Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–7, 2023
Received: 15 November 2022 | Revised: 27 January 2023 | Accepted: 2 May 2023

Hazard/Risk Assessment

Toxicity of Coumaphos Residues in Beeswax Foundation to the Honey Bee Brood

Christina Kast,* Benoît Droz, and Verena Kilchenmann

Agroscope, Swiss Bee Research Centre, Bern, Switzerland

Abstract: Coumaphos is one of the most frequently detected pesticides in recycled beeswax. The objective was to assess the maximal level of coumaphos in foundation sheets that could exist without lethal effects on the honey bee larvae. Brood development was followed in cells drawn on foundation squares containing coumaphos ranging from 0 to 132 mg/kg. Furthermore, larval exposure was determined by measuring the coumaphos level in the drawn cells. Coumaphos levels in the initial foundation sheets up to 62 mg/kg did not increase brood mortality because the emergence rates of bees raised on these foundation squares were similar to controls (median of 51%). After a single brood cycle, coumaphos levels in the drawn cells were up to three times lower than the initial levels in foundation sheets. Hence, coumaphos levels of 62 mg/kg in the initial foundation sheets, almost the highest exposures, resulted in levels of 21 mg/kg in drawn cells. A significantly reduced emergence rate (median of 14%) was observed for bees raised on foundation sheets with initial coumaphos levels of 132 mg/kg, indicating increased brood mortality. Such levels resulted in coumaphos concentrations of 51 mg/kg in drawn cells, which is close to the median lethal concentration (LC50) as determined in previous in vitro experiments. In conclusion, brood mortality was increased on wax foundation sheets with initial coumaphos levels of 132 mg/kg, while no elevated mortality was observed for levels up to 62 mg/kg. *Environ Toxicol Chem* 2023;00:1–7. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Pesticides; Pharmaceuticals; Organophosphates; Invertebrate toxicology; Hazard/risk assessment

INTRODUCTION

Honey bees are exposed to various pesticides, such as veterinary drugs authorized for beekeeping as well as plant protection products used in agriculture. Veterinary products for treatment against *Varroa destructor* are directly applied into the bee colonies. Furthermore, bees bring pesticides into the hive when they forage for nectar and pollen. Lipophilic pesticides are especially problematic because they accumulate in beeswax (Bogdanov, 2004), thus exposing developing honey bee larvae to these contaminants (Droz et al., 2020; Fulton et al., 2019a; Murcia Morales et al., 2020).

So far, little is known about the levels of pesticides in beeswax that affect honeybee development. In the present study, coumaphos served as a model substance. Coumaphos was examined as a common contaminant because it has been shown to have multiple adverse effects on honey bees (Tihelka, 2018). Coumaphos-containing products are authorized in several countries for beekeeping (D'Ascenzi et al., 2019) and/or for plant protection. In particular, the annual use of coumaphos-containing products for *V. destructor* treatment can lead to accumulation of coumaphos in the combs (Kast et al., 2020). Not surprisingly, coumaphos is one of the most frequently detected pesticides in beeswax (Alkassab et al., 2020; Calatayud-Vernich et al., 2017; El Agrebi et al., 2020; Fulton et al., 2019b; Kast et al., 2021; Lozano et al., 2019; Marti et al., 2022; Murcia Morales et al., 2020; Perugini et al., 2018; Shimshoni et al., 2019). When beekeepers bring old combs that previously have been exposed to coumaphos-containing products to manufacturers for wax recycling, such residues will be present in the newly produced foundation sheets (Bogdanov et al., 1998). Coumaphos remains in the wax after sterilization of wax at 140 °C for 2 h (Bogdanov et al., 1998). This is a problem for beekeeping because coumaphos residues stay in the wax cycle for many years even if over time the residues are diluted by newly produced

This article includes online-only Supporting Information.

* Address correspondence to christina.kast@agroscope.admin.ch
Published online 5 May 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5645

wax from bees (Kast et al., 2021). Thus, honeybees are exposed to coumaphos residues in wax, even if no coumaphos-containing product is directly applied to the colonies.

Bees may accumulate coumaphos in their bodies when they come into contact with contaminated wax (Van Buren et al., 1992). Previous studies have shown that coumaphos residues in beeswax negatively affect developing queen (Collins et al., 2004; Pettis et al., 2004) and worker larvae (Kast & Kilchenmann, 2022). Coumaphos can migrate from the beeswax into the larval diet to concentrations up to one fourth of the initial concentration in beeswax (Kast & Kilchenmann, 2022). Consequently, larvae are exposed to coumaphos in the jelly in addition to exposure through contact with contaminated beeswax.

Previously, an in vitro model tested the effect of coumaphos in beeswax on honey bee larvae (Kast & Kilchenmann, 2022). However, toxicity could be underestimated in an in vitro assay because exposure is initiated at the larval stage, while eggs are not exposed. On the other hand, decreased exposure levels might be expected when bees relocate wax or apply new wax to build the combs on the foundation sheets. Thus, we aimed to complement the in vitro study with an in-hive study because there can be significant differences between these systems. Foundation sheets were produced containing various coumaphos levels. Several small foundation squares were placed in a Dadant Blatt brood frame from which the bees constructed the combs. Subsequently, the development was followed during a single brood cycle. In addition, the distribution of coumaphos from the initial foundation sheets into the drawn cells was investigated. Finally, the emergence rates of bees that had been exposed to coumaphos containing wax during development were correlated to the coumaphos levels of the initial foundation sheets.

MATERIAL AND METHODS

Test frames

The beeswax used for the production of the foundation sheets was our own wax, which had not been previously exposed to in-hive applied coumaphos-containing products and hence contained no coumaphos above the detection limit (0.08 mg/kg; Kast et al., 2020). Coumaphos (No. 45403, PESTANAL™, analytical standard; Sigma-Aldrich) was added as a powder to beeswax, aiming at six different coumaphos concentrations ranging from 0 to 160 mg/kg. The concentrations included the tested range of the in vitro model (up to 100 mg/kg; Kast & Kilchenmann, 2022) and the maximal levels reported for brood combs (92 mg/kg; Mullin et al., 2010). To dissolve the coumaphos in the wax, the wax was melted at 85 °C for approximately 15 min and shaken by hand, followed by cooling to room temperature. Next, the wax was liquefied for a second time for pouring into the silicone forms to produce the foundation sheets.

The final coumaphos concentrations of the foundation sheets were determined by gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis (Kast et al., 2020; recoveries for most levels ~ 85%). They ranged from 0 to 132 mg/kg. These

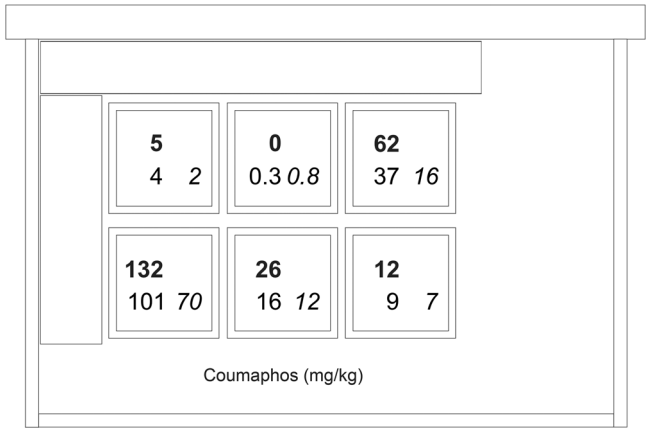

FIGURE 1: Example of a test frame containing six squares of wax foundation sheets (64 cm^2) at various coumaphos concentrations. The inner lines of the squares represent the part that was collected for chemical analysis. The numbers printed in bold indicate concentrations in the initially molded foundation sheets, while not bold numbers and numbers in italic specify average concentrations in the remaining foundations and in drawn cells after one brood cycle, respectively.

measured concentrations were used in the present study for comparison of the coumaphos concentrations of the initially molded foundation sheets to the measured concentrations in wax after one brood cycle (drawn cells and the remaining foundations). One sheet per concentration was subdivided for all frames. A square of 8 × 8 cm (64 cm^2) was cut from each sheet, and the squares were placed into the central part of a single Dadant Blatt brood frame (with a foundation strip placed as a template on the top and the right). The squares were 2 cm apart from each other and positioned side by side and fixed in the frames by electric heating of the wires. In total, 12 test frames with six squares each were produced. A small positional effect was expected because bees that come into contact with coumaphos-containing wax may accumulate coumaphos in their cuticles and subsequently transfer coumaphos into the newly constructed wax (Van Buren et al., 1992). Hence, the positions of the squares containing the six different coumaphos concentrations were altered in each test frame to minimize potential positional effects. An example of such a test frame is shown in Figure 1.

Honeybee colonies

The honeybee (*Apis mellifera*) colonies used in the present study were located in Bern, Switzerland (GPS coordinates: 46°55′57.192″N, 7°25′27.007″E). The queens were of various genetic backgrounds. Before the experiment, the colonies were never exposed to coumaphos-containing products. The colonies were in 12-frame Dadant Blatt hives on six to eight frames and treated against *V. destructor* infestation using organic acids (August and December 2018). The test frames were placed in the colonies for the bees to construct the combs. In total, 12 combs were constructed. The test series were staggered during the months of June and July 2019. Six colonies were available for our study. Thus, each colony served for two test series. The second test series in a colony was initiated

7–10 days after the start of the first series. The queens were caged for up to 24 h on the combs until there were eggs on all foundation squares. To avoid further egg laying, the queens were caged on another frame. The eggs were inspected 1–3 days later on both sides of the comb and marked on a transparent foil (data of both sides of the combs were later combined for the evaluation). Three to 4 days later, the numbers and positions of the developing larvae were monitored and marked on the test foils. The next inspection was performed a few days later when the brood was sealed and the frames were placed in an incubator until the bees emerged approximately 7 days later. The empty cells from where bees had emerged were marked. The number of eggs, larvae, sealed brood cells, and emerged bees (empty cells) were counted on the test foils, which allowed the calculation of the percentage of overall survival. In total, we evaluated 10 test series (out of 12). Two test series (two combs from different colonies) could not be evaluated (no eggs, honey storage).

Sample preparation for analysis

The wax squares were cut out from the test frames for the determination of the coumaphos concentration in the squares after a single brood cycle. For this, 7.5 × 7.5 cm of each square (inner part) was cut, and the drawn wax cells were scraped down to the foundation. The scraped wax and the remaining foundation were analyzed separately.

For purification, the wax samples were wrapped in silk organza cloth that had been previously washed with water to remove any possible residue and dried at room temperature. The wrapped samples were placed for 30 min in a small beaker containing distilled water at a temperature of 85 °C. Next, the wax was squeezed from the cloth before letting the water cool. The hardened wax was then collected from the surface of the water. To ensure homogeneity, all the samples were melted once again at 85 °C. Subsequently, the samples were processed according to a modified Quick Easy Cheap Effective Rugged Safe (QuEChERS) method, as previously described (Kast et al., 2020). Briefly, coumaphos was extracted from 1.0 g of beeswax with 10 mL of acetonitrile at a temperature of ca. 80 °C. Subsequently, the wax was precipitated by placing the sample in a deep freezer at a temperature of −18 °C overnight, followed by centrifugation at 1620*g* (Eppendorf Centrifuge 5804) the next day. Next, 2 mL of the supernatant was purified with 50 mg of primary-secondary amine (PSA; Bondesil-PSA 40 µm, Part No 12213024; Agilent Technologies) and 50 mg of Bondesil-C18 40 µm sorbent (Part No 12213012; Agilent Technologies), and the wax was precipitated in the deep freezer once more. After centrifugation, the sample was filtered (0.45 µm) to obtain the final extract for analysis.

GS-MS/MS analysis

Analysis was performed using a Thermo Trace Ultra 2000 gas chromatograph equipped with a Deans heartcut switching system coupled with a MS/MS triple quadrupole (Thermo Quantum) and a flame ionization detector (FID) with a 15-m transfer column. Chromatography analysis was performed as previously described (Kast et al., 2020). A retention capillary column deactivated with OV-1701-OH (0.53 mm internal diameter [ID]) of 50 cm and a DB-1 analytical capillary column (J + W, 0.25 mm ID, 0.25 µm film thickness) of 30 m as well as a transfer column Rxi®-5 Sil MS (0.25 mm ID, 0.25 µm film thickness) were used. One microliter of the final beeswax extract was injected on the column using an autosampler (CTC Combi PAL Systems). Helium was used as the carrier gas. The gas chromatograph temperature program was 2.0 min at 75 °C, 75 °C to 250 °C at 5 °C/min, and 250 °C to 300 °C at 3 °C/min, where it was held for 50 min. The source temperature (TSQ Quantum) and the temperature of the transfer column were 250 °C. To prevent pollution of the MS system, the Deans heartcut switching system was used allowing the detection of the coumaphos peak at RT window 43.0–48.5 min on the MS/MS triple quadrupole, while the rest of the chromatogram was directed to an FID. The transitions of *m/z* 362–334 (CE10), *m/z* 362–109 (CE25), and *m/z* 226–163 (CE18) were used for identification and the transition *m/z* 226–163 was used for quantification (external calibration using coumaphos PESTANAL™). The standard solutions were prepared in blank matrix extract to compensate for matrix effects. The limit of detection was 0.008 mg/L, which corresponded to 0.08 mg/kg wax, and the limit of quantification (LOQ) was 0.01 mg/L, which corresponded to an LOQ of 0.1 mg/kg wax. The recovery at spiking levels between 0.1 and 400 mg/kg were in the range 80%–96%.

RESULTS

First, the effect of coumaphos in foundation sheets on the developing bees was studied. The median emergence rate was 51% for bees raised in cells drawn on control foundation sheets (Figure 2). Similar emergence rates were observed for bees grown in cells drawn on foundation squares containing up to 62 mg of coumaphos per kg of wax. Their median values ranged from 41% (coumaphos at 26 mg/kg) up to 53% (coumaphos at 5 mg/kg). These emergence rates did not differ significantly from the controls (Mann–Whitney *U*-test: $p > 0.05$), suggesting that coumaphos levels in foundation sheets up to 62 mg/kg did not affect mortality rate up to the imago stage (Figure 2). However, the emergence rates with a median value of 14% were significantly lower for bees grown in cells drawn on foundation squares containing coumaphos at 132 mg/kg (Mann–Whitney *U*-test: $p < 0.05$, e.g. $U = 14$, $z = 2.684$, $p = 0.007$ for 62 vs. 132 mg/kg), suggesting that this coumaphos level increased overall mortality (Figure 2).

The development of the bees from egg to emergence was monitored to investigate which developing stage was mainly affected by coumaphos residues in foundation sheets. For all the tested coumaphos concentrations, larvae hatched from 74% of the eggs or above (mean value of controls 76%; Figure 3), suggesting that coumaphos residues in beeswax at the tested concentrations had no major effect on the hatching rate from the eggs. The present study suggests that

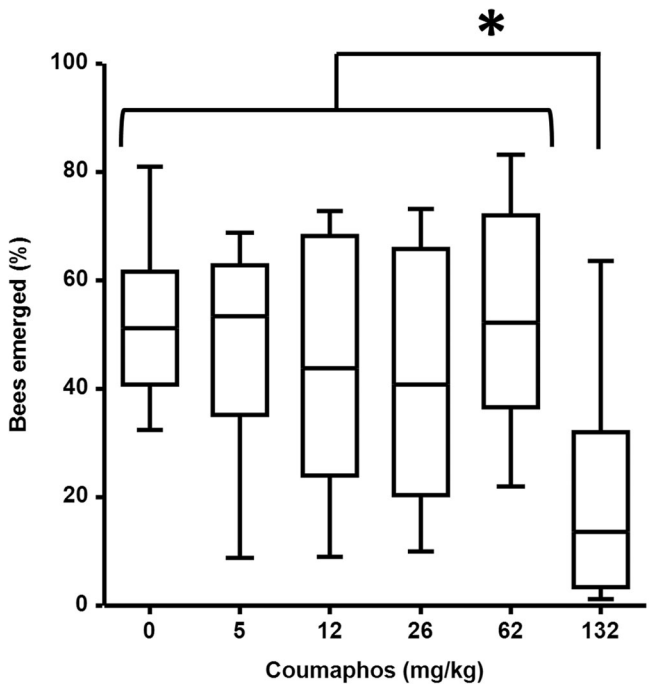

FIGURE 2: Emergence rate of bees raised on foundation sheets containing coumaphos at concentrations ranging from 0 to 132 mg/kg ($n = 10$ for each tested concentration). The line at the center of each box indicates the median, while the edges of the boxes indicate the upper and lower quartiles. The edge of the whiskers represent the minimal and maximal values. *Mann–Whitney *U*-test; $p < 0.05$.

coumaphos in foundation sheets affected brood survival mainly during the larval stage. The mean numbers of sealed brood cells corresponded to 48%–57% of the initial eggs for all coumaphos levels in foundation sheets up to 62 mg/kg, while the mean number of sealed brood cells was 24% for a coumaphos level of 132 mg/kg (Figure 3). Hence, bees capped a lower rate of cells drawn on these foundation sheets, suggesting that coumaphos increased mortality at the larval stage. Finally, adult bees emerged from most capped cells of all tested conditions, suggesting no markedly further effect of coumaphos on pupation.

Second, the distribution of coumaphos at comb positions built on the experimental foundations squares were analyzed to estimate the levels of exposure of the developing bees to coumaphos residues. After a single brood cycle, coumaphos levels in the remaining foundations of the experimental squares were approximately 70% of the concentrations in the initial molded foundation sheets. The coumaphos levels were reduced between 21% and 36%, as compared with the initial concentrations (Table 1). The coumaphos concentrations in the drawn cells were between 45% and 66% lower than in the initial molded foundation sheets (Table 1), as measured at the end of a single brood cycle. Hence, the coumaphos levels in the drawn cells on the experimental foundation squares were approximately 40% of the initial levels in the molded foundation sheets.

DISCUSSION

The present study aimed to assess at what level coumaphos residues in recycled beeswax pose a risk for brood development in an in-hive environment. After one brood cycle, coumaphos levels in the drawn cells were between 45% and 66% below the concentrations of the initial foundation sheets. A coumaphos level at 62 mg/kg in foundation sheets led to a coumaphos concentration of 21 mg/kg in the drawn cells. This level did not affect brood mortality. On the other hand, a coumaphos level of 132 mg/kg in foundation sheets resulted in a level of 51 mg/kg in drawn cells. At this concentration, mortality rates were significantly increased, suggesting negative effects on brood development at the colony level. Based on the results of the present study focusing on brood development, maximal coumaphos levels up to 60 mg/kg for recycled

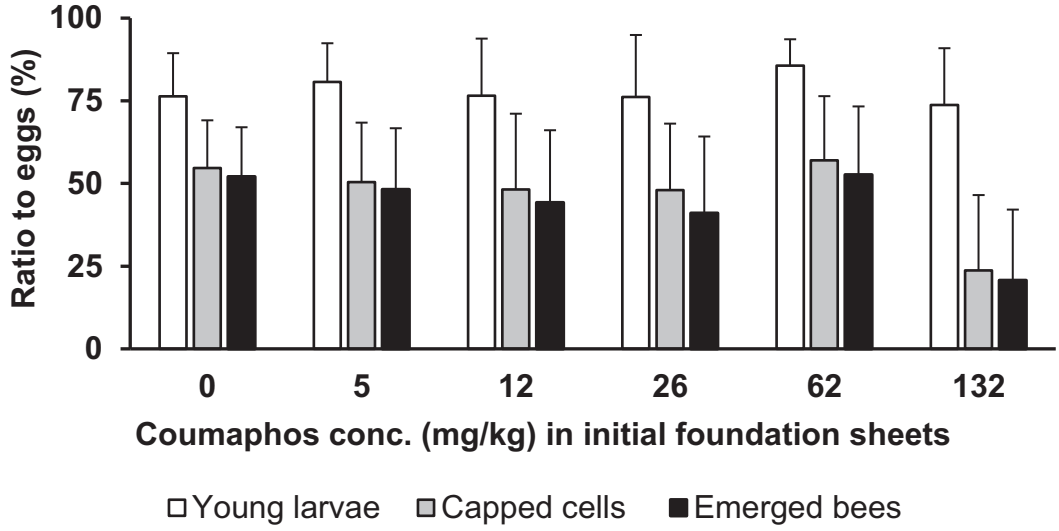

FIGURE 3: Developmental stages of honeybees raised on foundation sheets containing various coumaphos concentrations. The initial number of eggs was set to 100%, and the percentages (%) of young larvae (white boxes), capped cells (gray boxes), and emerged bees (black boxes) were calculated with respect to the initial number of eggs. The graphic shows means and standard deviations for 10 repetitions.

TABLE 1: Coumaphos concentrations in the initial foundation sheets and after one brood cycle in the remaining foundations and drawn cells

Coumaphos concentration in foundation sheets	Coumaphos concentration after one brood cycle											
	Remaining foundations						Drawn cells					
	Mean	SD	Min[a]	Max[b]		Reduction[d]	Mean	SD	Min[a]	Max[b]		Reduction[d]
(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	n[c]	(%)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	n[c]	(%)
0	0.4	0.2	0.2	0.7	10		1.2	0.6	0.4	2.6	10	
5	4	1	3	6	10	21	3	2	2	7	10	45
12	9	2	6	11	10	29	5	2	3	8	10	56
26	18	3	14	23	10	32	12	4	5	20	10	56
62	40	8	30	55	10	36	21	9	11	42	10	66
132	92	21	49	113	10	30	51	20	15	76	10	61

[a]Minimal value (min).
[b]Maximal value (max).
[c]Number (n) of samples.
[d]Reduction calculated in terms of the concentration in the initial foundation sheet.

foundation wax might be acceptable. However, one has to keep in mind that an additive effect of coumaphos in combination with other residue types was not considered in the present study, nor were additional nonlethal effects, such as the longevity of the adult bee.

As reported in most international studies on residues in wax foundation sheets, coumaphos levels were usually below 20 mg/kg (Alkassab et al., 2020; El Agrebi et al., 2020; Fulton et al., 2019b; Lozano et al., 2019; Murcia Morales et al., 2020; Perugini et al., 2018). For example, levels up to 4.3 mg/kg have been measured in Swiss (Marti et al., 2022), up to 10.9 mg/kg in German (Shimshoni et al., 2019), and up to 17.4 mg/kg in Spanish foundation beeswax (Calatayud-Vernich et al., 2017). According to the present study, these levels would result in coumaphos levels in the combs that would not increase brood mortality. Therefore, it can be concluded that the exposure route of coumaphos through beeswax in most cases does not affect brood survival. It is beneficial that residues in the foundation sheets are diluted when bees produce new wax to construct the combs or else when bees transfer wax from other locations with lower residue levels.

The developing larvae seem to be especially sensitive to coumaphos exposure, which is in line with the observations in our in vitro test series preceding the present study (Kast & Kilchenmann, 2022). Coumaphos levels up to 20 mg/kg were nonlethal, whereas 55.9 mg/kg was the concentration killing 50% of the individuals (Kast & Kilchenmann, 2022). Thus, the mortality rate at a given coumaphos level obtained in the in vitro test series correlates well with the rates obtained in an in-hive environment with respect to the actual concentrations measured in the drawn cells. Based on the results of the in vitro model, a maximal coumaphos level of 20 mg/kg for beeswax has been previously proposed. This maximal value may remain valid for the interpretation of residue data in brood combs after the application of coumaphos-containing products for mite control.

Coumaphos is the active substance in veterinary products such as Perizin or CheckMite+. These products are authorized in several countries to treat honeybees against the parasitical mite *V. destructor* (D'Ascenzi et al., 2019). Levels up to 91.9 mg/kg have been previously reported for comb wax (Mullin et al., 2010). Other studies have reported coumaphos levels in beeswax of up to 35.1 mg/kg after application of CheckMite+ in two consecutive years (Premrov Bajuk et al., 2017). Levels of 36–159 mg/kg were measured 7 months after a single CheckMite+ application in central brood combs that were close to the strips (Kast et al., 2020). Hence, treatment against the *V. destructor* mite can lead to coumaphos levels (above 20 mg/kg) in brood combs that negatively affect brood development, especially in comb areas that are close to the treatment strips. On the other hand, this effect might be restricted to the first brood cycle because honey bee cocoons can act as barriers to coumaphos exposure in subsequent brood cycles (Fries et al., 1998).

Subtle effects on bee development due to residues in beeswax are best studied in in vitro studies because this permits following each larva under standardized conditions from an early age to the emergence of the bee (Kast & Kilchenmann, 2022), while in an in-hive study it is much more challenging to obtain standardized conditions leading to higher variability of the data. Eggs and young larvae might be removed by bees for reasons unrelated to mortality. Brood cannibalism in free-flying colonies occurs in response to environmental conditions and for seasonal regulation of the colony size (Woyke, 1977). A comprehensive study revealed a large variability in overall brood termination rates until emergence of the bees, especially for experiments initiated after the end of June (Pistorius et al., 2011), which is in line with our study. In this respect, it might be preferable to perform future studies a bit earlier in the season.

Impacts of temperature and relative humidity on brood development have been reviewed by Abou-Shaara et al. (2017). An optimal relative humidity is especially important for the hatching rate of young larvae from eggs (Doull, 1976). For successful in vitro rearing of honey bee larvae, a narrow range of temperature and relative humidity has to be maintained, also showing the importance of these parameters on brood development (Aupinel et al., 2005). In our study, the combs were kept outside the optimal microclimate of the hive on several occasions for marking the eggs, larvae, and sealed brood on the test foils. Given the importance of an optimal temperature and humidity, this fact may have had an impact on control

mortality during the egg and larval stages. Nevertheless, the obtained brood termination rates (unsuccessful development of larvae from the eggs) of up to 26% in our study were in line with previous studies (Lückmann & Tänzler, 2020; Szczesniak et al., 2018). Furthermore, we obtained in our experimental setting an average control mortality of 28% (hatched larvae vs. capped cells), which is comparable to that in other studies reporting a mortality of 22% for controls during the larval stage (Schott et al., 2021).

In conclusion, commercially recycled beeswax with coumaphos residues at the currently reported levels is not expected to lead to coumaphos levels in combs that substantially increase brood mortality. Whether this is also true for coumaphos residues in combination with other residue types or pathogens needs further investigation. Additional studies may also include sublethal effects related to exposure to residues in beeswax during development, such as the longevity of the bees as adults, their foraging capabilities, the size of their hypopharyngeal glands, and their effect on brood care.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5645.

Acknowledgments—No funding was received. Open access funding provided by Agroscope.

Conflict of Interest—The authors declare no conflict of interest.

Author Contributions Statement—**Christina Kast:** Conceptualization; Data curation; Methodology; Project administration; Writing—original draft; Writing—review & editing. **Benoît Droz:** Conceptualization; Investigation; Methodology; Writing—review & editing. **Verena Kilchenmann:** Conceptualization; Data curation; Investigation; Methodology; Writing—review & editing.

Disclosure Statement—No financial interest or benefit that has arisen from the direct applications of this research.

Data Availability Statement—Data and associated metadata is available as supplementary material.

REFERENCES

Abou-Shaara, H. F., Owayss, A. A., Ibrahim, Y. Y., & Basuny, N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, *64*, 455–463. https://doi.org/10.1007/s00040-017-0573-8

Alkassab, A. T., Thorbahn, D., Frommberger, M., Bischoff, G., & Pistorius, J. (2020). Effect of contamination and adulteration of wax foundations on the brood development of honeybees. *Apidologie*, *51*, 642–651. https://doi.org/10.1007/s13592-020-00749-2

Aupinel, P., Fortini, D., Dufour, H., Tasei, J. N., Michaud, B., Odoux, J. F., & Delegue, M. H. P. (2005). Improvement of artificial feeding in a standard in vitro methods for rearing *Apis mellifera* larvae. *Bulletin of Insectology*, *58*, 107–111.

Bogdanov, S. (2004). Beeswax: Quality issues today. *Bee World*, *85*(3), 46–50. https://doi.org/10.1080/0005772X.2004.11099623

Bogdanov, S., Kilchenmann, V., & Imdorf, A. (1998). Acaricide residues in some bee products. *Journal of Apicultural Research*, *37*(2), 57–67. https://doi.org/10.1080/00218839.1998.11100956

Calatayud-Vernich, P., Calatayud, F., Simó, E., & Picó, Y. (2017). Occurrence of pesticide residues in Spanish beeswax. *Science of the Total Environment*, *605–606*, 745–754. https://doi.org/10.1016/j.scitotenv.2017.06.174

Collins, A. M., Pettis, J. S., Wilbanks, R., & Feldlaufer, M. F. (2004). Performance of honey bee (*Apis mellifera*) queens reared in beeswax cells impregnated with coumaphos. *Journal of Apicultural Research*, *43*(3), 128–134. https://doi.org/10.1080/00218839.2004.11101123

D'Ascenzi, C., Formato, G., & Martin, P. (2019). Chemical hazards in honey. In F. J. Smulders, I. M. Rietjens, & M. D. Rose (Eds.), *Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health* (pp. 443–475). Wageningen Academic Publishers.

Doull, K. M. (1976). The effects of different humidities on the hatching of the eggs of honeybees. *Apidologie*, *7*, 61–66.

Droz, B., Kilchenmann, V., & Kast, C. (2020). Coumaphos im Wachs: Ein Risiko für die Bienengesundheit. *Schweizerische Bienenzeitung*, *2*, 13–15.

El Agrebi, N., Traynor, K., Wilmart, O., Tosi, S., Leinartz, L., Danneels, E., de Graaf, D. C., & Saegerman, C. (2020). Pesticide and veterinary drug residues in Belgian beeswax: Occurrence, toxicity, and risk to honey bees. *Science of the Total Environment*, *745*, 141036. https://doi.org/10.1016/j.scitotenv.2020.141036

Fries, I., Wallner, K., & Rosenkranz, P. (1998). Effects on *Varroa jacobsoni* from acaricides in beeswax. *Journal of Apicultural Research*, *37*(2), 85–90. https://doi.org/10.1080/00218839.1998.11100959

Fulton, C. A., Huff Hartz, K. E., Fell, R. D., Brewster, C. C., Reeve, J. D., & Lydy, M. J. (2019b). An assessment of pesticide exposures and land use of honey bees in Virginia. *Chemosphere*, *222*, 489–493. https://doi.org/10.1016/j.chemosphere.2019.01.156

Fulton, C. A., Huff Hartz, K. E., Reeve, J. D., & Lydy, M. J. (2019a). An examination of exposure routes of fluvalinate to larval and adult honey bees (*Apis mellifera*). *Environmental Toxicology and Chemistry*, *38*(6), 1356–1363. https://doi.org/10.1002/etc.4427

Kast, C., & Kilchenmann, V. (2022). An in vitro model for assessing the toxicity of pesticides in beeswax on honey bee larvae. *Chemosphere*, *287*, 132214. https://doi.org/10.1016/j.chemosphere.2021.132214

Kast, C., Kilchenmann, V., & Charrière, J. D. (2021). Long-term monitoring of lipophilic acaricide residues in commercial Swiss beeswax. *Pest Management Science*, *77*(9), 4026–4033. https://doi.org/10.1002/ps.6427

Kast, C., Kilchenmann, V., & Droz, B. (2020). Distribution of coumaphos in beeswax after treatment of honeybee colonies with CheckMite® against the parasitical mite *Varroa destructor*. *Apidologie*, *51*(1), 112–122. https://doi.org/10.1007/s13592-019-00724-6

Lozano, A., Hernando, M. D., Uclés, S., Hakme, E., & Fernández-Alba, A. R. (2019). Identification and measurement of veterinary drug residues in beehive products. *Food Chemistry*, *274*, 61–70. https://doi.org/10.1016/j.foodchem.2018.08.055

Lückmann, J., & Tänzler, V. (2020). Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: Is there a difference of the brood termination rate? Hazards of pesticides to bees–14th international symposium of the ICP-PR Bee protection group, October 23–25 2019, Bern (Switzerland). *Julius-Kühn-Archiv*, *465*, 91–95. https://doi.org/10.5073/jka.2020.465.043

Marti, J. N. G., Kilchenmann, V., & Kast, C. (2022). Evaluation of pesticide residues in commercial Swiss beeswax collected in 2019 using ultra-high performance liquid chromatographic analysis. *Environmental Science and Pollution Research*, *29*, 32054–32064. https://doi.org/10.1007/s11356-021-18363-9

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., van Engelsdorp, D., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. *PLoS One*, *5*, e9754. https://doi.org/10.1371/journal.pone.0009754

Murcia Morales, M., Ramos, M. J. G., Vázquez, P. P., Galiano, F. J. D., Valverde, M. G., López, V. G., Flores, J. M., & Fernández-Alba, A. R. (2020). Distribution of chemical residues in the beehive compartments and their transfer to the honeybee brood. *Science of the Total Environment*, *710*, 136288. https://doi.org/10.1016/j.scitotenv.2019.136288

Perugini, M., Tulini, S. M., Zezza, D., Fenucci, S., Conte, A., & Amorena, M. (2018). Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013–2015. *Science of the Total Environment*, *625*, 470–476. https://doi.org/10.1016/j.scitotenv.2017.12.321

Pettis, J. S., Collins, A. M., Wilbanks, R., & Feldlaufer, M. F. (2004). Effects of coumaphos on queen rearing in the honey bee, *Apis mellifera*. *Apidologie*, *35*(6), 605–610. https://doi.org/10.1051/apido:2004056

Pistorius, J., Becker, R., Lückmann, J., Schur, A., Barth, M., Jeker, L., Schmitzer, S., & von der Ohe, W. (2011). *Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions*. 11th International Symposium of the ICP-BR Bee Protection Group, Wageningen, the Netherlands, November 2–4, 2011. https://doi.org/10.5073/jka.2012.437.033

Premrov Bajuk, B., Babnik, K., Snoj, T., Milčinski, L., Pislak Ocepek, M., Škof, M., Jenčič, V., Filazi, A., Štajnbaher, D., & Kobal, S. (2017). Coumaphos residues in honey, bee brood, and beeswax after Varroa treatment. *Apidologie*, *48*(5), 588–598. https://doi.org/10.1007/s13592-017-0501-y

Schott, M., Sandmann, M., Cresswell, J. E., Becher, M. A., Eichner, G., Brandt, D. T., Halitschke, R., Krueger, S., Morlock, G., Düring, R. A., Vilcinskas, A., Meixner, M. D., Büchler, R., & Brandt, A. (2021). Honeybee colonies compensate for pesticide-induced effects on royal jelly composition and brood survival with increased brood production. *Scientific Reports*, *11*, 62. https://doi.org/10.1038/s41598-020-79660-w

Shimshoni, J. A., Sperling, R., Massarwa, M., Chen, Y., Bommuraj, V., Borisover, M., & Barel, S. (2019). Pesticide distribution and depletion kinetic determination in honey and beeswax: Model for pesticide occurrence and distribution in beehive products. *PLoS One*, *14*(2), e0212631. https://doi.org/10.1371/journal.pone.0212631

Szczesniak, B., Pilling, E., Bocksch, S., Becker, R., & Lückmann, J. (2018). ICP-PR Bee Brood Working Group – Variability of brood termination rates in reference to validity criteria and limited effectiveness of method improvement in honeybee semi-field studies (OECD GD 75). Hazards of pesticides to bees–13th international symposium of the ICP-PR Bee protection group, October 18–20 2017, Valencia (Spain), *Julius-Kühn-Archiv*, *462*, 111–115. https://doi.org/10.5073/jka.2018.462.030

Tihelka, E. (2018). Effects of synthetic and organic acaricides on honey bee health: A review. *Slovenian Veterinary Research*, *55*(3), 119–140. https://doi.org/10.26873/SVR-422-2017

Van Buren, N. W., Mariën, J., Velthuis, H. H., & Oudejans, R. C. (1992). Residues in beeswax and honey of Perizin, an acaricide to combat the mite *Varroa jacobsoni* Oudemans (Acari: Mesostigmata). *Environmental Entomology*, *21*(4), 860–865. https://doi.org/10.1093/ee/21.4.860

Woyke, J. (1977). Cannibalism and brood-rearing efficiency in the honey bee. *Journal of Apicultural Research*, *16*(1), 84–94. https://doi.org/10.1080/00218839.1977.11099866

