrease in concentration was observed over a storage period of 9 months. This demonstrates that flumethrin does not degrade in honey. It is noted that the Committee has not evaluated any data regarding the disposition of flumethrin in other commodities derived from beehives, such as propolis, royal jelly, etc.

Comparative Metabolism

Since flumethrin is not metabolised when used in beehives, no comparative metabolism can be determined.

Residue depletion studies in honey

Radiolabelled residue depletion studies

No radiolabelled residues depletion study has been conducted or provided.

Residues depletion studies with non-radiolabelled drug

Between-comb strips:

Six non-GLP compliant residues depletion studies of flumethrin in honey produced by honeybees were conducted in various locations in Germany and the UK. All studies used Bayvarol strips, which are LDPE strips impregnated with 3.6 mg flumethrin per strip.

In one study, 24 honeybee colonies were treated with 4 strips each, corresponding to a total dose of 14.4 mg flumethrin, for a duration of 6 months, over the winter period. One sample was analysed, no residues were detected (LOQ = 3 µg/kg; LOD = 1 µg/kg). At another site, 12 honeybee colonies were treated for 6 weeks with 4 strips per hive, from the beginning of September to the middle of October, over 2 years. Four samples were analysed, no residues were detected (LOQ = 3 µg/kg; LOD = 1 µg/kg).

In the second study, honeybee colonies were treated with 4 strips per brood chamber for six weeks, after the honey harvest. One sample was taken, during the spring honey flow. The results of the analysis showed no detectable residues of flumethrin in the honey (LOQ = 3 µg/kg; LOD = 1 µg/kg).

In the third study, six honeybee colonies were treated for 6 weeks, from September to October, with 4 strips per frame. Six samples were taken in June the next year and combined in pairs to form three samples for analysis. The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

In the fourth study, six honeybee colonies were treated for 5 months from October to March with 4 strips per frame. Six samples of freshly capped honeycomb were taken in June of the following year, after the early nectar flow. The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

In the fifth study, seven honeybee colonies were treated from early March to mid-April with 4 strips per frame. At the end of the treatment period, these combs were labelled and suspended in the honey chamber until the last brood had hatched and fresh honey had been inserted and capped. The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

In the final non-GLP study, four colonies of honeybees were treated for 4 months from May to September, during the honey flow period. The entire honey harvest of each treated colony was centrifuged separately in the last week of August. The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

Table 2. Summary of residues trials using between-comb strips

Honey Trial Country, year	Treatment					Time of sample collection	Flumethrin residue (µg/kg)	Reference
	Formulation and use rate	No of colonies	No of samples	Duration (weeks)	Treatment Period			
Lindlar, Germany 1992-1993	Bayvarol strips 3.6 mg/strip 4 strips/hive	24	1	23	12 Oct 1992-08 Mar 1993	15 Jun 1993	≤1	Krebber, R., 1994a Report RA-158/94 Bayer Ref: 014313
Leverkusen, Germany 1991-1992	Bayvarol strips 3.6 mg/strip 4 strips/hive	12	4	6	early Sept-mid Oct 1991 early Sept-mid Oct 1992	1993 (end of fruit and dandelion flowering)	≤ x 4	
UK 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	Not reported	Spring 1993	≤1	Krebber, R., 1994b Report RA-233/94 Bayer Ref: 014338
Rhineland, Germany 1987-88	Bayvarol strips 3.6 mg/strip 4 strips/hive	6	3 (each sample from 2 colonies)	6	early Sept - mid-Oct 1987 (pre-winter storage period)	June 1988 (after early nectar flow)	≤ 2 x 3	Riegner, K., Krieger, K., 1990a Report 90/13521 Bayer Ref: 015989

Description of individual residue trials in honey using between-comb strips

Reference AH ID 014313en.pdf (Report No. 158/94)

Residues of Flumethrin in Honey after Administration of Bayvarol Strips to Honeybee Colonies in Germany; Project number P65335008 (Krebber, 1994a).

At one site, in Lindlar, North Rhine-Westphalia, Germany, 24 colonies of honeybees were treated with 4 LDPE strips impregnated with flumethrin. Treatment commenced on 12th October 1992 and was completed (the strips were removed) on 8th March 1993, so these colonies were exposed to the product for 6 months, over winter. The honey was sampled on 15th June 1993 and stored at 4 °C until analysis. One sample was analysed.

At another site, in Leverkusen, North Rhine-Westphalia, Germany, 12 honeybee colonies were treated for 6 weeks with 4 strips per hive from the beginning of September to the middle of October over 2 years (1991 & 1992). Honey samples were taken in 1993, at the end of fruit and dandelion flowering (exact dates not stated). Four samples were analysed.

All samples were analysed using method RA-654/93 (HPLC-UV).

Table 3. Recovery from honey fortified with 3 µg/kg flumethrin

Recovery values (%) n = 4				Mean (%)	Relative Standard Deviation (RSD %)
93	93	83	80	87.25	7.7

The results of the analyses showed no detectable residues of flumethrin in the honey from the treated colonies (LOQ = 3 µg/kg; LOD = 1 µg/kg).

Reference AH ID 014338en.pdf (Report No. RA-233/94)

Residues of Flumethrin in Honey after Administration of Bayvarol Strips to Honeybee Colonies in Great Britain; Project number P65335009, Report number RA-233/94 (Krebber, 1994b).

Beehives in Great Britain were treated as recommended in the instructions for use. One sample was taken during the spring honey flow of 1993. No further information was provided.

The sample was analysed using method RA-654/93 (HPLC-UV).

The results of the analysis showed no detectable residues of flumethrin in the honey (LOQ = 3 µg/kg; LOD = 1 µg/kg).

Reference AH ID 015989en.pdf (Doc No. 90/13521)

Residues of Flumethrin in Honey Following the Use of Bayvarol Strips during the Pre-Winter Storage Period (Riegner & Krieger, 1990a)

Six commercial bee colonies belonging to a single beekeeper in the Rhineland, Germany were treated for 6 weeks, from early September to mid-October 1987, with 4 strips per frame (each frame contained 8-10 Freudenstein combs). Two strips were suspended in each of two gaps between the central combs of the brood nest, so that the bees could crawl over both sides.

In June 1988, after the early nectar flow, one freshly capped honeycomb from the honey chamber of each colony was removed and centrifuged. The samples obtained were stored at 4 °C until preparation for analysis.

Equal weights of samples 1 & 2, 3 & 4, and 5 & 6, respectively, were combined to give three samples, which were analysed on 12th August 1987, using method RA-197. The report number of the analysis part is RA-1104/RGK046.

The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

Reference AH ID 015990en.pdf (Doc No. 90/13522)

Residues of Flumethrin in Honey Following the Use of Bayvarol Strips During the Winter (Riegner & Krieger, 1990b).

Six experimental colonies of honeybees in Bergische Land, North Rhine-Westphalia, Germany, were treated for 5 months from late October 1987 to mid-March 1988 with 4 strips per frame. Two strips were suspended in each of two gaps between the central combs of the brood nest, so that the bees could crawl over both sides. Six samples of freshly capped honeycomb were taken in June of the following year, after the early nectar flow.

Samples 1 & 2, 3 & 4, and 5 & 6, respectively, were combined to give three samples, which were analysed on 21/22 Jun 1988, using method RA-197. The report number of the analysis part is RA-1106/RGK048.

The results of analysis from all samples showed no residues above the LOQ (LOQ = 2 µg/kg).

Reference AH ID 015991en.pdf (Doc number 90/13523)

Residues of Flumethrin in Honey Following the Use of Bayvarol Strips in Spring (Doc number 90/13523, Riegner & Krieger, 1990c)

Seven experimental honey bee colonies in Taunus, Hesse, Germany, were treated from early March to mid-April 1986 with 4 strips per frame (10 Zander combs per frame).

Two strips were suspended between combs 3 & 4 and between combs 7 & 8, so that the bees could crawl over both sides.

At the end of the treatment period, these combs were labelled and suspended in the honey chamber until the last brood had hatched and fresh honey had been inserted and capped.

The honey from combs 3 & 4 and combs 7 & 8 was centrifuged and analysed separately.

In order to compare two colonies (5 and 21), honey from the two combs furthest away from the strips (1 & 10) was centrifuged and analysed.

The samples were analysed in December 1985 using method RA-197. The report number of the analysis part is RA-273.

The results of analysis from all samples showed no residues above the Limit of Quantification (LOQ = 2 µg/kg).

Reference AH ID 015992en.pdf (Doc number 90/13524)

Residues of Flumethrin in Honey Following the Use of Bayvarol Strips During the Nectar Flow Period (Doc number 90/13524, Riegner & Krieger, 1990d)

Four experimental colonies of honey bees in Bergische Land, North Rhine-Westphalia, Germany, were treated from May to September (4 months) 1988, with 4 strips per brood fame (each containing 11 DN combs).

The strips were inserted into the honey chamber when the colony swarmed in the first week of May, and were suspended in the central gaps between the combs, so that the bees could crawl over both sides.

The entire honey harvest of each treated colony was centrifuged separately in the last week of August.

The samples were stored at 4 °C until being prepared for analysis. The samples were analysed on 14th October 1988, using method RA-197. The report number of the analysis part is RA-1110/RGK053.

The results of analysis from all samples showed no residues above the LOQ (LOQ = 2 µg/kg).

Table 4. Summary of residues studies using beehive gates

Honey		Treatment				Time of sample collection	Flumethrin residue (µg/kg)	Reference
Trial Country, year	**Formulation and use rate**	**No of colonies**	**No of samples**	**Duration (weeks)**	**Treatment Period**			
Apiary A1 63571 Gelnhausen, Germany 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	7	1	17	13 Aug 2014 – 11 Dec 2014	21 May 2015	≤ 3	Krebber, R. and Hoffend, J., 2015 Report MR-15/183 Bayer Ref: 41149 Altreuther, G., 2017, Supplementary information to study 41149.
Apiary A2 63543 Neuberg, Germany 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	17	13 Aug 2014 – 11 Dec 2014	21 May 2015	≤ 3	
Apiary A3 55131 Mainz, Germany 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	18	14 Aug 2014 – 15 Dec 2014	25 May 2015	≤ 3	
Apiary A4 63526 Erlensee, Germany 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	13	1	17	13 Aug 2014 – 11 Dec 2014	20 May 2015	≤ 3	
Apiary B1 6708 PB Wageningen, The Netherlands 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	17			≤ 3	
Apiary B2 5386 KR Geffen, The Netherlands 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	5	1	17	20 Aug 2014 – 16 Dec 2014	16 Jun 2015	≤ 3	

Honey			Treatment			Time of sample collection	Flumethrin residue (µg/kg)	Reference
Trial Country, year	**Formulation and use rate**	**No of colonies**	**No of samples**	**Duration (weeks)**	**Treatment Period**			
Apiary B3 4221 LH Hoogblokland, The Netherlands 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	7	1	17	20 Aug 2014 – 16 Dec 2014	17 Jun 2015	≤ 3	
Apiary C1 8349 Zalagyömörö, Hungary 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	15	25 Aug 2014 – 05 Dec 2014	11 May 2015	≤ 3	
Apiary C2 8330 Sümeg, Hungary 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	15	25 Aug 2014 – 05 Dec 2014	12 May 2015	≤ 3	
Apiary D1 19180 Marchamalo, Spain 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	13	21 Oct 2014 – 19 Jan 2015	08 Jun 2015	≤ 3	
Apiary D2 19180 Marchamalo, Spain 2014-2015	PolyVar Yellow strips 275 mg/strip 2 strips/hive	6	1	13	21 Oct 2014 – 19 Jan 2015	08 Jun 2015	≤ 3	

Description of residue trials in honey using Beehive gate strips.

Reference ID41149:

Determination of flumethrin in honey and wax. Report No.: MR-15/183. Study Number: P673155038 (Study No. 201394), (Krebber & Hoffend, 2015).

The applicant provided one GLP-compliant field study conducted in beehives, using beehive gate strips: Honeycombs were obtained from different test sites in Germany (4 apiaries), Hungary (2 apiaries), Spain (2 apiaries) and The Netherlands (3 apiaries) after application of Polyvar Yellow (flumethrin bee-hive gate). Both honey and beeswax were sampled and analysed.

Thirty-two honeycomb samples from four apiaries in Germany, 14 samples from two apiaries in Hungary, 12 samples from two apiaries in Spain and 18 samples from three apiaries in The Netherlands were analysed.

The maximum application time at hive entrance of 4 months is covered by data from Germany (122 or 120 days) and the Netherlands (119 days). In the other regions, application time was 92 days (Spain) and 102 days (Hungary).

For separation of honey and wax, honey-filled wax cells were scraped off from representative areas of the upper third from both sides of the collected honey combs and transferred to a plastic bag. Each of these sample bags was kept at 40 °C until wax has floated and separated from the honey. This procedure took up to five days. Then, one edge of the bottom of each plastic bag was cut and the honey was collected in a beaker. Aliquots of the samples were filled into glass vials. The remaining mixture in the bag was transferred to a sieve and the remaining honey was washed off thoroughly with water. The pure wax was then transferred to a glass dish and kept at 40 °C until it was dry and transferred to a new plastic bag for storage.

For residue analysis the preparation of honey and wax pooled samples from all hives of one apiary was necessary. Therefore, equal amounts of the previously purified honey samples were combined and homogenized by stirring at 40 °C. Wax samples were melted at around 63 °C, homogenised and purified from contamination by skimming off or passing it again through a sieve.

The honey and wax samples were analysed for concentrations of flumethrin based using a validated LC-MS/MS method.

Residue concentrations in honey were below the limit of quantification of 3 µg/kg and also below the lowest calibration standard which corresponds to 1 µg/kg.

Residue depletion studies in beeswax

Between-comb strips

Five non-GLP compliant residues depletion studies of flumethrin in beeswax produced by honeybees were conducted in various locations in Germany and Switzerland. All studies used between-comb strips, impregnated with 3.6 mg flumethrin per strip.

In the first study, 15 beehives were treated as per product instructions (4 strips per brood chamber for six weeks, after the honey harvest) in three Swiss cantons. Thirteen samples of beeswax were analysed for flumethrin and residues were found in the range < 26 µg/kg to 176 µg/kg. Mean residues were around 50 µg/kg. The LOQ for the analytical method used was 26 µg/kg.

In the second study, an unreported number of beehives were treated with ten times the recommended number of strips (40 strips per hive). The analysis of four samples was reported and the results were in the range 70 – 146 µg/kg; mean = 106 µg/kg.

In the third study, two beehives were treated with 4 strips per chamber for 6 weeks, just before the start of honey flow. Two samples per frame were analysed. Residues in the range < 15 to 40 µg/kg were reported. The LOQ of the analytical method used was 15 µg/kg.

In the fourth study, six beehives were treated with 4 strips per brood chamber for 6 weeks, from September to October, after the honey harvest. The following year, samples were taken in June, after the early honey flow. One honeycomb was taken from each hive and combined with one from another beehive to form three samples, which were then analysed for flumethrin. The results of the analyses showed residues in the range of < 20 – 50 µg/kg.

In the final study, four beehives were treated with 4 strips per brood chamber for six months, during the honey flow period. The strips were inserted in May and removed in September. Two combs from each hive that had been in the brood chamber were moved to the honey chamber in mid-August until the brood hatched. These combs were sampled and analysed. The results were in the range 30 – 130 µg/kg.

Beehive gates

With the beehive gates, four supervised trials were carried out on wax in 11 apiaries in Europe during 2014-2015. All trials were conducted according to proposed label with 2 strips applied at the entrance of each hive. Each apiary consisted of multiple colonies.

Residues of flumethrin in wax were in the range of <0.025 – 0.119 mg/kg. The results of the trials are presented. Samples were analysed using an HPLC/MS/MS method with an LOQ of 25 µg/kg. The mean procedural recovery was 99 % (fortification range 25-250 µg/kg, n = 18, RSD = 6.2 %).

Table 5. Summary of residues in beeswax

Beeswax	Treatment					Time of sample collection	Flumethrin residue (µg/kg)
Trial Country, year	**Formulation and use rate**	**No of colonies**	**No of samples**	**Duration (weeks)**	**Treatment Period**		
6482 Seedorf, Uri, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip ”Following instructions for use”	Not reported	1	Not reported	1992 and 1993	1993	53
6484 Wassen, Uri, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	26
1510 Moudon, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	≤26
1812 Ecoteaux, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	≤26
1142 Pampigny, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	26
1142 Eclepéns, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	≤26
1142 Eclepéns, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	72
1110 Morges, Waadt, Switzerland 1992-1993	Bayvarol strips 3.6 mg/strip Use rate not reported	Not reported	1	Not reported	1992 and 1993	1993	55

Description of individual residue trials in wax using between-comb strips

Reference AH ID 014583en.pdf

Residues of Flumethrin in Beeswax after Administration of Bayvarol Strips to Honeybee Colonies in Switzerland (Krebber, 1994 – Report No. RA-126/94; Project No. P65345009)

Honeybee colonies in the Swiss cantons Neuenburg, Uri and Waadt were treated with Bayvarol strips according to the instructions for use in the years 1992 and 1993. Wax samples were taken in 1993 and analysed for residues of flumethrin (in July 1994).

The samples were analysed using method RA-654/93.

Table 6. Concurrent QC recoveries:

Fortification level (µg/kg)	Recovered (%)	Mean value (%)
26*	92 62 77 104	84
100	85 74	80

*Determined as LOQ.

Analyses were performed from July 12 to July 26, 1994.The concentrations of residue were in the range of < LOQ (26 µg/kg) to 176 µg/kg. The results were not corrected with respect to the recovery rate.

Reference AH ID 014714en.pdf

Residues of Flumethrin in Beeswax after Treatment with a Tenfold Overdose of Bayvarol Strips to Honeybee Colonies in Germany (Krebber 1994, Report No. 286/94; Project No. P65335010).

Beehives in Freiburg, Germany were treated with a 10 x overdose of Bayvarol Strips (40 strips per hive) from 28th June 1991 to 23rd July 1991. Four 10 g samples of wax were taken on 13th August 1991 and analysed for residues of flumethrin (in August 1994).

The samples were analysed using method RA-654/93.

Table 7. Concurrent QC recoveries:

Fortification level (µg/kg)	Recovered (%)	Mean value (%)
26*	92 62 77 104 85	84
100	85 74 81	80

*Determined as LOQ.

The concentrations were between 103 and 146 µg/kg. Analyses were performed from 2nd to 4th August, 1994. The results were not corrected with respect to the recovery rate.

No raw data were provided.

Reference AH ID 015993en.pdf

Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips in Spring. (Riegner & Krieger 1990; Doc No. 90/13518)

Two experimental bee colonies in the Taunus region of Germany were treated with 4 strips per frame (10 Zander combs per frame) for 6 weeks, from early March to Mid-April 1986, immediately before the start of the nectar flow period. Two strips each were suspended between combs 3 and 4, and 7 and 8, respectively, so that the bees could crawl over both sides of the strips.

At the end of the treatment period, combs 3 and 8, and 4 and 7, respectively, were combined to form two samples per frame. Samples were analysed using method RA-197. The report number was RA-273. The LOQ for the method was stated as being 15 µg/kg wax.

Samples were analysed on 6th March 1986. One concurrent QC at 200 µg/kg was analysed (n=5).

The concentrations were between <LOQ and 40 µg/kg (corrected for recovery of 62 %).

Reference AH ID 015994en.pdf

Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips during the Pre-winter Storage Period (Riegner & Krieger, 1990; Doc No. 90/13519).

Six commercial bee colonies in the Rhineland, Germany, were each treated for 6 weeks with 4 strips per frame (8-10 Freudenstein combs per frame), from early September to mid-October 1987. The strips were suspended between the central combs of the brood nest, so that the bees could crawl over both sides of the strips.

In June the following year, after early nectar flow, one freshly capped honeycomb was removed from the honey chamber of each colony and centrifuged (to separate the honey from the comb). The comb samples were stored at 4 °C until preparation for analysis. Equal parts (w/w) of samples 1 & 2, 3 & 4 and 5 & 6, respectively, were combined to form three samples, which were then analysed using method RA-197 (report no RA-1104/RGK046, not provided).

Samples were analysed on 14th and 22nd October 1987

The concentrations were between < LOQ and 50 µg/kg (corrected for recovery of 62 %).

Concurrent QC samples were also analysed (n = 1 each) 473 µg/kg on the 14th, and 472 µg/kg on the 22nd. The LOQ was reported as 20 µg/kg.

Reference AH ID 015995en.pdf

Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips during the Nectar Flow Period (Riegner & Krieger, 1990; Doc No. 90/13520)

Four experimental colonies of domestic honey bees in Bergische Land were treated with 4 Bayvarol strips per brood frame (11 DN combs per frame) from May to September 1988. The strips were inserted around the honey chamber when the colony swarmed in the first week of May. The strips were suspended in the central passages of the brood chamber, between the combs, so that the bees could crawl over both sides of the strips. Two combs that had been in

the brood chamber until mid-August were moved to the honey chamber until the brood hatched. These combs were removed and stored at 4 °C until being prepared for analysis.

The residues analysis was performed using method RA-197 and the procedure was recorded under RA-1113/RGK073. The analyses took place on 9th December 1988 (samples B-2, B-3, & B-4), and 3rd December 1988 (sample B-5).

The results of the analyses were between 30 and 130 µg/kg (corrected for recovery of 62 %).

Concurrent QC samples (n=2) were analysed with each analytical run, spiked at 253 ppb. The mean results of the test samples were compared to the mean of two QC samples to determine the concentration of flumethrin in the test samples.

Description of individual residue trials in wax using beehive gates

Four supervised trials were carried out using beehive gates, in Germany (4 apiaries), Hungary (2 apiaries), Spain (2 apiaries) and the Netherlands (3 apiaries) during 2014-2015. All trials were conducted according to proposed label for PolyVar Yellow, with 2 strips applied at the entrance of each hive. Each apiary consisted of multiple colonies. Honeycomb samples were collected from each colony and stored at either ambient temperature or frozen prior to shipment to the analytical laboratory. Wax was separated from the honeycombs and pooled to give one wax sample per apiary. The pooled wax samples were stored frozen at < 18 °C prior to analysis.

Samples were analysed using method an HPLC/MS/MS method (01462) with an LOQ of 25 µg/kg. The mean procedural recovery was 99 % (fortification range 25-250 µg/kg, n = 18, RSD = 6.2 %).

Prior to separation of the wax from the honeycomb, samples were stored at ambient temperature for up to 56 days or frozen for up to 72 days. After separation from the honeycomb, wax samples were stored frozen at < 18 °C for up to 44 days before analysis. In the trials, therefore, all wax samples were analysed within 3.5 months after collection.

Residues of flumethrin in wax were in the range of < 25 – 119 µg/kg.

Methods of analysis for residues in honey and beeswax

Table 8. Analytical Methods

Reference Number	Author(s)	Year	Study Title
016001	Riegner, K.	1986	Analytical method to determine the content of Bayvarol (FCR-1622) in honey and beeswax. Report No. RA-197 Bayer Ref: 016001 Non-GLP; Unpublished 25 Mar 1986
013910	Heukamp, U.	1993	Analytical method for the determination of Bayvarol (active ingredient flumethrin) in honey and wax. Report No. RA- 411/92

			Bayer Ref: 013910 Non-GLP; Unpublished 02 Apr 1993
016000	Heukamp, Krebber, R.	U.,1993	Method for determining the residue of flumethrin in bees' honey and wax. Report No. RA-654/93 Bayer Ref: 016000 Non-GLP; Unpublished 02 Nov 1993
041150	Krebber, Hoffend, Anjard, O.	R.,2015 J.,	Analytical method for the determination of flumethrin in bees honey and wax by LC-MS/MS Report No. MR-15/101, Bayer Ref: 041150 Non-GLP; Unpublished 03 Dec 2015 Also referred to as 'Enforcement Method' Method 01462

Summary of Method Validation Data

Table 9. Methods based on HPLC/UV

Matrix	Analyte/ Method	Fortification µg/kg	n	Range Recovery (%)	Mean recovery (%)	% RSD	Method	Reference
Honey	Flumethrin	3.1	2	61-63	62	-	RA-197	Riegner, 1986, Report No. RA-197, Bayer Ref: 016001
		3.8	2	54-74	64	-		
		4.4	3	58-70	63	10		
Honey	Flumethrin	3	5	72-76	74	2	00317 and 00339	Heukamp, 1993, Report No. RA-411/92, Bayer Ref: 013910 Heukamp and Krebber, 1993, Report No. RA-654/93, Bayer Ref: 016000
		13	5	85-88	87	1		
		85	5	83-89	86	3		
Wax	Flumethrin	25	2	58-66	62	-	RA-197	Riegner, 1986, Report No. RA-197, Bayer Ref: 016001
		50	2	53-69	61	-		
		100	3	58-69	63	9		
Wax	Flumethrin	26	5	54-65	60	7	00317 and 00339	Heukamp, 1993, Report No. RA-411/92, Bayer Ref: 013910 Heukamp and Krebber, 1993, Report No. RA-654/93, Bayer Ref: 016000
		51	5	65-90	79	13		
		102	5	61-96	76	19		

Table 10. Methods based on LC-MS/MS

Matrix	Analyte	Fortification Level (µg/kg)	Range Recovery (%)	n	Mean (%)	RSD (%)	Reference
Honey (quantification m/z 527 → m/z 267)	Flumethrin (LC-MS/MS)	3	78-97	6	85	8.6	Method 01462 Krebber, Hoffend and Anjard, 2015, Report No. MR-15/101, Bayer Ref: 041150
		6	78-90	6	86	4.9	
		30	77-90	6	83	5.3	
		Overall	**77-97**	**18**	**85**	**6.4**	
Honey (confirmation m/z 527 → m/z 239)	Flumethrin (LC-MS/MS)	3	80-98	6	88	8.8	
		6	76-94	6	86	6.7	
		30	79-92	6	84	5.8	
		Overall	**76-98**	**18**	**86**	**7.1**	
Wax (quantification m/z 527 → m/z 267)	Flumethrin (LC-MS/MS)	25	87-103	6	96	6.7	
		50	98-104	6	101	2.1	
		250	87-108	6	101	7.4	
		Overall	**87-108**	**18**	**99**	**6.2**	
Wax (confirmation m/z 527 → m/z 239)	Flumethrin (LC-MS/MS)	25	66-96	6	83	19	
		50	87-113	6	99	9.4	
		250	85-103	6	96	7.0	
		Overall	**66-113**	**18**	**92**	**13**	

Method RA-654/93 summary:

Honey:

Flumethrin was extracted from honey with a mixture of toluene, dichloromethane and methanol. After evaporation to dryness, the residue was re-dissolved in ethylacetate/cyclohexane. Further clean-up was performed by gel permeation chromatography on silica gel, with an elution mixture of n-hexane and dichloromethane. Flumethrin was determined by HPLC-UV.

Beeswax:

Flumethrin was extracted from 10 g wax by boiling with a mixture of 2-propanol and methanol. After addition of water and cooling in an ice bath, the suspension was filtered and the extract was evaporated to a small volume. Further clean-up was performed by partitioning with a mixture of cyclohexane and ethyl acetate followed by column chromatography on silica gel. Flumethrin was determined by HPLC with UV detection. The limit of quantification was 26 µg/kg.

Table 11. Recovery from honey fortified with 3 µg/kg flumethrin

Recovery values (%) n = 4				Mean (%)	Relative Standard Deviation (RSD %)
93	93	83	80	87.25	7.7

LOQ = 3 µg/kg; LOD = 1 µg/kg.

Reference AH ID 016000en.pdf

Method for determining the residue on flumethrin in bees' honey and wax. Heukamp, U. and Krebber, R. (1993). Report No.RA-654/93 (Method 00339), Bayer AG.

Non-GLP compliant study

This method is a revised version of, and supersedes, Method RA-677/92 (Method no. 00317). The documentation of the raw data in the Appendix to Method RA-677/92 is also applicable to the method described here.

All the reagents and equipment were listed. Samples were separated into honey and wax fractions by heating to 90 °C, allowing the wax to set and scraping off the upper wax layer with a knife.

Honey residue extraction:

Test portions (50 g), were homogenised (2 min) with 100 ml toluene:dichloromethane (DCM):methanol (5:4:1 v/v/v), and the supernatant dried with 25 g sodium sulphate (10 min). The supernatant and 2 × 10 ml DCM salt washings of the precipitate were filtered, evaporated to dryness and resuspended in 7.5 ml ethylacetate:cyclohexane (1:1 v/v). 5 ml of the residue solution was added to a gel permeation chromatography column (GPC: Bio beads SX3, 5 ml/min) and resolved using a ethylacetate:cyclohexane (1:1 v/v) mobile phase. The first 60 ml of GPC eluate was discarded, the next 85 ml of eluate (residue fraction) was collected, evaporated to dryness and the residue resuspended in 1 ml toluene. Solid phase extraction (SPE) on silica gel (10 g; 70-230 mesh, Merck) was achieved by sequentially applying 50 ml n-hexane, the GPC residue, 5 × 2 ml n-hexane rinsings, 150 ml n-hexane:DCM (7:3 v/v), 50 ml n-hexane:DCM (eluate 1), 150 ml n-hexane:DCM (1:1 v/v; eluate 2). The pooled eluates were evaporated to *ca*. 1 ml, transferred to a 10 ml vessel with acetonitrile (ACN) washings, reduced to dryness and resuspended in 0.5 ml ACN. The concentration correction factor in this method for extracts was ×0.015.

Wax residue extraction:

Test portions (10 g), were extracted twice by warming to boiling in 50 ml 2-propanol and 50 ml methanol, cooling (ice bath) and removing the supernatant by filtration. The two filtrates and methanol wax washings were combined and reduced to *ca*. 5 ml by evaporation. The filtrate and 100 ml water were twice extracted with 100 ml ethylacetate:cyclohexane (1:1 v/v) and the organic phases pooled. The organic phases were dried with sodium sulphate (25 g, 15 min), filtered, and evaporated to dryness. The residue was resuspended in 50 ml ACN, washed with 50 ml n-hexane, reduced to dryness and resuspended in 1 ml toluene. Solid phase extraction (SPE) on silica gel (10 g; 70-230 mesh, Merck) was achieved as for honey samples. The residue was resuspended in 1 ml ACN, which corresponded to a correction factor of ×0.10 for concentrations in wax.

Flumethrin residues were determined quantitatively by high-pressure liquid chromatography and UV detection (HPLC-UV) at a wavelength of 266 nm.

The residue, i.e., the content as measured, is calculated from the areas or heights of the peaks obtained with the measurement solution, by reference to those obtained with the external standard.

Method Validation

Specificity: this area was not addressed.

Accuracy: flumethrin was spiked into honey and wax samples (n = 5; 50 g and 10 g respectively) and calibrated against external standards.

Precision: this was tested as indicated in the accuracy section above.

Table 12. The recoveries and CV % of flumethrin determinations in honey and wax

Matrix	Spike (µg/l)	n =	Mean recovery (%)	CV %
Honey	3	5	74	2
	13	5	86.6	1
	85	5	85.9	3
Wax	26	5	59.6	7
	51	5	79.2	13
	102	5	76.2	19

Limit of Detection: these were reported to have been 1 and 20 µg/kg for honey and wax samples respectively.

Limit of Quantification: these were reported to have been 3 and 26 µg/kg for honey and wax samples respectively.

Sensitivity: the linearity of the analytical method was not reported in this study.

Susceptibility to interference: The linearity of the method was demonstrated in the range 0-70 mg/l.

Reference AH ID 013910en.pdf

Analytical method for the determination of Bayvarol (active ingredient flumethrin) in honey and wax. Heukamp, U. (1993). Report No.RA-441/92 (**method 00317**), Bayer AG.

Non-GLP compliant study.

Honey is extracted with a mixture of toluene, dichloromethane and methanol (5:4:1 v/v). After evaporation to dryness the residue is re-dissolved 1n ethylacetate/cyclohexane (1:1 v/v) and further cleaned by gel-permeation-chromatography. This is followed by a clean-up with silica gel. The quantitative determination is performed by HPLC with UV-detection.

Wax is extracted by boiling with 2-propanol and methanol. After adding of water and cooling down the suspension is filtered and the extract is evaporated to a small volume. After clean-up

by partitioning, the extract is further cleaned with silica gel as in the case of honey. The quantitative determination is performed by HPLC with UV-detection.

Method validation

The method was validated in the laboratory of the author by conducting recovery experiments. Given amounts of standard substance were added to untreated samples of honey and wax. The samples were then analysed according to the method as described above.

Recoveries

Table 13. Honey

Amount added (µg/kg)	Recoveries (%)	Mean (%)	Relative standard deviation (RSD)
3	74, 75, 76, 72, 73	74	0.02
13	85, 87, 86, 88, 87	87	0.01
85	87, 86, 89, 84, 83	86	0.03

Table 14. Wax

Amount added (µg/kg)	Recoveries (%)	Mean (%)	Relative standard deviation (RSD)
26	60, 54, 65, 62, 57	60	0.07
51	78, 90, 65, 75, 89	79	0.13
102	63, 61, 82, 96, 80	76	0.19

Limit of Detection

Control samples of honey and wax were analysed. In the case of honey, there was no peak detected at the retention time of flumethrin, the noise of the baseline was used to determine the limit of detection, using the mean recovery and adding 3 x the standard deviation of the mean.

Two wax samples were used for method validation, there was a small blind value peak found at the retention time of flumethrin corresponding to 8-12 µg/kg of flumethrin.

Therefore, in this case the limit of detection was defined as twice the height of this blind value peak.

Table 15. LOD

Sample	No. of analyses	LOD (µg/kg)
Honey	3	1
Wax	2	20

Limit of Quantification

The LOQ, defined as the lowest concentration detected with good recovery results is 3 µg/kg for honey and 26 µg/kg for wax. No further details were given.

Linearity

The linearity of the detector used for method validation was checked in the range of 85 µg/l to 85 171 µg/l, corresponding to concentrations of:

Honey: 1 µg/kg to 1271 µg/kg in matrix.

Wax: 9 µg/kg to 8517 µg/kg in matrix.

Reference AH ID 0106001en.pdf

Liquid chromatographic method to determine the content of Bayvarol (FCR 1622) in honey and beeswax. Riegner, K. (1986). Report No.RA-197/86, Bayer AG.

Non-GLP compliant study.

Honey is extracted with a mixture of toluene, dichloromethane and methanol (5:4:1). The raw extract is then purified by gel permeation chromatography (GPC) and then over silica gel. The quantitative final determination is performed using high-pressure liquid chromatography (HPLC) with UV detection at 254 nm. The recovery rate from honey is 63 % and the determination limit 2 µg/kg.

Wax is extracted from combs by melting at 90 °C. This wax is dissolved in heated isopropanol and precipitated out again by adding methanol and water. Suctioning and repetition of the extraction procedure are followed by dispersion for further extract purification, using water and a mixture of ethyl acetate/cyclohexane (1:1) and subsequently using acetonitrile and hexane.

As for honey, the final determination is made after final purification over silica gel, using HPLC and UV detection.

The recovery rate from wax combs is 62 % and the determination limit (LOQ) is 25 µg/kg.

All the reagents and equipment were listed. Samples were separated into honey and wax fractions by heating to 90 °C, allowing the upper wax layer to set and scraping off the wax with a knife.

Residue extraction: honey and wax extractions were carried out as in method 654/93.

Method Validation

Recovery (in matrix)

Flumethrin was dissolved in acetone and added to the honey or wax. To determine the wax yield, the wax was melted once again.

Table 16. Honey

Amount added (µg/kg)	Recovery (%)	Mean (%)
3.1	61, 63	62
3.8	54, 74	64
4.4	58, 62, 70	63

The mean recovery rate from honey is 63 % of the added quantity of flumethrin with a confidence interval of ± 6.3 % and 95 % statistical certainty.

Table 17. Wax

Amount added (µg/kg)	Recovery (%)	Mean (%)
25	58, 66	62
50	53, 69	61
100	58, 63, 69	63

In the concentration range tested, the mean recovery rate from wax is 62 % of the added flumethrin, with a confidence interval of ± 5.7 % and 95 % statistical certainty.

Limit of Quantification

Honey: 2 µg/kg

Wax: 25 µg/kg (15 µg/kg when taken up in 1 ml acetonitrile)

Reference AH ID 041150en.pdf

Analytical method for the determination of flumethrin in bees honey and wax by LC-MS/MS. Report number: MR-15/101. Study number P 603 155025 (Krebber, Hoffend & Anjard, 2015). Method 01462.

LOQ: Honey: 3 µg/kg; Wax: 25 µg/kg

Honey is extracted by shaking with water for 10 minutes, and then acetonitrile is added and mixed. The extract is cleaned-up using a C18 SPE cartridge, and the analyte eluted with acetonitrile. The extract is evaporated to dryness, re-dissolved in acetonitrile and, if necessary, filtered prior to determination by LC-MS/MS.

Wax is extracted with ethanol by heating to 70 °C for 15 minutes, and then shaking for 30 minutes. The extract is placed in dry ice for 15 minutes (or in a freezer at -18 °C overnight), and then centrifuged. A portion of the supernatant is evaporated, redissolved in isohexane and cleaned-up by liquid-liquid partition with acetonitrile (saturated with isohexane). The acetonitrile extract is evaporated to dryness, redissolved in acetonitrile and filtered prior to determination by LC-MS/MS.

LC-MS/MS is performed in positive ionisation mode. Two mass transitions can be monitored; m/z 527 to 267 (quantification transition) and m/z 527 to 239 (confirmation transition).

The method has been validated with an acceptable range of recoveries (70 %-120 %) and relative standard deviations (RSD = < 20 %).

Table 18. Accuracy and Precision Data

Matrix	Analyte	Fortification Level (µg/kg)	Range (%)	Recovery	n	Mean (%)	RSD (%)
Honey (quantification m/z 527 → m/z 267)	Flumethrin	3	78-97		6	85	8.6
		6	78-90		6	86	4.9
		30	77-90		6	83	5.3
		Overall	**77-97**		**18**	**85**	**6.4**
Honey (confirmation m/z 527 → m/z 239)	Flumethrin	3	80-98		6	88	8.8
		6	76-94		6	86	6.7
		30	79-92		6	84	5.8
		Overall	**76-98**		**18**	**86**	**7.1**
Wax (quantification m/z 527 → m/z 267)	Flumethrin	25	87-103		6	96	6.7
		50	98-104		6	101	2.1
		250	87-108		6	101	7.4
		Overall	**87-108**		**18**	**99**	**6.2**
Wax (confirmation m/z 527 → m/z 239)	Flumethrin	25	66-96		6	83	19
		50	87-113		6	99	9.4
		250	85-103		6	96	7.0
		Overall	**66-113**		**18**	**92**	**13**

Reference ID 41149

Determination of flumethrin in honey and wax. Report No.: MR-15/183. Study Number: P673155038 (Krebber & Hoffend, 2015)

The honey and wax samples were analysed for concentrations of flumethrin by LC-MS/MS. Flumethrin was extracted from honey by shaking with water and acetonitrile. The extract was cleaned-up by solid phase extraction. Wax was extracted with ethanol at 70 °C and half of the extract was used for further clean-up. After elimination of the wax by freezing, the solvent was evaporated. The remainder is dissolved in iso-hexane and extracted by partition with acetonitrile. The extract was evaporated to dryness and dissolved in acetonitrile.

The quantitative determination was performed by HPLC with a tandem mass spectrometric detector. The limit of quantitation was 3 µg/kg in honey and 25 µg/kg in wax.

The measured concentration was calculated by comparison of the analyte response to a standard calibration curve obtained from matrix-matched standards.

Method validation

The method was validated for honey and wax before and during the analyses by concurrent recoveries.

Table 19. Recovery rates for flumethrin in honey

Sample material	Fortification level (µg/kg)	Recovery (%)						Mean value (%)	RSD (%)
Honey	3	80	82	83	97	91	78	85	8.6
	6	90	87	86	78	88	88	86	4.9
	30	85	90	81	77	82	81	83	5.3
	Mean	n = 18						85	6.4

RSD = Relative standard deviation

Table 20. Recovery rates for flumethrin in wax

Sample material	Fortification level (µg/kg)	Recovery (%)						Mean value (%)	RSD (%)
Wax	25	91	102	98	87	93	103	96	6.7
	50	102	100	103	101	104	98	101	2.1
	250	108	87	103	104	106	100	101	7.4
	Mean	n = 18						99	6.2

RSD = Relative standard deviation

Specificity:

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.

Linearity:

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for matrix-matched standard solutions ranging from 0.5 to 150 µg/l (corresponding to 1 to 300 µg/kg in the sample) for honey and between 1 and 50 µg/l for wax (corresponding to 10 to 500 µg/kg in the sample). The correlation coefficients were ≥ 0.996 for both matrices.

Accuracy and precision:

For precision, repeatability and within-laboratory reproducibility data were provided. The coefficient of variation (CV (= RSD)) was used as the measure.

Table 21. Recovery rates for flumethrin in honey for the quantitation ion (m/z 527 → m/z 267)

Sample material	Fortification level (µg/kg)	Recovery (%)						Mean value (%)	Accuracy (%)	CV (%)
Honey	3	80	82	83	97	91	78	85	-15	8.6
	6	90	87	86	78	88	88	86	-14	4.9
	30	85	90	81	77	82	81	83	-17	5.3
	Mean	n = 18						85	-15	6.4

Table 22. Recovery rates for flumethrin in wax for the quantitation ion (m/z 527 → m/z 267)

Sample material	Fortification level (µg/kg)	Recovery (%)						Mean value (%)	Accuracy (%)	CV (%)
Wax	25	91	102	98	87	93	103	96	-7	6.7
	50	102	100	103	101	104	98	101	1	2.1
	250	108	87	103	104	106	100	101	-1	7.4
	Mean	n = 18						99	-1	6.2

Limit of Quantitation (LOQ):

The limit of quantitation is 3 µg/kg in honey and 25 µg/kg in wax, based on the lowest fortification levels tested.

Limit of Detection (LOD):

The detection limits (LOD) were calculated based on a statistical approach for each sample material. The LODs were calculated to be 0.74 µg/kg for honey and 10 µg/kg for wax.

Table 23. Calculated LOD for honey

Sample material	Fortification level (µg/kg)	Residue detected (µg/kg)
Honey	0	0
	0	0
	0	0
	Replicates	3
	Average	0
	3	2.40
	3	2.46
	3	2.49
	3	2.91
	3	2.73
	3	2.56
	Replicates	6

	Average	2.56
	Standard Deviation (SD)	0.22
	Calculated LOD	0.74

Calculated LOD = (3.365 x SD + average residue in untreated controls), where 3.365 is Student t-factor for n = 6 replicates.

Table 24. Calculated LOD for wax

Sample material	Fortification level (µg/kg)	Residue detected (µg/kg)
Wax	0	0
	0	0
	0	0
	Replicates	3
	Average	0
	25	22.5
	25	25.5
	25	24.5
	25	17.5
	25	23.3
	25	25.8
	Replicates	6
	Average	23.2
	Standard Deviation (SD)	3.05
	Calculated LOD	10.3

Calculated LOD = (3.365 × SD + average residue in untreated controls), where 3.365 is Student t-factor for n = 6 replicates.

Matrix Effects:

The MS/MS detection of flumethrin is affected by the matrix. For honey, peak area for standard in solvent was about 84 % of the peak area obtained for matrix-matched standards. For wax the peak area decreased to about 20 % compared to the peak area found for standards in solvent.

Stability of residues

Reference AH ID 042560en.pdf

Study of Acaricide Stability in Honey. Characterization of Amitraz Degradation Products in Honey and Beeswax (Korta *et.al*., 2001).

Only the information relevant to the stability of flumethrin in honey has been reported below. No data on beeswax were included.

Honey:

Honey samples were spiked with flumethrin at 10 mg/kg. This spiked honey was kept in the closed glass container at room temperature (20-25 °C) for 9 months, away from exposure to direct sunlight. Samples were analysed (using an HPLC method) at day 0, 3 months and 9 months. There was no appreciable decline in concentration of flumethrin in the honey (results were 9.8, 10, And 9.9 mg/kg, respectively).

From Reference ID 41150:

Determination of flumethrin in honey and wax. Report No.: MR-15/183. Study Number: P673155038 (Krebber & Hoffend, 2015)

Stability:

Solutions of the reference substance flumethrin in solvent are stable for at least 3 months when stored in a refrigerator ≤ 7 °C.

Analytical solutions of honey and wax samples were stable for at least 5 days when stored in a refrigerator ≤ 7 °C.

The stability of flumethrin in stored samples was demonstrated in honey and wax samples for a period of one month at ambient temperature and ≤ -18 °C.

Appraisal

No radiolabeled residue depletion study has been conducted or provided. These data are not required for substances in honey, as there is no known metabolism of xenobiotics in honeybees and in this case there is also no degradation in honey.

Between-comb strips (honey):

Six non-GLP-compliant residues depletion studies of flumethrin in honey were conducted in various locations in Germany and the UK in the 1980s and 1990s. All these studies used LDPE between-comb strips impregnated with 3.6 mg flumethrin per strip.

In one study, 24 beehives were treated with 4 strips each, corresponding to a total dose of 14.4 mg flumethrin, for a duration of 6 months, over the winter period. One sample was analysed, no residues were detected (LOQ = 3 µg/kg; LOD = 1 µg/kg). Additionally, 12 beehives were treated for 6 weeks with 4 strips per hive, from the beginning of September to the middle of October, over 2 years. Four samples were analysed, no residues were detected.

In the second study, an unreported number of beehives were treated with 4 strips per brood chamber for six weeks, after the honey harvest. One sample was taken, during the spring honey flow. No residues were detected. (LOQ = 3 µg/kg; LOD = 1 µg/kg).

In the third study, six beehives were treated for 6 weeks, from September to October, with 4 strips per frame. Six samples were taken in June the next year and combined in pairs to form three samples for analysis. No residues were detected above the LOQ of 2 µg/kg.

In the fourth study, six beehives were treated for 5 months from October to March with 4 strips per frame. Six samples of freshly capped honeycomb were taken in June of the following year, after the early nectar flow. No residues were detected above the LOQ of 2 µg/kg.

In the fifth study, seven beehives were treated from early March to mid-April with 4 strips per frame. At the end of the treatment period, these combs were labelled and suspended in the honey chamber until the last brood had hatched and fresh honey had been inserted and capped. No residues were detected above the LOQ of 2 µg/kg.

In the final study of between-comb strips, four beehives were treated for 4 months from May to September, during the honey flow period. The entire honey harvest of each treated hive was centrifuged separately in the last week of August. No residues were detected above the LOQ of 2 µg/kg.

These studies demonstrate that even when used contrary to Good Beekeeping Practice (in doses higher than recommended, or over extended periods of time), or when used during honey flow, no residues of flumethrin were detected in honey. It should be noted, however, that these studies were not conducted to current GLP standards, did not have comprehensive study reports, used slightly different analytical methods (based on HPLC-UV), and had inconsistencies in reporting, that limit the usefulness of the results.

Between-comb strips (beeswax):

Five non-GLP-compliant residues depletion studies of flumethrin in beeswax were conducted in various locations in Germany and Switzerland. All studies used LDPE strips impregnated with 3.6 mg flumethrin per strip. The samples in all studies were analysed using HPLC-UV.

In the first study, 15 beehives were treated as per product instructions (4 strips per brood chamber for six weeks, after the honey harvest) in three Swiss cantons. Thirteen samples of beeswax were analysed for flumethrin and residues were found in the range < 26 µg/kg to 176 µg/kg. Mean residues were around 50 µg/kg. The LOQ for the analytical method used was 26 µg/kg.

In the second study, an unreported number of beehives were treated with ten times the recommended number of strips (40 strips per hive). The analysis of four samples was reported and the results were in the range 70 – 146 µg/kg; mean = 106 µg/kg. The LOQ of the analytical method used was reported as 26 µg/kg.

In the third study, two beehives were treated with 4 strips per chamber for 6 weeks, just before the start of honey flow. Two samples per frame were analysed. Residues in the range < 15 to 40 µg/kg were reported. The LOQ of the analytical method used was 15 µg/kg.

In the fourth study, six beehives were treated with 4 strips per brood chamber for 6 weeks, from September to October, after the honey harvest. The following year, samples were taken in June, after the early honey flow. One honeycomb was taken from each hive and combined with one from another beehive to form three samples, which were then analysed for flumethrin. The results of the analyses showed residues in the range of < 20 - 50 µg/kg. The LOQ of the analytical method used was 20 µg/kg.

In the final study, four beehives were treated with 4 strips per brood chamber for six months, during the honey flow period. The strips were inserted in May and removed in September. Two combs from each hive that had been in the brood chamber were moved to the honey chamber in mid-August until the brood hatched. These combs were sampled and analysed. The results were in the range 30 - 130 µg/kg. The LOQ of the analytical method used was reported as 20 µg/kg.

These studies demonstrate that flumethrin has more affinity for beeswax than the honey, which is to be expected considering the lipophilic nature of flumethrin (Log P_{ow} = 6.2). Again, it should be noted that these studies were not conducted to current GLP standards, did not have comprehensive study reports, used slightly different analytical methods (based on HPLC-UV), and had inconsistencies in reporting that limit the usefulness of the results.

Beehive gates (honey and wax):

The sponsor provided one GLP-compliant field study conducted in 2015 in beehives, using beehive gate strips. Honeycombs were obtained from different test sites in Germany (4 apiaries), Hungary (2 apiaries), Spain (2 apiaries) and The Netherlands (3 apiaries) after application of flumethrin bee-hive gates (275 mg per gate). Both honey and beeswax were sampled and analysed.

Thirty-two honeycomb samples from four apiaries in Germany, 14 samples from two apiaries in Hungary, 12 samples from two apiaries in Spain and 18 samples from three apiaries in The Netherlands were analysed.

The maximum recommended application time at the hive entrance of 4 months was covered by data from Germany (122 or 120 days) and the Netherlands (119 days). In the other regions, application time was 92 days (Spain) and 102 days (Hungary).

For residue analysis honey from all hives of one apiary were pooled, as was the wax. Equal amounts of the previously purified honey samples were combined and homogenised by stirring at 40 °C. Wax samples were melted at around 63 °C, homogenised and purified from contamination (e.g., hive detritus) by skimming off or passing it again through a sieve.

The honey and wax samples were analysed for flumethrin using a validated LC-MS/MS method.

Residue concentrations in honey were below the limit of quantification of 3 µg/kg. Concentrations in beeswax were above the LOQ of 25 µg/kg in three out of 11 samples. The highest residue in wax was 119 µg/kg.

Analytical methods

Several versions of the HPLC-UV method used to analyse the samples generated from the studies conducted using the between-comb strips in the 1980s-90s to determine residues of flumethrin in honey and wax were provided, although none of them were well-reported or validated according to current standards.

The sponsor also provided the details of an LC-MS/MS method that was used to determine residues of flumethrin in honey and wax after use of the beehive gates. This study was conducted in a GLP-accredited laboratory and the method validated according to international requirements. Flumethrin was extracted from honey and wax. The quantitative analysis was performed using LC-MS/MS. The limit of quantitation was 3 µg/kg in honey and 25 µg/kg in wax.

Dietary Exposure Assessment

Exposure to flumethrin residues may occur through its use as a pesticide as well as a veterinary drug.

Dietary exposure from pesticide residues (IEDI)

When used as a pesticide, the exposure of flumethrin was found to be below the upper bound of the ADI of 0.004 mg/kg bw (JMPR, 1996). The sources of exposure considered were cattle meat and milk.

Dietary exposure from veterinary drug residues (GECDE)

JECFA has proposed an ADI of 0-0.004 mg/kg bw and an ARfD of 0.005 mg/kg bw. The toxicological profile of flumethrin requires exposure estimates for children at the highest reliable percentile of food consumption based on consumers only.

When used as a veterinary drug, chronic and acute dietary exposure in the general population and in children was estimated based on the potential occurrence of residues in honey and beeswax. It was assumed that in all cases honey is consumed with beeswax (as occurs in comb honey and raw honey) and the honey to wax ratio is 9:1 (JECFA 2008). This is a conservative assumption, as most commercial honey is likely to contain much less beeswax. Other honeybee products, such as propolis, royal jelly and pollen, were not included in the exposure assessment.

The GECDE for the general population is 0.008 µg/kg bw per day (Table 25), which represents 0.2 % of the upper bound of the ADI of 0.004 mg/kg bw set by JECFA during this meeting. The GECDE for children is 0.006 µg/kg bw per day, which represents 0.2 % of the upper bound of the ADI.

In addition to the accepted GECDE methodology, further calculations were carried out. Instead of using the highest mean and the highest 97.5th percentile consumption across surveys, the calculations were carried out using the mean and the highest reliable percentile for each individual national survey from available data sets (CIFOCOss). The mean of all the GECDE calculations across surveys were reported. The mean of 21 estimates was 0.002 µg/kg bw per day (0.05 % of the upper bound of the ADI), with a range of <0.0001-0.008 (<0.003-0.2 % of the upper bound of the ADI). For children, chronic dietary exposure could be estimated from 43 individual studies. The mean of these 43 studies was 0.001 µg/kg bw per day (0.03% of the upper bound of the ADI), with a range of <0.0001-0.006 (<0.003-0.2 % of the upper bound of the ADI).

Dietary exposure from veterinary drug residues (GEADE)

The GEADE for the general population is 0.1 µg/kg bw per day (Table 26), based on consumption of wax contained in honey, which represents 2.2 % of the ARfD of 0.005 mg/kg bw per day. The GEADE for children is also 0.1 µg/kg bw per day, based on consumption of wax contained in honey, which represents 2.2 % of the ARfD.

Combined chronic dietary exposure from pesticide and veterinary drug residues (Extended GECDE)

Modified methods based on the GECDE were used to estimate combined chronic dietary exposure. The usual GECDE approach was extended to include additional commodities that were assessed for the compound by JMPR (Extended GECDE). It should be noted that this new exposure assessment methodology is still being piloted. It should further be noted that the median residues used as inputs were extracted from JMPR publications and have not been validated for this assessment.

Combined chronic dietary exposure from veterinary drug and pesticide residues was considered for the general population and in children, based on the potential occurrence of residues in honey, beeswax, cattle meat and cattle milk. Assumptions for honey and beeswax were the same as for the GECDE.

The Extended GECDE for the general population is 0.179 µg/kg bw per day (Table 27), which represents 5 % of the upper bound of the ADI of 0.004 mg/kg bw set by JECFA during this meeting. The Extended GECDE for children is 1.008 µg/kg bw per day, which represents 25 % of the upper bound of the ADI. For both populations cattle milk was the major contributor to chronic dietary exposure.

Table 25. Estimated chronic dietary exposure to flumethrin (GECDE) occurring in honey and associated beeswax

Category	Type	Median concentration[1] (µg/kg)	Mean consumption[2] (whole population, g/kg bw/day)	Highest reliable percentile consumption[3] (consumers only, g/kg bw) /[percentile used]	MR:TR ratio	Exposure (µg/kg bw/day)		GECDE[4]	
						mean	97.5th	µg/kg bw/day	ADI %
General Population									
Honey	Honey	2	0.036	1.9 [97.5]	1	*0.00007*	0.004		
Honey	Beeswax[5]	40	0.004	0.2 [97.5]	1	0.00016	*0.008*		
TOTAL						0.00007	0.008	0.008	0.2
Children									
Honey	Honey	2	0.136	1.3 [97.5]	1	*0.00027*	0.003		
Honey	Beeswax[5]	40	0.015	0.1 [97.5]	1	0.00061	*0.006*		
TOTAL						0.00027	0.006	0.006	0.2

[1]Median concentration at the end of treatment

[2]highest mean consumption figures based on whole population considered from the available dataset

[3]highest food consumption figures based on consumers only considered from the available dataset

[4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other foods

[5]Beeswax in honey only, calculated by assuming beeswax consumption is 1/9 of honey consumption

Table 26. Estimated acute dietary exposure to flumethrin (GEADE) occurring in honey and associated beeswax

Category	Type	95th centile concentration[1] (mg/kg)	Acute Consumption[2] (g/kg bw)	MR:TR ratio	GEADE[3]	
					mg/kg bw/day	ARfD %
General Population						
Honey	Honey	0.002	5.5	1	0.01	0.2
Honey	Beeswax[4]	0.176	0.6	1	*0.11*	*2.2*
Children						
Honey	Honey	0.002	5.5	1	0.01	0.2
Honey	Beeswax[4]	0.176	0.6	1	*0.11*	*2.2*

[1]concentration at the end of treatment

[2]highest food consumption figures based on the 97.5th percentile consumption from available data set

[3]GEADE is the product of the 97.5th level of consumption multiplied with the highest residue

[4]Beeswax in honey only, calculated by assuming beeswax consumption is 1/9 of honey consumption

Table 27. Estimated chronic dietary exposure to flumethrin (Extended GECDE) occurring in honey, associated beeswax, cattle meat and cattle milk

Category	Type	Median concentration[1] (µg/kg)	Mean consumption[2] (whole population, g/kg bw/day)	Highest reliable percentile consumption[3] (consumers only, g/kg bw) /[percentile used]	MR:TR ratio	Exposure (µg/kg bw/day)		GECDE[4]	
						mean	97.5th	µg/kg bw/day	ADI %
General Population									
Honey	Honey	2	0.036	1.9 [97.5]	1	*0.00007*	0.004		
Honey	Beeswax[5]	40	0.004	0.2 [97.5]	1	*0.00016*	0.008		
Mammalian meat	Beef and other bovines	10	0.959	4.4 [97.5]	1	*0.00959*	0.044		
Milk	Cattle Milk	10	4.4	16.9 [97.5]	1	0.04400	*0.169*		
TOTAL						0.00982	*0.169*	0.179	5
Children									
Honey	Honey	2	0.136	1.3 [97.5]	1	*0.00027*	0.003		
Honey	Beeswax[5]	40	0.015	0.1 [97.5]	1	*0.00061*	0.006		
Mammalian meat	Beef and other bovines	10	8.349	1.9 [97.5]	1	*0.01940*	0.084		
Milk	Cattle Milk	10	98.74	42.6 [97.5]	1	0.42590	*0.987*		
TOTAL						0.02028	*0.987*	1.008	25

[1]Median concentration at the end of treatment

[2]highest mean consumption figures based on whole population considered from the available dataset

[3]highest food consumption figures based on consumers only considered from the available dataset

[4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other foods

[5]Beeswax in honey only, calculated by assuming beeswax consumption is 1/9 of honey consumption

Risk management considerations

It is noted that beeswax may be present in food and originate from a variety of sources; because of this, and the fact that flumethrin accumulates in the wax, it is considered that risk management measures around the use of beeswax that may contain residues of flumethrin could be applied.

An example is where bee keepers reuse the wax combs season after season. This is common practice, as it takes a lot of energy for the bees to make the wax combs, so in order to maximise honey production, the combs are reused. It might therefore be prudent to advise beekeepers to limit reuse of their combs if they are using products that contain flumethrin on their hives. Another measure might be to recommend not using the same active ingredient in subsequent years, so to rotate the available products year by year. This may also reduce the likelihood of resistance to flumethrin of the target parasites.

No data on residues of flumethrin have been evaluated with regard to other products derived from beehives (e.g., propolis, royal jelly, etc.), therefore, no risk management proposals can be made by the Committee for these commodities.

Maximum Residue Limits

In recommending an MRL for flumethrin in honey, the Committee considered the following factors:

- An ADI for flumethrin of 0–0.004 mg/kg bw was established by the Committee.
- An ARfD of 0.005 mg/kg bw was established by the Committee.
- In view of the toxicological profile of flumethrin, specific exposure scenarios are required to address exposure of pregnant women, infants and young children and high percentile adult consumers.
- Flumethrin is used both as a pesticide and a veterinary drug.
- Flumethrin is authorised for use in beehives in several countries. The maximum recommended dose is 275 mg x 2, administered via PVC beehive gates, or $3.6\ \text{mg} \times 4$ administered via between-comb LDPE strips.
- The withdrawal period for all products is zero days.
- Flumethrin is the marker residue in honey.
- The ratio of the concentration of marker residue to the concentration of total residue of 1.0 was calculated in honey and beeswax.
- A validated analytical method for the determination of flumethrin in honey and beeswax is available and may be used for monitoring purposes.

Although there were no quantifiable residues found in honey after treatment with the flumethrin products evaluated, the Committee, in response to the request of the 23rd session of CCRVDF, could set an MRL for honey at twice the LOQ of the analytical method used in the residues studies. Because the most reliable method was the more recent LC-MS/MS method, which had an LOQ of 3 μg/kg, the Committee recommended an MRL of 6 μg/kg.

References

FAO/WHO. Report of the Twenty-third Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Houston, Texas, USA, 17–21 October 2016. Rome: Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (REP16/RVDF).

Heukamp, U. and Krebber, R. 1993. Method for determining the residue on flumethrin in bees' honey and wax. Bayer AG. Report No.RA-654/93 (Method 00339). Sponsor submitted.

Heukamp, U. 1993. Analytical method for the determination of Bayvarol (active ingredient flumethrin) in honey and wax. Bayer AG. Report No.RA-441/92 (method 00317). Sponsor submitted.

JMPR. 1996. Pesticide residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 140. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Reports_1991-2006/Report1996.pdf

Korta *et.al*., 2001. Study of Acaricide Stability in Honey. Characterization of Amitraz Degradation Products in Honey and Beeswax (2001).

Krebber, R. 1994a. Residues of Flumethrin in Honey after Administration of Bayvarol Strips to Honeybee Colonies in Germany; Bayer AG, Project number P65335008. Sponsor submitted.

Krebber, R. 1994b. Residues of Flumethrin in Honey after Administration of Bayvarol Strips to Honeybee Colonies in Great Britain. Bayer AG, Project number P65335009, Report number RA-233/94. Sponsor submitted.

Krebber, R. 1994. Residues of Flumethrin in Beeswax after Administration of Bayvarol Strips to Honeybee Colonies in Switzerland. Bayer AG. Report No. RA-126/94; Project No. P65345009. Sponsor submitted.

Krebber, R. 1994. Residues of Flumethrin in Beeswax after Treatment with a Tenfold Overdose of Bayvarol Strips to Honeybee Colonies in Germany. Bayer AG. Report No. 286/94; Project No. P65335010. Sponsor submitted.

Krebber, R. Hoffend, J. & Anjard. 2015. Analytical method for the determination of flumethrin in bees honey and wax by LC-MS/MS. Bayer CropScience AG. Report number: MR-15/101. Study number P 603 155025 (Method 01462). Sponsor submitted.

Krebber, R. & Hoffend, J. 2015. Determination of flumethrin in honey and wax. Report No.: MR-15/183. Bayer CropScience AG. Study Number: P673155038. Sponsor submitted.

Riegner, K. 1986. Liquid chromatographic method to determine the content of Bayvarol (FCR 1622) in honey and beeswax. Bayer AG. Report No.RA-197/86. Sponsor submitted.

Riegner, K. & Krieger, K. 1990a. Residues of Flumethrin in Honey Following the Use of Bayvarol Strips during the Pre-Winter Storage Period. Bayer AG. Report no. 90/13521. Sponsor submitted.

Riegner, K. & Krieger, K. 1990b. Residues of Flumethrin in Honey Following the Use of Bayvarol Strips During the Winter. Bayer AG. Report no. 90/13522. Sponsor submitted.

Riegner, K. & Krieger, K. 1990c. Residues of Flumethrin in Honey Following the Use of Bayvarol Strips in Spring. Bayer AG. Report no. 90/13523. Sponsor submitted.

Riegner, K. & Krieger, K. 1990d. Residues of Flumethrin in Honey Following the Use of Bayvarol Strips During the Nectar Flow Period. Bayer AG. Report no. 90/13524. Sponsor submitted.

Riegner, K. & Krieger, K. 1990. Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips in Spring. Bayer AG Doc No. 90/13518. Sponsor submitted.

Riegner, K. & Krieger, K. 1990. Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips during the Pre winter Storage Period. Bayer AG. Doc No. 90/13519. Sponsor submitted.

Riegner, K. & Krieger, K. 1990. Residues of Flumethrin in Beeswax Following the Use of Bayvarol Strips during the Nectar Flow Period. Bayer AG. Doc No. 90/13520. Sponsor submitted.