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Comparison of transfer of different sulphonamides from contaminated beeswax to honey

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Abstract

Introduction: No maximum residue limits in honey have been legislated in the EU for antimicrobial substances such as sulphonamides, and they are not permitted, therefore, for treating honey bees unless in a cascade system. Since sulphonamides are used illegally in apiculture to treat foulbrood, their residues can be found in honey and other apiculture products, including beeswax. The study aimed to assess the contamination of honey from beeswax containing residues of 10 sulphonamides (sulphadimethoxine (SDM), sulphadoxine (SDX), sulphamonomethoxine (SMM), sulphamethoxazole (SMX), sulphameter (SMT), sulphamethazine (SMZ), sulphamerazine (SMR), sulphadiazine (SDA), sulphathiazole (STZ) and sulphacetamide (SCA)). **Material and Methods:** Wax-based foundations fortified with 10 sulphonamides at 10,000 µg/kg were evaluated for sulphonamide concentrations and then placed in a beehive so that honey bees (*Apis mellifera* L.) could build honeycombs with them. Frames of capped honey were taken out of the hives one month later and honey was sampled from them. The honeycombs were subsequently incubated in a laboratory at 35°C for five months, and honey was sampled monthly. The honey sulphonamide concentrations were measured using liquid chromatography–tandem mass spectrometry and compared to the wax-based foundation concentrations. **Results:** The maximum transfers to honey of the initial amount of SDM, SDX, SMM, SMX, SMT, SMZ, SMR, SDA, STZ and SCA in the wax-based foundations were 42.6, 34.3, 31.7, 30.1, 29.5, 25.2, 18.7, 16.1, 9.5 and 8.6%, respectively. **Conclusion:** This study demonstrated that every tested sulphonamide could migrate from beeswax in antimicrobial-tainted honeycombs to honey, SDM having the highest migration potential and SCA the lowest.

Keywords: sulphonamides, beeswax, honey, transfer, LC-MS/MS.

Introduction

The honey bee (*Apis mellifera* L.) population, and thus the manufacture of honey and other apiculture products, may significantly decline because of bacterial or protozoal diseases in honey bees. American and European foulbrood are the diseases that affect adult honey bees most severely and widely. They cause significant losses in apiaries and are a grave economic problem. These infections in bee colonies have usually been controlled by using sulphonamides (1). However, no maximum residue limits (MRLs) in honey were set for sulfonamides or other antimicrobial substances in the Commission Regulation (EU) No. 37/2010 (3), and thus, they are not permitted for treating honey bees. They can only be applied in apiculture within a cascade system (6), according to which the veterinarian prescribes only a veterinary medicinal product with an allowed pharmacologically active substance and sets a withdrawal period long enough to guarantee that the honey does not contain residues in amounts harmful to human health. Although this rule has been adopted, 14 honey samples monitored under Directive 96/23/EC were non-compliant for antibacterial residues in 2021 (4). Honey was the most frequently non-compliant animal product with antibacterials (0.96%). The antibacterial classes with the highest instances in honey were sulphonamides and tetracyclines. Most of these non-compliant results were due to the presence of sulphamethazine, sulphathiazole, sulphacetamide, sulphachloropyrazine, sulphadimethoxine, and sulphamonomethoxine.

Depletion of residues of pharmacologically active substances in honey is not time-dependent, in contrast to their depletion in mammalian or avian organisms due to pharmacokinetic behaviour. When the residues are found in honey, they mostly stay there (5). Because of this, using sulphonamides illegally may cause their residue accumulation in honey and other apiculture products like beeswax (7, 10). Apart from honey, beeswax is a valuable beehive product. It is used by

the food, cosmetic and pharmaceutical industries in a wide range of applications that require high-quality beeswax. The product is classified as an authorised food additive (E 901) in the EU and is listed in the European Pharmacopoeia (2). Since it is a natural product, there should not be any additives in it.

However, beeswax can dissolve or integrate toxic compounds, which could be released long afterwards when the beeswax is used to produce pharmaceuticals or cosmetics, is eaten, or is given to honey bees as a wax foundation. Previous work has focused only on sulphamethazine, which can contaminate honey during the next season if it stays in the comb's wax after being used in the hive (11). To the best of our knowledge, no similar studies have been carried out for other sulphonamides. Therefore, the aim of the research was to compare the migration of 10 sulphonamides from contaminated beeswax to honey.

Material and Methods

Reagents and chemicals. All solvents and chemical compounds used were of analytical or liquid chromatography grade. JT Baker (Deventer, the Netherlands) provided acetonitrile, acetic acid, ammonium hydroxide solution, isopropanol, methanol and n-hexane. A Milli-Q plus water purification system from Millipore (Bedford, MA, USA) produced ultrapure water. Strata SCX solid-phase extraction (SPE) tubes (500 mg, 3 mL) were supplied by Phenomenex (Torrance, CA, USA). The PVDF syringe filters (0.45 µm, 13 mm) were provided by Restek (Bellefonte, PA, USA). High purity analytical standards (>98.5%) of sulphadimethoxine (SDM), sulphadoxine (SDX), sulphamonomethoxine (SMM), sulphamethoxazole (SMX), sulphameter (SMT), sulphamethazine (SMZ), sulphamerazine (SMR), sulphadiazine (SDA), sulphathiazole (STZ) and sulphacetamide (SCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulphadimethoxine-D_6 (SDM-D_6), sulphadoxine-D_3 (SDX-D_3), sulphamonomethoxine-$^{13}C_6$ (SMM-$^{13}C_6$), sulphamethoxazole-$^{13}C_6$ (SMX-^{13}C), sulphamethazine-$^{13}C_6$ hemihydrate (SMZ-$^{13}C_6$), sulphamerazine-$^{13}C_6$ (SMR-$^{13}C_6$), sulphadiazine-$^{13}C_6$ (SDA-$^{13}C_6$) and sulphathiazole-$^{13}C_6$ (STZ-$^{13}C_6$) were obtained from Witega Laboratorien Berlin-Adlershof (Berlin, Germany), and sulphameter-D_4 (SMT-D_4) was acquired from Toronto Research Chemicals (Toronto, ON, Canada). Isotopically labelled analytes used as internal standards (ISs) were of chemical and isotopic purity greater than 98%.

Beeswax foundation and treatment. Blank beeswax was melted at 80°C, and a mixture of 10 sulphonamides in methanol was added to produce wax foundations (n = 4) containing sulphonamides at a concentration of 10,000 µg/kg. After mixing, liquid sulphonamide-containing beeswax was poured into a wax foundation mould and allowed to cool down. Next, a small portion of every beeswax foundation was removed for liquid chromatography–tandem mass spectrometry (LC-MS/MS) sulphonamide analysis. Subsequently, the sulphonamide-contaminated wax foundations were put into wooden frames (260 × 360 × 35 mm). In mid-June, when it was the summer flowering season, each frame was placed near the brood nest in a separate hive so that the honey bees (*Apis mellifera* L.) could build their combs from the contaminated wax foundations. A week later, the frames were moved from a brood box of the hives to a super box separated with a queen excluder so that the queen could not lay eggs in the combs. After three weeks, the capped honey frames were taken from the beehives for the initial honey sampling. After that, the frames were kept in the laboratory at 35°C for 5 months, and aliquots of honey (n = 4) were sampled every month. The concentration of sulphonamides in the honey samples was determined using LC-MS/MS and compared to those in the contaminated wax foundations before they were put into the frames. Sampling and analysis of residues of sulphonamides were also carried out in honey from negative control honeycombs: those present in the hives investigated in this study and those located within the same apiary.

Standard solutions. Stock solutions of individual analytical standards (1,000 µg/mL) were prepared in acetonitrile (stable at −20°C for at least a year). Next, the solutions were combined and diluted with 0.1% acetic acid to create sulphonamide and IS working standard solutions, which, when not in use, were kept in amber glass at 4°C where they were stable for at least six months.

Sample preparation. Honey analysis was performed as previously reported by Mitrowska *et al.* (9). A 2g sample of honey was weighed into a 50 mL centrifugal polypropylene tube and spiked with ISs at 25 µg/kg. After adding 15 mL of 2% acetic acid, the mixture was vortexed and placed into an ultrasonic water bath at 40°C for 10 min. Next, following centrifugation at 2,200 × *g* and −4°C for 10 min, the supernatant was loaded onto a Strata SCX SPE tube previously preconditioned with 5 mL of methanol and 5 mL of 2% acetic acid. The column was washed with 5 mL of 2% acetic acid and 5 mL of methanol and dried for 5 min. A 5 mL-volume mixture of acetonitrile and ammonium hydroxide (95:5, v/v) was used to elute sulphonamides. The eluate was evaporated at 35°C, 90% vortex speed and 110 mbar vacuum for 30 min with a RapidVap Vacuum Dry Evaporation system (Labconco, Kansas City, MO, USA). After reconstitution with 400 µL of 0.1% acetic acid, the honey extract was filtered, and 10 µL was analysed in the LC-MS/MS system. Beeswax sample preparation was carried out as earlier reported by Mitrowska *et al.* (8) with minor changes. A 1g sample of beeswax was weighed into a 50 mL polypropylene centrifuge tube and spiked with ISs at 5,000 µg/kg. After adding 10 mL of a mixture of n-hexane and isopropanol (8:2, v/v), the tube was vortexed and put into an ultrasonic water bath at 40°C for 10 min. After melting the beeswax, 10 mL of 2% acetic acid was added, and the tube was vortexed and subjected to ultrasonication for 10 min. Following centrifugation at 2,200 × *g* at −4°C for 10 min, a 100 µL volume of the aqua phase was diluted with 900 µL

of 0.1% acetic acid, and 10 µL was analysed in the LC-MS/MS system.

Liquid chromatography–tandem mass spectrometry and quantification. An LCMS-8050 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) with an electrospray ionisation source and Lab Solutions LCMS 5.60 SP2 software (Shimadzu) was used to analyse honey and beeswax extracts. Sulphonamides were separated chromatographically in gradient mode on a Luna analytical column of 150 × 2 mm with 3 µm particle size, in which the stationary phase was pentafluorophenyl (Phenomenex). Mobile phase A contained 0.01% acetic acid in acetonitrile, and mobile phase B contained 0.01% acetic acid in water. More information about LC-MS/MS conditions and ion transitions monitored can be found in Mitrowska *et al.* (8).

Spiked and blank quality control honey and beeswax samples were analysed with each sample series. The sulphonamide concentrations in honey and beeswax samples were determined by comparing the ratio of a sulphonamide peak area to its corresponding IS peak area with the same ratio in the calibration curves (2–200 µg/kg for honey and 400–40,000 µg/kg for beeswax).

Statistical analysis. The significance of the differences was determined using analysis of variance.

Results

Quantification and method validation. The methods used to quantify sulphonamides in honey and beeswax were validated by determining specificity, linearity, intermediate precision, recovery, limit of detection and limit of quantification. The specificity of the methods was assessed by examining 20 blank honey and beeswax samples. During the retention time of the target compounds, no interfering peaks from natural substances were observed. The calibration curves demonstrated high linearity for each analyte in the concentration range of 2–200 µg/kg for honey and 400–40,000 µg/kg for beeswax (correlation coefficient > 0.99). For each sulphonamide in honey, the limit of detection and limit of quantification were 1 and 2 µg/kg, respectively, whereas the corresponding values in beeswax were 200 and 400 µg/kg. The recoveries of sulphonamides from honey (at 2, 25 and 100 µg/kg) and from beeswax (at 1,000, 5,000 and 10,000 µg/kg) ranged from 68.1 to 99.9% with a coefficient of variation < 16.6% under intermediate precision conditions (Table 1). The data showed that the methods for quantification of sulphonamides in honey and beeswax were accurate, precise and fit for use in these studies.

Table 1. Validation parameters calculated for determination of sulphonamides in honey and beeswax samples (n = 18)

	Honey			Beeswax		
Sample	Concentration (µg/kg)	Recovery (%)	Intermediate precision (CV, %)	Concentration (µg/kg)	Recovery (%)	Intermediate precision (CV, %)
SCA	2	69.7	15.1	1,000	71.9	12.7
	25	68.1	14.9	5,000	73.1	14.6
	100	72.4	16.6	10,000	69.1	13.2
SDA	2	96.7	12.9	1,000	98.2	11.4
	25	98.4	11.7	5,000	99.2	12.3
	100	99.3	13.2	10,000	97.7	12.9
STZ	2	93.3	13.4	1,000	98.9	10.4
	25	93.2	11.8	5,000	99.1	12.3
	100	94.2	14.2	10,000	97.2	10.9
SMR	2	97.2	14.8	1,000	97.4	10.1
	25	99.7	13.2	5,000	98.6	10.9
	200	98.3	14.6	10,000	97.7	12.3
SMZ	2	97.4	11.9	1,000	99.1	12.9
	25	99.7	13.2	5,000	98.6	10.4
	200	98.1	12.8	10,000	99.7	12.1
SMT	2	98.0	12.3	1,000	97.1	11.7
	25	96.7	11.1	5,000	98.5	13.2
	200	99.3	12.8	10,000	97.9	10.5
SMM	2	96.9	13.7	1,000	99.9	12.1
	25	98.0	12.9	5,000	97.0	10.3
	200	99.6	14.1	10,000	99.1	12.2
SDX	2	97.0	10.4	1,000	98.2	13.2
	25	99.9	11.2	5,000	97.9	11.5
	200	98.3	10.7	10,000	99.7	13.3
SMX	2	96.1	14.5	1,000	98.0	11.8
	25	97.8	13.8	5,000	96.6	11.9
	200	99.7	13.2	10,000	98.8	12.8
SDM	2	95.6	15.9	1,000	96.3	12.9
	25	94.2	13.2	5,000	99.3	12.6
	200	97.8	15.2	10,000	97.0	10.2

CV – coefficient of variation; SCA – sulphacetamide; SDA – sulphadiazine; STZ – sulphathiazole; SMR – sulphamerazine; SMZ – sulphamethazine; SMT – sulphameter; SMM – sulphamonomethoxine; SDX – sulphadoxine; SMX – sulphamethoxazole; SDM – sulphadimethoxine

Table 2. The concentrations and recoveries of sulphonamides calculated for the beeswax foundation fortified with 10 sulphonamides at 10,000 µg/kg (n = 4)

Analyte	Concentration (µg/kg)	Recovery (%)	Analyte	Concentration (µg/kg)	Recovery (%)
SDM	4,625 ± 287	46	SMR	4,358 ± 260	44
SMM	5,326 ± 399	53	SDA	5,027 ± 318	50
SDX	4,541 ± 383	45	SMT	1,993 ± 156	20
SMX	3,824 ± 406	38	SCA	4,409 ± 370	44
SMZ	4,138 ± 152	41	STZ	3,072 ± 236	31

SDM – sulphadimethoxine; SMM – sulphamonomethoxine; SDX – sulphadoxine; SMX – sulphamethoxazole; SMZ – sulphamethazine; SMR – sulphamerazine; SDA – sulphadiazine; SMT – sulphameter; SCA – sulphacetamide; STZ – sulphathiazole

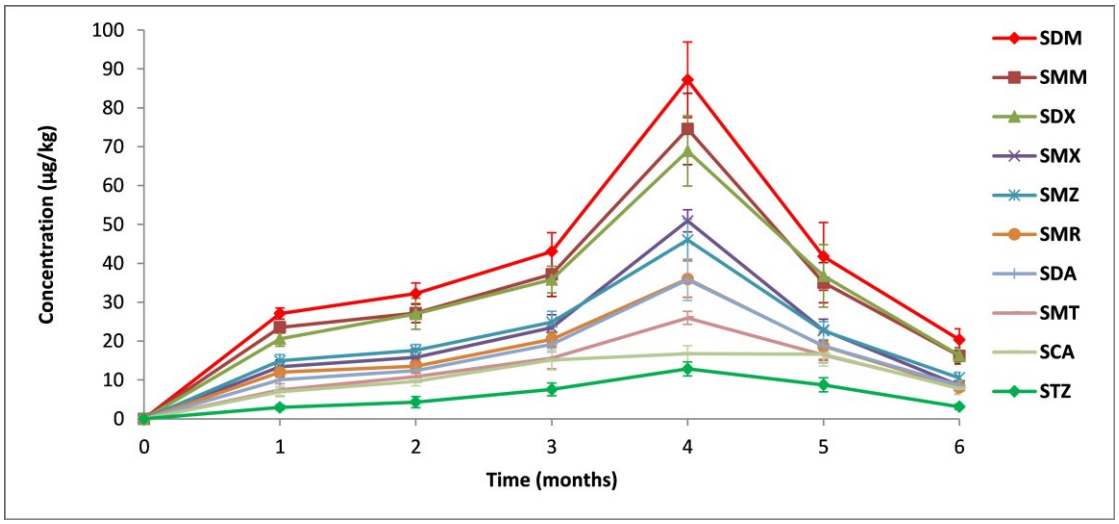

Fig. 1. Concentrations of sulphadimethoxine (SDM), sulphamonomethoxine (SMM), sulphadoxine (SDX), sulphamethoxazole (SMX), sulphamethazine (SMZ), sulphamerazine (SMR), sulphadiazine (SDA), sulphameter (SMT), sulphacetamide (SCA) and sulphathiazole (STZ) in honey sampled from a comb drawn out on a wax foundation contaminated with these antimicrobials at 4,625, 5,326, 4,541, 3,824, 4,138, 4,358, 5,027, 1,993, 4,409 and 3,072 µg/kg, respectively (n = 4)

Table 3. The maximum transfer (%) of sulphonamides from tainted beeswax to the honey stored in the comb

Analyte	The maximum transfer (%) of sulphonamides					
	1 month	2 months	3 months	4 months	5 months	6 months
SDM	13.2	15.7	21.1	42.6	20.4	9.9
SDX	10.3	13.4	17.9	34.3	18.3	8.1
SMM	10.0	11.5	15.8	31.7	14.9	6.9
SMX	7.9	9.3	13.9	30.1	13.5	5.1
SMT	8.4	12.3	17.7	29.5	18.6	9.0
SMZ	8.2	9.6	13.6	25.2	12.3	5.8
SMR	6.2	7.0	10.6	18.7	9.7	4.2
SDA	4.5	5.6	8.6	16.1	8.4	3.9
STZ	2.2	3.2	5.6	9.5	6.4	2.3
SCA	3.6	5.0	7.8	8.6	8.5	4.0

SDM – sulphadimethoxine; SDX – sulphadoxine; SMM – sulphamonomethoxine; SMX – sulphamethoxazole; SMT – sulphameter; SMZ – sulphamethazine; SMR – sulphamerazine; SDA – sulphadiazine; STZ – sulphathiazole; SCA – sulphacetamide. Combs were completely filled with honey on both sides, thus the ratio of honey (2,448 g) to beeswax (110 g) at each sampling point on the comb was always 22.25 : 1

Table 4. Predicted pKa and lipophilicity of sulphonamides

Analyte	pKa_1[a] pKa_2[b]	% particles in the unionised form at pH 4.2	log*P*	Analyte	pKa_1[a] pKa_2[b]	% particles in the unionised form at pH 4.2	log*P*
SDM	1.95 6.91	99.3	1.26	SMZ	2.00 6.99	99.2	0.65
SDX	2.11 6.12	98.0	0.58	SMR	2.00 6.99	99.2	0.52
SMM	2.17 7.15	99.0	0.74	SDA	2.01 6.99	99.2	0.39
SMX	1.97 5.86	97.3	0.79	STZ	2.04 5.73	96.4	0.98
SMT	1.98 7.06	99.3	0.23	SCA	2.14 5.60	95.3	-0.26

[a] — strongest basic; [b] — strongest acidic; SDM – sulphadimethoxine; SDX – sulphadoxine; SMM – sulphamonomethoxine; SMX – sulphamethoxazole; SMT – sulphameter; SMZ – sulphamethazine; SMR – sulphamerazine; SDA – sulphadiazine; STZ – sulphathiazole; SCA – sulphacetamide

Transfer of sulphonamides from contaminated beeswax to honey. The results of the sulphonamide analysis of wax foundations made from beeswax fortified with 10 sulfonamides at 10,000 µg/kg revealed that some quantity of the added substances had been lost as a result of thermal degradation occurring when the wax was heated to 80°C. In these conditions, the sulphonamides were stable in a range from 20 to 53%. The mean concentrations of SDM, SMM, SDX, SMX, SMZ, SMR, SDA, SMT, SCA and STZ present in the wax foundation were 4,625, 5,326, 4,541, 3,824, 4,138, 4,358, 5,027, 1,993, 4,409 and 3,072 µg/kg, respectively (Table 2).

The results indicated that each sulphonamide could be transferred from contaminated beeswax to honey. The highest concentrations of SDM (87.2 µg/kg), SMM (74.6 µg/kg), SDX (68.9 µg/kg), SMX (50.9 µg/kg), SMZ (46.0 µg/kg), SMR (36.0 µg/kg), SDA (35.8 µg/kg), SMT (26.0 µg/kg), SCA (16.7 µg/kg) and STZ (12.8 µg/kg) were found in honey from the comb built of the contaminated wax-based foundations fortified at 10,000 µg/kg four months from the beginning of the experiment (Fig. 1). The frames used in the study were completely filled with honey on both sides of the comb. Thus, the ratio of honey (2,448 g) to beeswax (110 g) at each sampling point on the comb was always 22.25:1. Applying this proportionality, the maximum transfers of the initial actual amount of SDM, SDX, SMM, SMX, SMT, SMZ, SMR, SDA, STZ and SCA from sulphonamide-containing wax-based foundations to honey were 42.6, 34.3, 31.7, 30.1, 29.5, 25.2, 18.7, 16.1, 9.5 and 8.6%, respectively (Table 3).

Sulphonamides were not detected in the honey samples taken from negative control honeycombs in experimental hives or other hives in the same apiary, indicating that the contaminated beeswax was the source of all sulphonamides observed in the honey samples.

Discussion

In order to have comparable results, the sulphonamide-tainted wax foundations were prepared in the same way as drug-containing wax foundations used in similar experiments (10, 12) by adding analytes at 10,000 µg/kg to blank beeswax melted at 80°C. Although the melting point for beeswax is 62–64°C, increasing the temperature to 80°C was necessary to contaminate the beeswax homogeneously without subjecting the added substances to high temperatures over an extended period. As it transpired, some portions of the added sulphonamides were lost nevertheless, because of thermal degradation. Sulphamonomethoxine was the most stable sulphonamide and degraded 47%, while SMT was revealed to be the least stable, with a loss of 80%. Our results are in line with those obtained by Reybroeck *et al.* (11), in which 62% of SMZ was lost in the preparation of wax foundation because of thermal degradation: in our experiment, 59% SMZ was degraded (Table 2). Sulphonamides were less stable than nitroimidazoles such as metronidazole (MNZ), dimetridazole (DMZ), ronidazole (RNZ) and ipronidazole (IPZ), which only degraded in the range from 3 to 28% in an analogous study (9).

The analysis of sulphonamides in honey indicated that all the tested compounds could be transferred from contaminated beeswax to honey. During the experiment, the sulphonamide levels in honey appeared to be relatively stable despite their quantities declining subsequent to the four-month point after reaching earlier maximum concentrations. The observed decrease in sulphonamide concentrations after four months could be attributed to the possible degradation of compounds in both honey and beeswax. The stability data obtained by Posyniak *et al.* (10) showed that the concentration of STZ, SCA and SMZ in honey was not significantly affected by an incubation period of at least 28 days at 34°C, but the stability of sulphonamides in beeswax is unknown. In a similar experiment that lasted four months, the SMZ concentration increased during the first month that the frames were kept in the incubator, while from the second month, the SMZ residues in honey were rather constant (11). When comparing the initial quantity of sulphonamides that was transferred from contaminated beeswax foundations to honey, SDM showed the largest transfer rate (42.6%), whilst SCA showed the lowest (8.6%) (Table 3). Although Reybroeck *et al.* (11) found that SMZ transfer to honey from beeswax contaminated at the same initial spiking level was 56.9%, we found a lower transfer rate for this antimicrobial of 25.5%. It was also found that in the same conditions, the maximum transfer of the tested sulphonamides was lower than that of MNZ (89.4%), RNZ (54.6%), 2-hydroxymethyl-1-methyl-5-nitroimidazole (79.4%) and hydroxymetronidazole (99.5%) and higher than that of DMZ (2.7%) and IPZ (2.0%) (9). It should be taken into account that the calculations assumed no analyte losses in honey and beeswax during all the transfer studies. However, the different stability levels among the sulphonamides at 35°C might have impacted calculated transfer values, just as the low level of sulphonamides in honey might be due to low thermal resistance.

According to Martinello *et al.* (7), when sulphonamides were applied to honey bee colonies, beeswax was more contaminated than honey and the honey bees. The physicochemical properties of sulphonamides determine their accumulation in the different parts of the hive. For the prediction of sulphonamides' physicochemical properties such as pKa and lipophilicity, Calculators & Predictors software was used (Chemaxon, Budapest, Hungary). Sulphonamides have an amphoteric nature with at least two pKa values, meaning that they can behave either as an acid or a base, depending on the pH of the medium. The predicted sulphonamide values of pKa_1, the strongest basic value, were in a range from 1.95 (SDM) to 2.17 (SMM), while those of pKa_2, the strongest acidic value, were in a range from 5.60 (SCA) to 7.15 (SMM) (Table 4). At the pH of honey, which in the study was 4.2, 95.3–99.4% of the particles of the sulphonamides

were in unionised form (Table 4). Honey is a hydrophilic sugar solution, and beeswax is a hydrophobic substance; therefore, sulphonamide lipophilicity is influential upon their redistribution between the two. The predicted values of the logarithm of the n-octanol/water partition coefficient (log*P*) of the sulphonamides were in a range from −0.26 (SCA) to 1.26 (SDM) (Table 4). Since the sulphonamides in honey are dominantly present in their neutral forms, their lipophilicity in this matrix is not affected by the pH. The values of the logarithm of the n-octanol/water distribution coefficient (log*D*), which quantifies the degree of ionisation at a given pH, are consequently nearly the same as log*P*. A compound with a negative log*P* value is more hydrophilic, while a positive value means a more lipophilic compound. By their predicted log*P* values, all the tested sulphonamides except SDM were considered hydrophilic and could be expected to accumulate in honey, whereas lipophilic SDM should tend to accumulate in beeswax. However, in our experiment, lipophilic SDM with log*P* of 1.26 transferred in the highest proportion (42.6%) from contaminated beeswax foundations to honey, while hydrophilic SCA with log*P* of −0.26 did so in the lowest (8.6%) (Table 3). This might be explained by sulphonamides having different stabilities and by the possible loss of analytes in honey and beeswax that could have occurred during the experiment. Additionally, since there is not an experimentally determined log*P* value available for every sulphonamide and the calculated log*P* values were a wide span, it was impossible to predict these compounds' beeswax/honey partitioning correctly.

Because residues of sulphonamides, as well as other veterinary substances and plant protection products, could be transferred from contaminated wax combs to stored honey and pose a health risk to consumers, Wilmart *et al.* (12) suggested that action limits should be applied to the presence of residues in beeswax in order to regulate possible dangers in the food chain. This suggestion is supported by the present researchers. It is also recommended that beekeepers either recycle their beeswax for use in the production of wax foundations or demand a certificate when purchasing wax foundations from commercial operations that convert wax, as most of these facilities utilise wax with unknown origins.

Conclusion

This investigation's findings lead us to conclude that every tested sulphonamide can migrate from beeswax in combs to honey stored in those combs, with the highest migration potential for SDM and the lowest for SCA. Consequently, honey contamination may occur through the use of sulphonamide-contaminated beeswax in wax foundations. Therefore, sulphonamides need to be monitored in this matrix to guarantee the high safety and quality of beeswax as a product, beeswax as comb foundation, and honey.

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