Science of the Total Environment 772 (2021) 145533

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Honey bee exposure scenarios to selected residues through contaminated beeswax

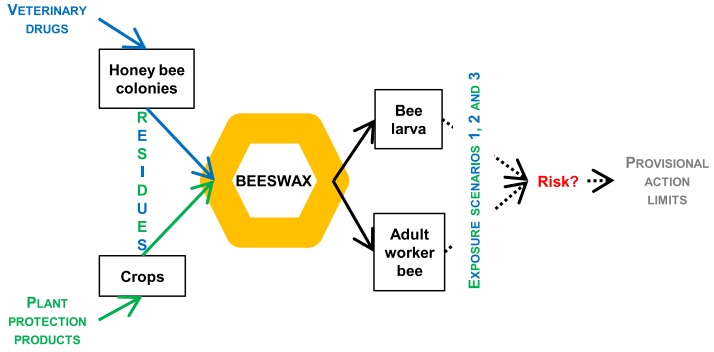
Olivier Wilmart [a,*], Anne Legrève [b], Marie-Louise Scippo [c,d], Wim Reybroeck [e], Bruno Urbain [f], Dirk C. de Graaf [g], Pieter Spanoghe [c,h], Philippe Delahaut [c,i], Claude Saegerman [c,j]

[a] Federal Agency for the Safety of the Food Chain (FASFC), Directorate Control Policy, Staff Direction for Risk Assessment, 55 Boulevard du Jardin Botanique, B-1000 Brussels, Belgium
[b] Université catholique de Louvain (UCL), Faculty of Bioscience Engineering, Earth & Life Institute (ELI), 2 bte L7.05.03 Croix du Sud, B-1348 Louvain-la-Neuve, Belgium
[c] Scientific Committee, Federal Agency for the Safety of the Food Chain, 55 Boulevard du Jardin Botanique, B-1000 Brussels, Belgium
[d] University of Liège (ULiège), Faculty of Veterinary Medicine, Department of Food Sciences – Laboratory of Food Analysis, Fundamental and Applied Research for Animals & Health (FARAH) Center, 10 Avenue de Cureghem, B43bis, B-4000 Liège, Sart-Tilman, Belgium
[e] Research Institute for Agriculture, Fisheries and Food (ILVO), Technology and Food Science Unit, 370 Brusselsesteenweg, B-9090 Melle, Belgium
[f] Federal Agency for Medicines and Health Products (FAMHP), Eurostation II, 40/40 Place Victor Horta, B-1060 Brussels, Belgium
[g] Ghent University (UGent), Faculty of Sciences, Laboratory of Molecular Entomology and Bee Pathology, 281 S2 Krijgslaan, B-9000 Ghent, Belgium
[h] Ghent University (UGent), Faculty of Bioscience Engineering, Department of Plants and Crops, 653 Coupure links, B-9000 Ghent, Belgium
[i] Centre d'Economie Rurale (CER), Département Santé, 8 Rue de la Science, B-6900 Aye, Belgium
[j] University of Liège (ULiège), Faculty of Veterinary Medicine, Research Unit of Epidemiology and Risk analysis applied to Veterinary sciences (UREAR-ULiège), Fundamental and Applied Research for Animal and Health (FARAH) Center, Quartier Vallée 2, 7A Avenue de Cureghem, B42, B-4000 Liège, Sart-Tilman, Belgium

HIGHLIGHTS

- Risk posed by residues in beeswax was assessed based on three exposure scenarios.
- Maximum concentrations were calculated in order to protect honey bee health.
- Provisional action limits were proposed for marketed beeswax for beekeeping.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 May 2020
Received in revised form 25 January 2021
Accepted 26 January 2021
Available online 1 February 2021

Editor: Yolanda Picó

Keywords:
Beeswax
Pesticide/veterinary drug residues
Honey bee (*Apis mellifera*)
Bee health risk
Exposure scenarios
Provisional action limits

ABSTRACT

Twenty-two pesticides and veterinary drugs of which residues were detected in beeswax in Europe were selected according to different criteria. The risk to honey bee health posed by the presence of these residues in wax was assessed based on three exposure scenarios. The first one corresponds to the exposure of larvae following their close contact with wax constituting the cells in which they develop. The second one corresponds to the exposure of larvae following consumption of the larval food that was contaminated from contact with contaminated wax. The third one corresponds to the exposure of adult honey bees following wax chewing when building cells and based on a theoretical worst-case scenario (= intake of contaminants from wax). Following these three scenarios, maximum concentrations which should not be exceeded in beeswax in order to protect honey bee health were calculated for each selected substance. Based on these values, provisional action limits were proposed. Beeswax exceeding these limits should not be put on the market.

* Corresponding author.
E-mail address: olivier.wilmart@afsca.be (O. Wilmart).

https://doi.org/10.1016/j.scitotenv.2021.145533
0048-9697/

1. Introduction

Within the colony, wax is secreted by worker honey bees (*Apis mellifera*) and its production reaches a maximum when they are 10–18 days old (Hepburn et al., 2014). Beeswax is essential to the colony. Within the hive, beeswax is used by worker honey bees to build combs consisting of hexagonal cells that will serve to store food resources, beebread (pollen added with honey, nectar and honey bee secretions) and honey, and to shelter brood (eggs, larvae and pupae of honey bees) during its development.

Beeswax can be contaminated by residues of veterinary drugs applied by beekeepers to treat beehives, notably to control the parasite *Varroa destructor* (e.g. Bogdanov et al., 1998; Boi et al., 2016; Calatayud-Vernich et al., 2017; Kast et al., 2020; Lozano et al., 2019; Martel et al., 2007; Rosenkranz et al., 2010). Over time, repeated application of varroacides can result in accumulation of residues in beeswax given that they are mostly fat-soluble and non-volatile (Johnson et al., 2010; Lozano et al., 2019; Thompson, 2012; Wallner, 1999). From their environment, honey bees themselves are likely to bring pesticide residues, in particular of plant protection products used in agriculture, back to hives through pollen, nectar, water, honeydew and/or propolis they collect (e.g. Böhme et al., 2018; Calatayud-Vernich et al., 2018; Daniele et al., 2018; Mullin et al., 2010; Piechowicz et al., 2018; Simon-Delso et al., 2014; Tong et al., 2018; Traynor et al., 2016). Within the hive, both types of residues can end up in beeswax of the existing combs (e.g. Chauzat and Faucon, 2007; Herrera López et al., 2016; Ostiguy et al., 2019; Perugini et al., 2018; Ravoet et al., 2015).

Throughout their lives, honey bees can be affected by many stressors, different in nature and origin (ANSES, 2015; Rortais et al., 2017). Next to biotic stressors, and in particular the ectoparasitic *V. destructor* mite (Boecking and Genersch, 2008), but also *Nosema ceranae* (*Microsporidia*) (Higes et al., 2009), viruses (e.g. *Black queen cell virus* (BQCV) or *Deformed wing virus* (DWV) (Cornman et al., 2012)), and/or predators (e.g. Asian hornet *Vespa velutina* (Rortais et al., 2010)), honey bees can also be exposed to abiotic stressors like the residues of a broad range of chemicals that affect the honey bee (colony) health (Johnson et al., 2010; Sánchez-Bayo and Goka, 2014).

This study focuses on the assessment of honey bee health risk posed by the presence of pesticide and veterinary drug residues in beeswax and, to prevent and/or control this potential health risk, aimed to calculate maximum concentrations for several residues following a three-scenario analysis. Beeswax exceeding the provisional action limits based on these maximum concentrations should not be put on the market.

2. Materials and methods

Wilmart et al. (2016) listed pesticides and veterinary drugs of which residues were detected in beeswax in Europe. This list was then completed with results of more recent studies (Herrera López et al., 2016; Calatayud-Vernich et al., 2017; Daniele et al., 2018; Perugini et al., 2018; Lozano et al., 2019; Shimshoni et al., 2019; El Agrebi et al., 2019, 2020a, 2020b). Table 1 summarizes, for each of these chemical substances, (contact/oral) acute median lethal doses (LD_{50}) to honey bees (adults and/or larvae) and octanol/water partition coefficients at pH 7 and 20 °C (=Log K_{ow} (with 'ow' meaning 'octanol/water') = Log P).

From that list, chemical substances were selected based on their acute toxicity to honey bees (LD_{50} values), their occurrence, their fat solubility and the fact that their use in beekeeping within the EU is currently authorized (veterinary drugs).

Regarding contact exposure and based on the LD_{50} 48 h after exposure (according to the PPDB/VSDB, see Table 1), the five most toxic active substances in descending order are cyfluthrin (0.001 μg adult honey bee^{-1}), deltamethrin (0.0015 μg adult honey bee^{-1}), fipronil (0.0059 μg adult honey bee^{-1}), pyrethrins (0.013 μg adult honey bee^{-1}) and cypermethrin (0.023 μg adult honey bee^{-1}). In addition, Stoner and Eitzer (2013) reported a contact acute toxicity value of 0.01 μg adult honey bee^{-1} for chlorpyrifos (-ethyl).

Regarding oral exposure and based on the LD_{50} 48 h after exposure (according to the PPDB/VSDB, see Table 1), the five (+1 *ex aequo*) most toxic active substances in descending order are imidacloprid (0.0037 μg adult honey bee^{-1}), fipronil (0.00417 μg adult honey bee^{-1}), thiamethoxam (0.005 μg adult honey bee^{-1}), lindane (γ-HCH) (0.011 μg adult honey bee^{-1}), cyfluthrin (0.05 μg adult honey bee^{-1}) and carbofuran (0.05 μg adult honey bee^{-1}).

In addition to the selection criteria of the active substances based on their respective toxicity (contact and oral), it was also appropriate to select active substances which most frequently occur in beeswax. They may also pose a risk to honey bee health. However, occurrence frequencies are often calculated based on a limited set of analysed samples and/or a non-random sampling. In Belgium, El Agrebi et al. (2020b) have analysed 182 beeswax samples randomly collected from apiaries located all over the Belgian territories (sampling stratified by province). According to this study, the five most frequently detected active substances in descending order are tau-fluvalinate (89.6% (=163/182)), coumaphos (78.6% (=143/182)), propargite (53.3% (=97/182)), diethyltoluamide (DEET) (36.3% (=66/182)) and piperonyl butoxide (29.1% (=53/182)).

It was also appropriate to focus on active substances which are likely to be present in high concentrations in beeswax. They may also pose a risk to honey bee health. Therefore, the most lipophilic active substances among the residues already detected in beeswax (Table 1) were also selected. Indeed, these active substances accumulate in wax, given the lipophilic nature of beeswax. Hydrophilic active substances are present in wax infrequently and in negligible concentrations. They were therefore not considered when estimating the transfer of residues from wax to honey bee larvae and to the larval food. Based on the Log P values mentioned in Table 1 (according to the PPDB/VSDB), the five most lipophilic active substances in descending order were tau-fluvalinate (Log P = 7.02), dichlorodiphenyltrichloroethane (DDT) (Log P = 6.91), dichlorodiphenyldichloroethylene (DDE) (Log P = 6.51), pyridaben (Log P = 6.37) and acrinathrin (Log P = 6.30).

It was also appropriate to select active substances currently authorized as veterinary drugs (varroacids) in beekeeping within the EU (HMA, 2019). Indeed, following their use, these active substances should be detected more frequently and/or in higher quantities in beeswax compared to some active substances present in plant protection products. The active substances selected according to this criterion were amitraz, coumaphos, flumethrin, tau-fluvalinate and thymol.

Of course, if necessary for legislation purposes for instance, the selection made can be extended to all residues detected in contaminated beeswax, and not only limited to the five most (orally/per contact) toxic, the five most frequently detected and the five most lipophilic ones.

3. Calculation

Honey bee's exposure to each of these twenty-two selected residues through beeswax was then assessed following a three-scenario analysis:

- Scenario 1 corresponds to the exposure of worker larvae following their close contact with wax constituting the cells in which they develop;
- Scenario 2 corresponds to the exposure of worker larvae following consumption of the larval food that was contaminated from contact with contaminated wax;
- Scenario 3 corresponds to the exposure of adult worker honey bees following wax chewing when cells building and based on a theoretical worst-case scenario which considers that wax chewing leads to the intake of contaminants contained in the contaminated beeswax.

Table 1
Overview of (contact/oral) acute median lethal doses (LD_{50}) to honey bees, in μg/honey bee (adult/larva), and of octanol/water partition coefficients (Log P) of pesticide/veterinary drug residues (alphabetically ordered) detected in beeswax in Europe according to various references/sources.

Chemical substance	Toxicity group	Detected in beeswax according to:								Honey bee larva		Adult honey bee						Octanol/water partition coefficient at pH 7, 20 °C (Log P) according to PPDB/VSDB	Note
										Oral acute LD_{50} (μg honey bee larva^{-1}) according to:		Contact acute LD_{50} (μg adult honey bee^{-1}) according to:			Oral acute LD_{50} (μg adult honey bee^{-1}) according to:					
		Wilmart et al. (2016)	Herrera López et al. (2016)	Calatayud-Vernich et al. (2017)	Daniele et al. (2018)	Perugini et al. (2018)	Lozano et al. (2019)	Shimshoni et al. (2019)	El Agrebi et al. (2019 and 2020a-b)	Dai et al. (2017)	Charpentier et al. (2014)	PPDB/ VSDB	Stoner and Eitzer (2013)	Sánchez-Bayo and Goka (2014)	PPDB/ VSDB	Stoner and Eitzer (2013)	Sánchez-Bayo and Goka (2014)	Oruc et al. (2012)		
2,4-D	LT		x									>100			94				−0.82	
Acetamiprid	MT	x	x									8.09			14.53				0.8	
Acrinathrin	HT	x		x		x		x	x			0.084		0.17	0.077		0.12		6.3	
Amitraz (metabolites included)	LT	x	x	x		x	x	x	x	14.83		50			/				5.5	
Atrazine	LT	x										>100	>97		>100				2.7	
Azinphos-methyl	HT	x		x								0.42	0.42		/	0.15			2.96	
Azoxystrobin	LT		x					x	x			>200	>200		>25				2.5	
Benalaxyl	LT						x					>100			>22.6				3.54	
Biphenyl									x			/			/				3.98	
Bitertanol	NT	x										>200			>104.4				4.1	
Boscalid	NT	x			x			x	x			>200	>200		>166	>166			2.96	
Bromophos	HT	x										0.44			/				5.21	
Bromopropylate		x				x		x	x			/			/				5.4	
Bupirimate	LT		x									>50			>200				3.68	
Captan	NT	x							x			>200			>100				2.5	
Carbaryl	HT		x									0.14			>0.21				2.36	
Carbendazim	LT	x		x				x	x			>50	>50		>756				1.48	
Carbofuran	HT	x						x				0.036	0.16	0.16	0.05				1.8	
Chloramphenicol		x										/			/				1.14	
Chlorantraniliprole	NT		x									>100			>104.1				2.86	
Chlordane	MT					x						6.0			/				2.78	
Chlordimeform	NT	x										>120			/				2.89	
Chlorfenvinphos	HT	x		x		x	x	x	x			/		4.1	0.55				3.8	
Chlorobenzilate						x		x				/			/				4.58	
Chloropropylate						x			x			/			/				4.41	
Chlorothalonil	LT	x				x			x			>40		135.3	>40				2.94	
Chlorpropham	LT	x							x			96.1			505				3.76	
Chlorpyrifos (-ethyl)	HT	x		x		x		x	x	0.46		0.059	0.01	0.07	0.25	0.25	0.24		4.7	
Chlorpyrifos-methyl	HT						x					0.15			0.18				4.00	
Coumaphos	MT	x		x		x	x	x	x	2.70		/	24	20.29	/		4.61		3.86	
Cyfluthrin	HT	x						x				0.001		0.03	0.05		0.05		6	[a]
λ-Cyhalothrin	HT							x				0.038			0.91				5.5	
Cymiazol		x				x						/			/				0.6	
Cypermethrin	HT	x				x	x	x	x			0.023		0.03	0.172		0.06		5.55	
Cyprodinil	NT	x				x		x	x			>784	>784		112.5				4	
DDD						x		x				/			/				6.02	
DDE		x				x		x	x			/			/				6.51	
DDT (sum of isomers, expressed as DDT)	MT	x				x		x	x			/			5		5.08		6.91	
DEET (diethyltoluamide)		x							x			/			/				2.18	
Deltamethrin	HT	x			x			x	x			0.0015		0.02	0.07				4.6	
Diazinon	HT		x			x			x			0.13	0.22	0.38	0.09	0.2	0.21		3.69	

(continued on next page)

Table 1 (*continued*)

Chemical substance	Toxicity group	Detected in beeswax according to:								Honey bee larva		Adult honey bee						Octanol/water partition coefficient at pH 7, 20 °C (Log P) according to PPDB/VSDB	Note
										Oral acute LD_{50} (μg honey bee larva^{-1}) according to:		Contact acute LD_{50} (μg adult honey bee^{-1}) according to:			Oral acute LD_{50} (μg adult honey bee^{-1}) according to:					
		Wilmart et al. (2016)	Herrera López et al. (2016)	Calatayud-Vernich et al. (2017)	Daniele et al. (2018)	Perugini et al. (2018)	Lozano et al. (2019)	Shimshoni et al. (2019)	El Agrebi et al. (2019 and 2020a-b)	Dai et al. (2017)	Charpentier et al. (2014)	PPDB/ VSDB	Stoner and Eitzer (2013)	Sánchez-Bayo and Goka (2014)	PPDB/ VSDB	Stoner and Eitzer (2013)	Sánchez-Bayo and Goka (2014)	Oruc et al. (2012)		
Dibromo-benzophenone		x							x			Non-listed			Non-listed				4.93	b
Dichlofenthion				x								/			/				5.14	
Dichlofluanid	LT								x			16			/				3.7	
Dichloro-benzophenone							x		x			Non-listed			Non-listed				4.44	b
Diethofencarb	NT	x										>100			>100				2.89	
Difenoconazole	NT		x									>100			>177				4.36	
Dimethoate	HT	x										0.1	0.16		0.1	0.056	0.17		0.75	
Dimethomorph	MT								x			>102	>10		>32.4				2.68	
Dimoxystrobin	LT							x	x			>100			>79.4				3.59	
Endosulfan	MT	x										>7.81		6.35	>15.6				4.75	
Ethion				x								/			/				5.07	
Etridiazole									x			/			/				3.37	
Fenbuconazole	MT		x									>5.5			>5.2				3.79	
Fenbutatin oxide	NT							x				>200			>200				5.15	
Fenhexamid	NT		x									>200			>102.07				3.51	
Fenitrothion	HT	x										0.16			0.20				3.32	
Fenpyroximate	LT		x					x	x			>15.8			>118.5				5.70	
Fenthion (sulfoxide)	HT			x								>0.308	0.308	0.22	/				1.92	c
Fenvalerate (sum of isomers)	HT							x				0.23			/				5.01	
Fipronil	HT		x									0.0059			0.00417				3.75	
Fludioxonil	NT					x						>100			>100				4.12	
Flufenacet	NT	x										>109.2			>100				3.5	
Flufenoxuron	NT		x									>100			>109.1				5.11	
Flumethrin	HT	x		x		x		x	x			/			/			0.178	6.2	
Fluopyram	NT							x				>100			>102.3				3.3	
Flusilazole	LT	x										165			33.8				3.87	
τ-Fluvalinate	HT	x		x		x	x	x	x	0.83		12	0.2	8.66	12.6				7.02	d
Glyphosate	LT								x			>100			100				−3.2	
Heptachlor	HT					x						>0.526			/				5.44	
Hexachloro-benzene (HCB)		x										/			/				3.93	
Hexachlorocyclo-hexane (HCH, sum of isomers α, β and δ)		x				x		x				/			/				3.82	
Hexythiazox	NT			x				x	x			>200			>112				2.67	
Imazalil	LT			x								39	39		35.1	35.1			2.56	
Imidacloprid	HT	x	x		x					4.17		0.081	0.0439	0.06	0.0037	0.0039	0.013		0.57	
5-hydroxy-imidacloprid (5-OH)	HT				x							Non-listed			Non-listed	0.159				
Indoxacarb	HT	x	x									0.08	0.07	0.59	0.232	0.194			4.65	
Iodofenphos		x										/			/				5.51	
Iprodione	NT	x				x		x	x			>100			>100				3.0	
Kresoxim-methyl	NT		x									>100			>110				3.4	
Lindane (γ-HCH)	HT	x				x		x	x			0.23			0.011		0.05		3.50	

Linuron	LT	x											>97.8			>112.1			3.0	
Malathion	HT	x		x				x					0.16	0.2	0.47	0.40	0.38	9.17	2.75	
Metalaxyl	NT								x				200	>100		269			1.75	
Metazachlor	LT	x											>100			>72.2			2.49	
Methoxychlor	MT								x				>23.6			/		5.02	5.83	
Metolachlor	NT							x					>110			110			3.4	
Mevinphos		x											/			/			0.127	
Myclobutanil	LT		x										33.9			>33.9			2.89	
Paraoxon-methyl							x						Non-listed			Non-listed			1.33	b
Parathion		x							x				/			/			3.83	
Parathion-methyl	LT	x											19.5			/			3	
Penconazole	LT		x			x							>30			>112			3.72	
Pendimethalin	LT		x			x			x				100	49.8		>101.2			5.4	
Pentachloro-anisole		x							x				Non-listed			Non-listed			5.45	b
Permethrin (sum of isomers)	HT	x				x		x	x				0.024		0.06	0.13		0.13	6.1	
Phenthoate			x										/			/			3.69	
2-Phenylphenol		x							x				/			/			3.18	
Phosmet							x						0.22			0.37			2.8	
Phthalamide							x						/			/			1.15	b
Piperonyl butoxide	NT	x				x		x	x				294			/			4.75	
Pirimicarb	MT	x							x				17.8	12.56		4.0	3.01	3.84	1.7	
Pirimiphos-methyl	HT		x										/			>0.22			4.2	
Procymidone	NT	x											>100			>100			3.3	
Profenofos	HT		x										0.095			/			1.7	
Propamocarb	LT								x				>100			>84			0.84	
Propargite	LT	x					x	x	x				47.9			>100			5.7	
Propiconazole	LT						x		x				>100	>25		>100			3.72	
Propoxur	HT							x					<0.112			/			0.14	
Propyzamide	NT		x										>136			>100			3.27	
Pyrazophos	HT	x											>0.25			/			3.8	
Pyrethrins	HT					x							0.013			/			5.9	
Pyridaben	HT								x				0.024		0.05	0.535			6.37	
Pyrimethanil	LT	x				x			x				>100	100		>100	100		2.84	
Pyriproxyfen	LT			x									74			>100			5.37	
Rotenone	HT	x				x							>0.24			>12			4.16	
Spirodiclofen	NT					x							>200			>196			5.83	
Spiroxamine	MT		x			x							4.2			>100			2.89	
Sulfonamides		x											/			/			−0.09	e
Tebuconazole	LT	x				x		x	x				>200			>83.05			3.7	
Tebufenozide	NT	x											>234			>100			4.25	
Terbuthylazine	LT	x	x			x							>32			>22.6			3.4	
Terbuthylazine-2-hydroxy		x											/			/			/	
Tetraconazole	LT					x	x						63			>130			3.56	
Tetradifon	LT	x				x			x				>11			/			4.61	
Tetramethrin						x		x	x				/			/			4.6	
Thiacloprid	LT		x		x				x				38.82	37.83		17.32	17.32		1.26	
Thiamethoxam	HT	x			x								0.024	0.024	0.02	0.005	0.005	0.005	−0.13	
Thymol	NT	x										44	>200			/			3.96	
Tolylfluanid	NT					x							>196			>197			3.9	
Trifloxystrobin	NT	x							x				>100	200		>110			4.5	
Vinclozolin	LT	x							x				/			>100			3.02	

High toxicity (HT): $LD_{50} < 2$ μg honey bee^{-1}. Moderate toxicity (MT): $LD_{50} = 2$–10.99 μg honey bee^{-1}. Low toxicity (LT): $LD_{50} = 11$–100 μg honey bee^{-1}. Negligible toxicity (NT): $LD_{50} > 100$ μg honey bee^{-1}. PPDB: Pesticide Properties DataBase (http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm). VSDB: Veterinary Substances DataBase (http://sitem.herts.ac.uk/aeru/vsdb/atoz.htm).

[a] LD_{50} values for β-cyfluthrin according to Sánchez-Bayo and Goka (2014).

[b] Solubility value according to ChemID*plus*.

[c] LD_{50} value for fenthion.

[d] LD_{50} values for fluvalinate according to Stoner and Eitzer (2013), Mullin et al. (2010) and Dai et al. (2017).

[e] Value for sulfadiazine.

To estimate the honey bees exposure following these 3 scenarios, the below assumptions were made. Accumulation of pesticide/veterinary drug residues in beeswax is directly related to their lipophilicity (Johnson et al., 2010; Lozano et al., 2019; Thompson, 2012; Wallner, 1999). From beeswax, part of these residues migrates to the honey bee larva or to the food reserves stored in cells, as demonstrated for fluvalinate between wax and pollen by Fulton et al. (2019) and for seventeen different pesticides between wax and honey by Shimshoni et al. (2019). Although the larva consists of ~80% water (respectively 74.4 ± 0.33% and 79.3 ± 0.19% for larvae and pupae of *Apis mellifera ligustica* according to Ghosh et al. (2016)), it was assumed that the most lipophilic molecules in wax migrate to the larva. Indeed, even though the cuticle could protect the larva against the transfer of a part of the contamination present in the wax, the larva is nevertheless covered with cuticular wax (Hepburn et al., 1991), composed mainly of lipids, which should favor transfer of most lipophilic molecules. Ghosh et al. (2016) have determined a fat content of 14.5 ± 0.15% on a dry matter basis for larvae of *A. m. ligustica*. To estimate this transfer, the octanol/water partition coefficients of substances listed in Table 1 were used as surrogate data and then standardized on a scale ranging from 0, corresponding to the lowest coefficient (=most hydrophilic substance), to 100, corresponding to the highest coefficient (=most lipophilic substance). The estimated transfer rate of each substance therefore corresponds to the standardized coefficient of partition between octanol and water expressed as a percentage. Like residues migration from wax to larva, the same transfer rate was used to estimate residues migration from wax to the larval food.

Regarding scenario 1 (worker larvae: contact with wax), a larval stage of 6 days was considered. Indeed, Winston (1987) stated that the average duration of larval stage is 5.5 days. During this period, contaminants gradually diffuse from the wax to the larvae. It was therefore assumed that one-sixth of the quantity of each of the considered contaminants migrates from wax to the larva daily. It is noteworthy that during its larval stage, worker larvae gain about 900 times their egg weight to reach approximately 140 mg at capping (Winston, 1987). It was also assumed that the larva, due to its small size, is only in contact with the bottom of the cell (=source of exposure) and, therefore, that there is only contact with the embossed wax foundation placed on the frame before honey bees build cells. Knowing that a sheet of embossed wax foundation fixed on a body frame of a Simplex hive measures 34.6 cm by 19.9 cm (=6.88 dm^2), represents 65 g of wax and allows the construction of 5504 cells, i.e. 800 cells per dm^2, the bottom of each cell represents 11.8 mg of wax.

Regarding scenario 2 (worker larvae: larval food consumption), worker honey bee larvae are fed by nurse honey bees during their first three days of development with a jelly similar to royal jelly, which is provided to queen honey bee larvae (Crailsheim et al., 2013; Haydak, 1943, 1970). From day four to day six, this worker jelly is added with honey which can contain very small amounts of pollen (Babendreier et al., 2004; Brodschneider and Crailsheim, 2010; Crailsheim et al., 2013; Haydak, 1970; Rembold and Dietz, 1966). *In vitro*, Rembold and Lackner (1981) were among the first to rear larvae successfully by means of a balanced diet. Their protocol was further improved and standardized by Vandenberg and Shimanuki (1987), Aupinel et al. (2005), Crailsheim et al. (2013) and more recently by Schmehl et al. (2016). According to these last authors, *in vitro* rearing of honey bee workers requires a daily maximum of 50 μl (on the sixth day) of a diet composed with 50%, 9%, 9%, 2% and 30% of royal jelly, glucose, fructose, yeast extract and water respectively. Under this exposure scenario, it was considered that a transfer from wax, containing mainly lipophilic active substances, to the larvae diet occurs only to its lipid part. Within the larvae diet of Schmehl et al. (2016) only royal jelly contains lipids. This diet corresponds to a maximum daily intake of approximately 28 mg of royal jelly (=50 μl of diet * 50% (percentage of royal jelly in diet) * 1.125 mg/μl). Royal jelly contains about 3 to 8% lipids (Bogdanov, 2017; EFSA, 2020; Wright et al., 2018). Therefore, regarding the calculations, a mean lipid concentration of 5% was considered for royal jelly. This diet corresponds therefore to a maximum daily intake of 1.40 mg (= 28 mg * 5%) of lipids. It should also be remembered that pollen and nectar brought back to hive by honey bees are potentially already contaminated by pesticide residues, or even by veterinary drug residues. So, royal jelly, produced by nurse honey bees from beebread (fermented pollen) and honey (converted nectar) (Wright et al., 2018), may also be already contaminated. The initial contamination of this matrix was not considered in this exposure scenario. Contrary to scenario 1, it was assumed in scenario 2 that the total mass of an uncapped built cell, i.e. 21.5 mg (de Graaf D.C. and Reybroeck W., personal communication; El Agrebi et al., 2019), contributed to the exposure. This is because it is considered that the cell is filled with the larval food and that the contact surface is therefore maximum, in contrast to that for the larvae. On the other hand, similarly to scenario 1, a larval stage duration equal to 6 days was also considered for scenario 2. Indeed, during this period, contaminants also progressively diffuse from the wax to lipids contained in royal jelly in contact with this wax. Here again, assumption was made that one sixth of the quantity of each of the considered contaminants migrates daily from wax to royal jelly.

Regarding scenario 3 (adult worker honey bees: wax chewing), we have considered that an adult worker honey bee chews 38.3 mg wax per day (El Agrebi et al., 2019) and we have assumed, as a worst case scenario, that wax chewing leads to the intake of the total amount of contaminants contained in the contaminated beeswax. Indeed, worker honey bees use their mandibles to manipulate the wax in order to shape the hexagonal cells during the comb-building sequence (Bauer and Bienefeld, 2013; Snodgrass, 1910). But their mandibles are also used when eating pollen and are considered to be part of the honey bee mouth parts (Snodgrass, 1910).

In addition, there are very few toxicity data on the above residues to honey bee larvae. Larval survival is reduced following chronic oral exposure to acetamiprid, amitraz, chlorothalonil, chlorpyrifos, coumaphos, fluvalinate, glyphosate, imidacloprid and thiamethoxam (Dai et al., 2018, 2019; Shi et al., 2020; Tavares et al., 2017; Tomé et al., 2020; Zhu et al., 2014). When acute toxicity data (LD_{50}) specific to larvae were available (see Table 1), these were considered in the calculations below for scenarios 1 and 2. Otherwise, lowest acute toxicity values determined on adult honey bee (Table 1) were used, as a first approach.

Moreover, although some interactions between active substances have been demonstrated (e.g. Colin and Belzunces, 1992; Johnson et al., 2009, 2013; Pilling et al., 1995; Thompson, 2012; Wade et al., 2019; Wang et al., 2020; Yao et al., 2018; Zhu et al., 2014), the above selected substances were considered separately when setting the provisional action limits below.

Finally, in order to compensate uncertainties related to the above-mentioned assumptions (not taking into account the initial contamination of pollen and royal jelly, fragmented LD_{50} data for larvae and not taking into account possible interactions between active substances), it was also assumed that exposure of honey bees to residues migrating from wax may not exceed 10% of the LD_{50} values 48 h after exposure (acute toxicity). This threshold was determined on the basis of the Hazard Quotient (HQ) threshold of 1000 calculated by Traynor et al. (2016) for a nurse honey bee through pollen consumption. Indeed, according to these authors a HQ threshold of 1000, corresponding with potential for some initial bee acute toxicity, is reached for a bee consuming 1% of their LD_{50} daily through pollen, which adds up to 10% of their LD_{50} during the 10 day nursing phase.

On the basis of above assumptions, the maximum concentration of a residue in beeswax not to be exceeded following scenario 1 was therefore proportional to one tenth of the LD_{50} value per contact (48 h after exposure) of the considered residue and to the exposure duration (= 6 days), and inversely proportional to the provisional 'wax/larva' transfer rate and to the exposure source (=11.8 mg wax). The maximum concentration 1 was therefore calculated based on the following formula (Eq. (1)):

$$\text{Maximum concentration 1} = \left(\left(\left(\frac{\left(\text{DL50 contact} \times \left(\frac{10}{100} \right) \right)}{\text{Transfer rate}} \right) \times \text{Exposure duration} \right) \over \text{Exposure source} \right) \times 1000 \quad (1)$$

With "Exposure source" = the amount of wax that makes up the bottom of the cell with which the larva is in contact.

On the basis of above assumptions, the maximum concentration of a residue in beeswax not to be exceeded following scenario 2 was therefore proportional to one tenth of the oral LD_{50} value (48 h after exposure) of the considered residue and to the exposure duration (= 6 days), and inversely proportional to the daily lipid intake via consumption of royal jelly (=1.40 mg), the provisional 'wax/royal jelly' transfer rate and the exposure source (=21.5 mg wax). The maximum concentration 2 was therefore calculated based on the following formula (Eq. (2)):

$$\text{Maximum concentration 2} = \left(\left(\left(\frac{\left(\text{DL50 oral} \times \left(\frac{10}{100} \right) \right)}{\frac{\text{Lipids intake through royal jelly consumption}}{\text{Transfer rate}}} \right) \times \text{Exposure duration} \right) \over \text{Exposure source} \right) \times 1000 \quad (2)$$

With "Exposure source" = the amount of wax that makes up an entire cell which is filled and in contact with the larval food.

On the basis of above assumptions, the maximum concentration of a residue in beeswax not to be exceeded following scenario 3 was therefore proportional to one tenth of the oral LD_{50} value (48 h after exposure) of the considered residue and inversely proportional to the amount of daily chewed wax (=38.3 mg). The maximum concentration 3 was therefore calculated based on the following formula (Eq. (3)):

$$\text{Maximum concentration 3} = \left(\frac{\left(\text{DL50 oral} \times \left(\frac{10}{100} \right) \right)}{\text{Amount of daily chewed wax}} \right) \times 1000 \quad (3)$$

4. Results

The maximum concentrations calculated following the three scenarios considered above for the 22 selected active substances are shown in Tables 2, 3 and 4 respectively. The maximum concentrations calculated following scenario 1 range from 0.056 mg/kg wax for cyfluthrin to 19218 mg/kg wax for piperonyl butoxide. The maximum concentrations calculated following scenario 2 range from 0.122 mg/kg wax for fipronil to 7534 mg/kg wax for piperonyl butoxide. The maximum concentrations calculated following scenario 3 range from 0.010 mg/kg wax for imidacloprid to 768 mg/kg wax for piperonyl butoxide. It is noteworthy that maximum concentrations for diethyltoluamide (DEET) could not be calculated for any of the three scenarios, due to the lack of LD_{50} value.

As they concern either larvae or adult honey bees and exposure either by contact or via the oral route, the three above scenarios should be considered separately. On the basis of Tables 2, 3 and 4, the lowest values are therefore retained as provisional action limits in order to protect honey bee health. These calculated values are then rounded according to mathematical rules and with reference to the values mentioned by the OECD (2014). In other words, the provisional action limits are rounded to one significant number, as a multiple of the decimal order of magnitude of the calculated value, unless the calculated value is between 12.5 and 17.4 (or by analogy, in another decimal order of magnitude), in which case rounding to 15 is used (or by analogy, in another decimal order of magnitude). The resulting provisional action limits are shown in Table 5. These range from 0.010 mg/kg wax for fipronil and imidacloprid to 800 mg/kg wax for piperonyl butoxide. The implementation of these provisional action limits by food safety authorities should help them to prevent harmful effects of pesticide and veterinary drug residues possibly present in beeswax on honey bee health.

5. Discussion

When we compare the proposed provisional action limits (Table 5) to actual residue levels found by El Agrebi et al. (2019, 2020b) in beeswax samples from Belgian apiaries (Table 6), we see that most of these limits are met on average. Only for cypermethrin, the mean concentration of 2.34 mg/kg determined in brood comb wax samples (El Agrebi et al., 2020b) exceeds the provisional action limit of 0.150 mg/kg. Compared to other recent European studies (Table 6), the proposed provisional action limits are exceeded on average for pyrethrins and cypermethrin in Italy (respective mean values of 1.14 and 0.18 mg/kg compared to respective limits of 0.200 and 0.100 mg/kg), for acrinathrin (mean value of 1.02 mg/kg compared to limit of 0.200 mg/kg) in Spain and, for acrinathrin, cyfluthrin and deltamethrin in Germany (respective mean values of 0.85, 6.08 and 0.76 mg/kg compared to respective limits of 0.200, 0.060 and 0.100 mg/kg). Note that the proposed provisional action limit for cypermethrin is also exceeded on average in Germany: mean value of 0.360 mg/kg compared to limit of 0.150 mg/kg (Shimshoni et al., 2019). Reported mean value for cyfluthrin in Shimshoni et al. (2019) is doubtful given that the reported maximum concentration is equal to 2.3 mg/kg at the same time. However, this value as well as the reported minimum concentration (0.400 mg/kg) exceed the proposed provisional action limit of 0.060 mg/kg. Given that they are detected in high concentrations in beeswax (Table 6), highly toxic to honey bees and highly lipophilic (Table 1), residues of pyrethroid insecticides, including acrinathrin, cyfluthrin, cypermethrin and deltamethrin in particular, and residues of pyrethrin insecticides could lead to many non-conformities if the proposed provisional action limits are applied to marketed beeswax. More generally, it is noteworthy that residues of insecticides and/or acaricides constitute the most important contamination load of beeswax (Table 6), and the majority of these active substances are highly toxic to honey bees.

Residues of veterinary drugs which are currently authorized in beekeeping within the EU (HMA, 2019) and which are detected in beeswax (Table 6) will probably meet the proposed provisional action limits in most cases (limits of 150, 10.0, 15.0 and 500 mg/kg respectively for amitraz, coumaphos, tau-fluvalinate and thymol), with the possible exception of flumethrin (limit of 0.500 mg/kg). Indeed, this active substance can be administered for the control of varroosis in conventional beekeeping but belongs to pyrethroid insecticides, which are highly toxic to honey bees. Residues of other veterinary drugs are also detected in beeswax (Table 6). These residues are a priori brought back to hives by honey bees themselves from their environment or are present in beeswax due to a former authorized use in beekeeping and following

Table 2
Maximum concentrations (mg active substance/kg beeswax) in beeswax calculated for the 22 selected active substances following scenario 1 (exposure of larvae following their close contact with wax constituting the cells in which they develop).

Active substance (a.s.)	10% contact LD_{50} (μg bee^{-1} or μg $larva^{-1}$)	Transfer rate (%)	Maximum concentration (mg a.s./kg wax)[a]	LD_{50} values reference	Remark
Acrinathrin	0.0084	92.95	4.60	PPDB/VSDB	LD_{50} for adult honey bees
Amitraz	1.483	85.13	886	Dai et al. (2017)	Oral LD_{50}
Carbofuran	0.0036	48.92	3.74	PPDB/VSDB	LD_{50} for adult honey bees
Chlorpyrifos (-ethyl)	0.046	77.30	30.3	Dai et al. (2017)	Oral LD_{50}
Coumaphos	0.27	69.08	199	Dai et al. (2017)	Oral LD_{50}
Cyfluthrin	0.0001	90.02	0.056	PPDB/VSDB	LD_{50} for adult honey bees
Cypermethrin	0.0023	85.62	1.37	PPDB/VSDB	LD_{50} for adult honey bees
DDE	0.5	95.01	268	PPDB/VSDB	Oral LD_{50} of DDT for adult honey bees
DDT	0.5	98.92	257	PPDB/VSDB	Oral LD_{50} for adult honey bees
Deltamethrin	0.00015	76.32	0.100	PPDB/VSDB	LD_{50} for adult honey bees
Diethyltoluamide (DEET)	[b]	52.64	[c]		
Fipronil	0.00059	68.00	0.441	PPDB/VSDB	LD_{50} for adult honey bees
Flumethrin	0.0178	91.98	9.84	Oruc et al. (2012)	Oral LD_{50} for adult honey bees
tau-Fluvalinate	0.083	100.00	42.2	Dai et al. (2017)	Oral LD_{50} of fluvalinate
Imidacloprid	0.417	36.89	575	Dai et al. (2017)	Oral LD_{50}
Lindane (γ-HCH)	0.023	65.56	17.8	PPDB/VSDB	LD_{50} for adult honey bees
Piperonyl butoxide	29.4	77.79	19,218	PPDB/VSDB	LD_{50} for adult honey bees
Propargite	4.79	87.08	2797	PPDB/VSDB	LD_{50} for adult honey bees
Pyrethrins	0.0013	89.04	0.742	PPDB/VSDB	LD_{50} for adult honey bees
Pyridaben	0.0024	93.64	1.30	PPDB/VSDB	LD_{50} for adult honey bees
Thiamethoxam	0.0024	30.04	4.06	PPDB/VSDB	LD_{50} for adult honey bees
Thymol	4.4	70.06	3193	Charpentier et al. (2014)	Oral LD_{50}

[a] Calculated on the basis of an exposure duration of 6 days and an exposure source of 11.8 mg of wax.
[b] Undetermined.
[c] Not calculated due to the lack of a LD_{50} value.

recycling of beeswax, but an unauthorized use of some active substances in beekeeping cannot be excluded. Comparing residues of veterinary drugs with residues of plant protection products in beeswax (Table 6) is challenging because some active substances can be used as both (e.g. tau-fluvalinate). In terms of quantities, we could assume that residues of plant protection products should be less present in beeswax than residues of veterinary drugs, given that these last resulting of a voluntary application within the hive itself. It seems to

Table 3
Maximum concentrations (mg active substance/kg beeswax) in beeswax calculated for the 22 selected active substances following scenario 2 (exposure of worker larvae following consumption of the larval food that was contaminated from contact with contaminated wax).

Active substance (a.s.)	10% oral LD_{50} (μg bee^{-1} or μg $larva^{-1}$)	Transfer rate (%)	Maximum concentration (mg a.s./kg wax)[a]	LD_{50} values reference	Remark
Acrinathrin	0.0077	92.95	1.65	PPDB/VSDB	LD_{50} for adult honey bees
Amitraz	1.483	85.13	347	Dai et al. (2017)	
Carbofuran	0.005	48.92	2.04	PPDB/VSDB	LD_{50} for adult honey bees
Chlorpyrifos (-ethyl)	0.046	77.30	11.9	Dai et al. (2017)	
Coumaphos	0.27	69.08	77.9	Dai et al. (2017)	
Cyfluthrin	0.005	90.02	1.11	PPDB/VSDB	LD_{50} for adult honey bees
Cypermethrin	0.006	85.62	1.40	Sánchez-Bayo and Goka (2014)	LD_{50} for adult honey bees
DDE	0.5	95.01	105	PPDB/VSDB	LD_{50} of DDT for adult honey bees
DDT	0.5	98.92	101	PPDB/VSDB	LD_{50} for adult honey bees
Deltamethrin	0.007	76.32	1.83	PPDB/VSDB	LD_{50} for adult honey bees
Diethyltoluamide (DEET)	[b]	52.64	[c]		
Fipronil	0.000417	68.00	0.122	PPDB/VSDB	LD_{50} for adult honey bees
Flumethrin	0.0178	91.98	3.86	Oruc et al. (2012)	LD_{50} for adult honey bees
tau-Fluvalinate	0.083	100.00	16.5	Dai et al. (2017)	LD_{50} of fluvalinate
Imidacloprid	0.417	36.89	225	Dai et al. (2017)	
Lindane (γ-HCH)	0.0011	65.56	0.334	PPDB/VSDB	LD_{50} for adult honey bees
Piperonyl butoxide	29.4	77.79	7534	PPDB/VSDB	Contact LD_{50} and for adult honey bees
Propargite	10	87.08	2289	PPDB/VSDB	LD_{50} for adult honey bees
Pyrethrins	0.0013	89.04	0.291	PPDB/VSDB	Contact LD_{50} and for adult honey bees
Pyridaben	0.0535	93.64	11.4	PPDB/VSDB	LD_{50} for adult honey bees
Thiamethoxam	0.0005	30.04	0,332	PPDB/VSDB	LD_{50} for adult honey bees
Thymol	4.4	70.06	1252	Charpentier et al. (2014)	

[a] Calculated on the basis of a lipids intake through royal jelly consumption of 1.40 mg, an exposure duration of 6 days and an exposure source of 21.5 mg of wax.
[b] Undetermined.
[c] Not calculated due to the lack of a LD_{50} value.

Table 4
Maximum concentrations (mg active substance/kg beeswax) in beeswax calculated for the 22 selected active substances following scenario 3 (exposure of adult honey bees following wax chewing when cells building and based on a theoretical worst-case scenario which considers that wax chewing leads to the intake of contaminants contained in the contaminated beeswax).

Active substance (a.s.)	10% oral LD_{50} (μg bee^{-1})	Maximum concentration (mg a.s./kg wax)[a]	LD_{50} values reference	Remark
Acrinathrin	0.0077	0.201	PPDB/VSDB	
Amitraz	5	131	PPDB/VSDB	Contact LD_{50}
Carbofuran	0.005	0.131	PPDB/VSDB	
Chlorpyrifos (-ethyl)	0.025	0.653	PPDB/VSDB	
Coumaphos	0.461	12.0	Sánchez-Bayo and Goka (2014)	
Cyfluthrin	0.005	0.131	PPDB/VSDB	
Cypermethrin	0.006	0.157	Sánchez-Bayo and Goka (2014)	
DDE	0.5	13.1	PPDB/VSDB	LD_{50} of DDT
DDT	0.5	13.1	PPDB/VSDB	
Deltamethrin	0.007	0.183	PPDB/VSDB	
Diethyltoluamide (DEET)	[b]	[c]		
Fipronil	0.000417	0.011	PPDB/VSDB	
Flumethrin	0.0178	0.465	Oruc et al. (2012)	
tau-Fluvalinate	1.26	32.9	PPDB/VSDB	
Imidacloprid	0.00037	0.010	PPDB/VSDB	
Lindane (γ-HCH)	0.0011	0.029	PPDB/VSDB	
Piperonyl butoxide	29.4	768	PPDB/VSDB	Contact LD_{50}
Propargite	10	261	PPDB/VSDB	
Pyrethrins	0.0013	0.034	PPDB/VSDB	Contact LD_{50}
Pyridaben	0.0535	1.40	PPDB/VSDB	
Thiamethoxam	0.0005	0.013	PPDB/VSDB	
Thymol	20	522	PPDB/VSDB	Contact LD_{50}

[a] Calculated considering that an adult worker honey bee chews 38.3 mg wax per day.
[b] Undetermined.
[c] Not calculated due to the lack of a LD_{50} value.

be the case for coumaphos, which is only used as veterinary drug (in beekeeping), but it is noteworthy that some active substances only used as plant protection products, like captan and iprodione (fungicides) and chlorpyrifos and acrinathrin (insecticides), are detected in high concentrations (Table 6). However, Table 6 should be interpreted with caution given that beeswax samples were collected and analysed in different ways between studies and that some studies reported residues concentrations based on a (very) limited set of samples.

The method we used to estimate the residues transfer rates from wax should be considered as a preliminary approach, due to the current lack of scientific evidence on this topic. For each residue detected in beeswax, a transfer rate to each of the hive matrices should have been determined experimentally. To our knowledge, this work has only been done for fluvalinate between wax and pollen by Fulton et al. (2019) and for seventeen different pesticides between wax and honey by Shimshoni et al. (2019). Fulton et al. (2019) have determined a Log K_{wp} value (with 'wp' meaning 'wax/pollen') of −0.54 for fluvalinate. It is noteworthy that this value should be compared to 3.85, the Log K_{ow} (=Log P) for fluvalinate. In our study, we took into account tau-fluvalinate, instead of fluvalinate, given that only the use of this substance is allowed in Europe (both as plant protection product and as a veterinary drug). Therefore, the Log P value of 7.02 for tau-fluvalinate was used and then standardized. Fulton et al. (2019) also concluded that the use of octanol/water partition coefficients to estimate transfer from wax into beebread instead of wax/pollen partition coefficients could lead to an underestimation of the risk to a hive. Shimshoni et al. (2019) have determined Log D (=Log distribution ratio, calculated as the logarithmic ratio of pesticide concentration in beeswax to honey at equilibrium) values between wax and honey ranging from −2.06 for thiamethoxam to 2.75 for chlorpyrifos. In our study, Log P values of −0.13 for thiamethoxam and of 4.7 for chlorpyrifos were used and then standardized.

Other uncertainties were identified during this risk assessment and these should be resolved by further research on this topic. These uncertainties were related to: (i) the fact that median lethal doses of substances found in beeswax are not always known for honey bee larvae and/or adult honey bees, which might influence the selection of pesticide/veterinary drug residues (see Materials and methods); (ii) the fact that, as there are few data on the impact of chronic exposure to sub-lethal doses on honey bee health, available data on acute toxicity of active substances, i.e. their LD_{50} 48 h after exposure, were used as a first approach in order to assess the risk to honey bee health of their presence in wax; and (iii) the fact that honey bees could be exposed to different residues at the same time through contaminated beeswax and that adverse synergistic effects could eventually occur. These potential "cocktail effects" were not taken into account in this paper and these should be further studied.

Another element which could be taken into account when setting action limits is the more or less long persistence of residues in beeswax.

Table 5
Provisional action limits (mg active substance/kg beeswax) in beeswax for the 22 selected active substances.

Active substance (a.s.)	Provisional action limit (mg a.s./kg wax)	Scenario considered
Acrinathrin	0.200	3
Amitraz	150	3
Carbofuran	0.150	3
Chlorpyrifos (-ethyl)	0.700	3
Coumaphos	10.0	3
Cyfluthrin	0.060	1
Cypermethrin	0.150	3
DDE	15.0	3
DDT	15.0	3
Deltamethrin	0.100	1
Diethyltoluamide (DEET)	[a]	[a]
Fipronil	0.010	3
Flumethrin	0.500	3
tau-Fluvalinate	15.0	2
Imidacloprid	0.010	3
Lindane (γ-HCH)	0.030	3
Piperonyl butoxide	800	3
Propargite	300	3
Pyrethrins	0.030	3
Pyridaben	1.50	1
Thiamethoxam	0.015	3
Thymol	500	3

[a] No provisional action limit could be proposed due to the lack of a LD_{50} value.

Table 6
Major mean residues concentrations (mg active substance/kg beeswax) in beeswax reported in recent European studies (in descending order).

According to and scope	Serra-Bonvehí and Orantes-Bermejo (2010)			Simon-Delso et al. (2014)			Boi et al. (2016)			Perugini et al. (2018)		
	15 different acaricides			99 different pesticides (acaricide, fungicide, herbicide, insecticide)			5 different acaricides			247 different agrochemicals (acaricide, fungicide, herbicide, insecticide)		
	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)[a]	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)
1	**Tau-fluvalinate**	**IN, AC (PPP + VD)**	**1.31**	Captan	FU (PPP)	3.10	**Coumaphos**	**IN, AC (VD)**	**0.31**	**Pyrethrins**	**IN, AC (PPP + VD)**	**1.14**
2	**Chlorfenvinphos**	**IN, AC (PPP + VD)**	**1.16**	Iprodione	FU (PPP)	0.87	**Tau-fluvalinate**	**IN, AC (PPP + VD)**	**0.26**	**Permethrin**	**IN (PPP + VD)**	**0.28**
3	**Endosulfan**	**IN, AC (PPP)**	**0.19**	**Tau-fluvalinate**	**IN, AC (PPP + VD)**	**0.50**	**Chlorfenvinphos**	**IN, AC (PPP + VD)**	**0.23**	**Tetramethrin**	**IN (PPP + VD)**	**0.26**
4	**Malathion**	**IN, AC (PPP + VD)**	**0.17**	**Coumaphos**	**IN, AC (VD)**	**0.37**	**Amitraz**	**IN, AC (PPP + VD)**	**0.12**	**Cypermethrin**	**IN (PPP + VD)**	**0.18**
5	**Chlorpyrifos**	**IN, AC (PPP)**	**0.17**	Boscalid	FU (PPP)	0.29	**Cymiazol**	**AC (VD)**	**0.02**	**Heptachlor**	**IN (PPP)**	**0.16**
5 *Ex aequo*										Piperonyl butoxide	SY (PPP + VD)	0.16

Calatayud-Vernich et al. (2018)			Shimshoni et al. (2019)			El Agrebi et al. (2020b)			El Agrebi et al. (2020b)		
63 different pesticides (acaricide, fungicide, herbicide, insecticide)			Dozens of pesticides (acaricide, fungicide, herbicide, insecticide)			294 different pesticides (acaricide, fungicide, herbicide, insecticide)			294 different pesticides (acaricide, fungicide, herbicide, insecticide)		
Active substance	A.s. type	Mean concentration (mg a.s./kg wax)	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)[b]	Active substance	A.s. type	Mean concentration (mg a.s./kg wax)[c]
Coumaphos	**IN, AC (VD)**	**5.41**	**Cyfluthrin**	**IN, AC (PPP + VD)**	**6.08**	**Chlorpyrifos**	**IN, AC (PPP)**	**0.64**	**Cypermethrin**	**IN (PPP + VD)**	**2.34**
Chlorfenvinphos	**IN, AC (PPP + VD)**	1.32	Iprodione	FU (PPP)	2.93	**Coumaphos**	**IN, AC (VD)**	**0.55**	**Amitraz**	**IN, AC (PPP + VD)**	**0.74**
Acrinathrin	**IN, AC (PPP)**	**1.02**	**Fenvalerate**	**IN, AC (PPP + VD)**	**1.90**	**Tau-fluvalinate**	**IN, AC (PPP + VD)**	**0.50**	Captan	FU (PPP)	0.65
Fluvalinate	**IN, AC (PPP + VD)**	**0.74**	**Acrinathrin**	**IN, AC (PPP)**	**0.85**	**Tetramethrin**	**IN (PPP + VD)**	**0.46**	**Tau-fluvalinate**	**IN, AC (PPP + VD)**	**0.53**
Amitraz	**IN, AC (PPP + VD)**	**0.18**	**Deltamethrin**	**IN, AC (PPP + VD)**	**0.76**	Diethyltoluamide (DEET)	RE (VD)	0.19	Propiconazole	FU (PPP)	0.38

a.s. = active substance; PPP = plant protection product; VD = veterinary drug; AC = acaricide; IN = insecticide; FU = fungicide; RE = repellent; SY = synergist; in bold = insecticide and/or acaricide active substance.
[a] Mean values of positive samples.
[b] Mean values for recycled comb wax.
[c] Mean values for brood comb wax.

For instance, Shimshoni et al. (2019) have demonstrated that amitraz is completely degraded within 1 min incubation time in beeswax to its two major metabolites, N-(2,4-Dimethylphenyl)-formamide (DMF) and N′-(2,4-Dimethylphenyl)-N-methylformamidine (DMPF). Conversely, these authors have demonstrated a long persistence for cypermethrin, tau-fluvalinate and fenbutatin oxide with respective half-life times ($t_{1/2}$) of 96.3, 48.1 and 32.1 days.

Contaminated beeswax can lead to exposure of honey bee larvae, in particular, to residues of chemicals. Therefore, residues can affect honey bee colony health directly, e.g. through reducing larval survival, but some residues can also affect it indirectly by reducing the colony immune response against some diseases and/or parasites (Sánchez-Bayo et al., 2016; Wu et al., 2012). This is the reason why it is necessary to reduce as much as possible the contamination load of beeswax used in beekeeping. Beekeepers should sufficiently renew beeswax they use, professional beeswax foundation manufacturers should purify beeswax they use as raw material and food safety authorities should impose maximum residue limits on marketed beeswax, for instance the provisional action limits we proposed.

6. Conclusions

Twenty-two pesticides and veterinary drugs of which residues were detected in beeswax in Europe have been selected according to different criteria. The risk to honey bee health posed by the presence of these substances in wax was assessed based on three exposure scenarios. Following these scenarios, maximum concentrations which should not be exceeded in beeswax in order to protect honey bee health were calculated for each selected substance. Based on these values, provisional action limits were proposed. Beeswax exceeding these limits should not be put on the market.

Abbreviations

FASFC Federal Agency for the Safety of the Food Chain

Oral/contact LD_{50} (median lethal dose) is a statistically derived single dose of a substance that can cause death in 50 per cent (50%) of animals when administered by the oral route (OECD, 1998a)/per contact (OECD, 1998b). The LD_{50} value is expressed in mg of test substance per bee

PPDB Pesticide Properties DataBase (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm)

VSDB Veterinary Substances DataBase (http://sitem.herts.ac.uk/aeru/vsdb/index.htm)

CRediT authorship contribution statement

Olivier Wilmart: Data curation, Writing – original draft, Writing – review & editing. **Anne Legrève:** Conceptualization, Methodology, Validation. **Marie-Louise Scippo:** Conceptualization, Methodology, Validation. **Wim Reybroeck:** Conceptualization, Methodology, Validation. **Bruno Urbain:** Conceptualization, Methodology, Validation. **Dirk C. de Graaf:** Conceptualization, Methodology, Validation. **Pieter Spanoghe:** Conceptualization, Methodology, Validation. **Philippe Delahaut:** Conceptualization, Methodology, Validation. **Claude Saegerman:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors acknowledge the members of the working group (SciCom, 2018) for their collaboration and of the Scientific Committee of the FASFC for their supervision and validation of the approach on which this study is based.

Funding sources

None.

References

ANSES, 2015. Co-exposition des abeilles aux facteurs de stress. Avis de l'ANSES. Rapport d'expertise collective. Saisine n° 2012-SA-0176. ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 252 p. https://www.anses.fr/fr/system/files/SANT2012sa0176Ra.pdf.

Aupinel, P., Fortini, D., Dufour, H., Tasei, J., Michaud, B., Odoux, F., 2005. Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. Bull. Insectol. 58 (2), 107–111. http://www.bulletinofinsectology.org/pdfarticles/vol58-2005-107-111aupinel.pdf.

Babendreier, D., Kalberer, N., Romeis, J., Fluri, P., Bigler, F., 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. Apidologie. 35 (3), 293–300. https://doi.org/10.1051/apido:2004016.

Bauer, D., Bienefeld, K., 2013. Hexagonal comb cells of honeybees are not produced via a liquid equilibrium process. Naturwissenschaften. 100, 45–49. https://doi.org/10.1007/s00114-012-0992-3.

Boecking, O., Genersch, E., 2008. Varroosis – the ongoing crisis in bee keeping. J. Verbr. Lebensm. 3, 221–228. https://doi.org/10.1007/s00003-008-0331-y.

Bogdanov, S., 2017. Royal Jelly, Bee Brood: Composition, Health, Medicine: A Review. Bee Product Science.

Bogdanov, S., Kilchenmann, V., Imdorf, A., 1998. Acaricide residues in some bee products. J. Apic. Res. 37 (2), 57–67. https://doi.org/10.1080/00218839.1998.11100956.

Böhme, F., Bischoff, G., Zebitz, C.P.W., Rosenkranz, P., Wallner, K., 2018. Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. PLoS One 13 (7), e0199995. https://doi.org/10.1371/journal.pone.0199995.

Boi, M., Serra, G., Colombo, R., Lodesani, M., Massi, S., Costa, C., 2016. A 10 year survey of acaricide residues in beeswax analysed in Italy. Pest Manag. Sci. 72 (7), 1366–1372. https://doi.org/10.1002/ps.4161.

Brodschneider, R., Crailsheim, K., 2010. Nutrition and health in honey bees. Apidologie. 41 (3), 278–294. https://doi.org/10.1051/apido/2010012.

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

Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2018. Pesticide residues in honey bees, pollen and beeswax: assessing beehive exposure. Environ. Pollut. 241, 106–114. https://doi.org/10.1016/j.envpol.2018.05.062.

Charpentier, G., Vidau, C., Ferdy, J.B., Tabart, J., Vetillard, A., 2014. Lethal and sub-lethal effects of thymol on honeybee (*Apis mellifera*) larvae reared in vitro. Pest Manag. Sci. 70 (1), 140–147. https://doi.org/10.1002/ps.3539.

Chauzat, M.P., Faucon, J.P., 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. Pest Manag. Sci. 63 (11), 1100–1106. https://doi.org/10.1002/ps.1451.

Colin, M.-E., Belzunces, L.P., 1992. Evidence of synergy between prochloraz and deltamethrin in *Apis mellifera* L.: a convenient biological approach. Pestic. Sci. 36 (2), 115–119. https://doi.org/10.1002/ps.2780360206.

Cornman, R.S., Tarpy, D.R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J.S., vanEngelsdorp, D., Evans, J.D., 2012. Pathogen webs in collapsing honey bee colonies. PLoS One 7 (8), e43562. https://doi.org/10.1371/journal.pone.0043562.

Crailsheim, K., Brodschneider, R., Aupinel, P., Behrens, D., Genersch, E., Vollmann, J., Riessberger-Gallé, U., 2013. Standard methods for artificial rearing of *Apis mellifera* larvae. J. Apic. Res. 52 (1), 1–16. https://doi.org/10.3896/IBRA.1.52.1.05.

Dai, P., Jack, C.J., Mortensen, A.N., Ellis, J.D., 2017. Acute toxicity of five pesticides to *Apis mellifera* larvae reared in vitro. Pest Manag. Sci. 73 (11), 2282–2286. https://doi.org/10.1002/ps.4608.

Dai, P., Jack, C.J., Mortensen, A.N., Bustamante, T.A., Ellis, J.D., 2018. Chronic toxicity of amitraz, coumaphos and fluvalinate to *Apis mellifera* L. larvae reared in vitro. Sci. Rep. 8 (1), 5635. https://doi.org/10.1038/s41598-018-24045-3.

Dai, P., Jack, C.J., Mortensen, A.N., Bustamante, T.A., Bloomquist, J.R., Ellis, J.D., 2019. Chronic toxicity of clothianidin, imidacloprid, chlorpyrifos, and dimethoate to *Apis mellifera* L. larvae reared in vitro. Pest Manag. Sci. 75 (1), 29–36. https://doi.org/10.1002/ps.5124.

Daniele, G., Giroud, B., Jabot, C., Vulliet, E., 2018. Exposure assessment of honeybees through study of hive matrices: analysis of selected pesticide residues in honeybees, beebread, and beeswax from French beehives by LC-MS/MS. Environ. Sci. Pollut. Res. 25, 6145–6153. https://doi.org/10.1007/s11356-017-9227-7.

EFSA, 2020. Risk assessment of beeswax adulterated with paraffin and/or stearin/stearic acid when used in apiculture and as food (honeycomb). EFSA Support. Publ. https://doi.org/10.2903/sp.efsa.2020.EN-1859 2020:17(5):EN-1859.

El Agrebi, N., Wilmart, O., Urbain, B., Danneels, E.L., de Graaf, D.C., Saegerman, C., 2019. Belgian case study on flumethrin residues in beeswax: possible impact on honeybee

and prediction of the maximum daily intake for consumers. Sci. Total Environ. 687 (2019), 712–719. https://doi.org/10.1016/j.scitotenv.2019.05.493.
El Agrebi, N., Tosi, S., Wilmart, O., Scippo, M.-L., de Graaf, D.C., Saegerman, C., 2020a. Honeybee and consumer's exposure and risk characterisation to glyphosate-based herbicide (GBH) and its degradation product (AMPA): residues in beebread, wax, and honey. Sci. Total Environ. 704 (2020), 135312. https://doi.org/10.1016/j.scitotenv.2019.135312.
El Agrebi, N., Traynor, K., Wilmart, O., Tosi, S., Leinartz, L., Danneels, E., de Graaf, D.C., Saegerman, C., 2020b. Pesticide and veterinary drug residues in Belgian beeswax: occurrence, toxicity, and risk to honey bees. Sci. Total Environ. 745 (2020), 141036. https://doi.org/10.1016/j.scitotenv.2020.141036.
Fulton, C.A., Huff Hartz, K.E., Reeve, J.D., Lydy, M.J., 2019. An examination of exposure routes of fluvalinate to larval and adult honey bees (*Apis mellifera*). Environ. Toxicol. Chem. 38, 1356–1363. https://doi.org/10.1002/etc.4427.
Ghosh, S., Jung, C., Meyer-Rochow, V.B., 2016. Nutritional value and chemical composition of larvae, pupae, and adults of worker honey bee, *Apis mellifera* ligustica as a sustainable food source. J. Asia Pac. Entomol. 19 (2), 487–495. https://doi.org/10.1016/j.aspen.2016.03.008.
Haydak, M.H., 1943. Larval food and development of castes in the honeybee. J. Econ. Entomol. 36 (5), 778–792. https://doi.org/10.1093/jee/36.5.778.
Haydak, M.H., 1970. Honey bee nutrition. Annu. Rev. Entomol. 15 (1), 143–156. https://doi.org/10.1146/annurev.en.15.010170.001043.
Hepburn, H.R., Bernard, R.T.F., Davidson, B.C., Muller, W.J., Llyod, P., Kurstjens, S.P., Vincent, S.L., 1991. Synthesis and secretion of beeswax in honeybees. Apidologie. 22, 21–36. https://doi.org/10.1051/apido:19910104.
Hepburn, H.R., Pirk, C.W.W., Duangphakdee, O., 2014. Honeybee Nests: Composition, Structure, Function. Springer-Verlag, Berlin Heidelberg 389 p. (ISBN:978-3-642-54327-2).
Herrera López, S., Lozano, A., Sosa, A., Hernando, M.D., Fernández-Alba, A.R., 2016. Screening of pesticide residues in honeybee wax comb by LC-ESI-MS/MS. A pilot study. Chemosphere. 163 (2016), 44–53. https://doi.org/10.1016/j.chemosphere.2016.07.008.
Higes, M., Martín-Hernández, R., Garrido-Bailón, E., González-Porto, A.V., García-Palencia, P., Meana, A., Del Nozal, M.J., Mayo, R., Bernal, J.L., 2009. Honeybee colony collapse due to Nosema ceranae in professional apiaries. Environ. Microbiol. Rep. 1, 110–113. https://doi.org/10.1111/j.1758-2229.2009.00014.x.
HMA, 2019. Authorised bee products: situation in Europe. EMA/CMDv/497311/2009 rev. 15. Co-ordination Group for Mutual Recognition and Decentralised Procedures – Veterinary (CMDVv). European Medicines Agency (EMA) https://www.hma.eu/fileadmin/dateien/Veterinary_medicines/CMDv_Website/Procedural_guidance/Miscellaneous/Bee_products_available_in_Europe2019.pdf.
Johnson, R.M., Pollock, H.S., Berenbaum, M.R., 2009. Synergistic interactions between in-hive miticides in *Apis mellifera*. J. Econ. Entomol. 102 (2), 474–479. https://doi.org/10.1603/029.102.0202.
Johnson, R.M., Dahlgren, L., Siegfried, B.D., Ellis, M.D., 2013. Acaricide, fungicide and drug interactions in honey bees (*apis mellifera*). PLoS One 8 (1), e54092. https://doi.org/10.1371/journal.pone.0054092.
Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010. Pesticides and honey bee toxicity – USA. Apidologie. 41 (2010), 312–331. https://doi.org/10.1051/apido/2010018.
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, 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 Chem. 274 (2019), 61–70. https://doi.org/10.1016/j.foodchem.2018.08.055.
Martel, A.-C., Zeggane, S., Aurières, C., Drajnudel, P., Faucon, J.-P., Aubert, M., 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol®50. Apidologie. 38 (06), 534–544. https://doi.org/10.1051/apido:2007038.
Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One 5 (3), e9754. https://doi.org/10.1371/journal.pone.0009754.
OECD, 2014. MRL Calculator: Users Guide and White Paper, Series on Pesticides and Biocides, No. 56. OECD Publishing, Paris https://doi.org/10.1787/9789264221567-en.
OECD, 1998a. Test no. 213: honeybees, acute oral toxicity test. Guidelines for the testing of chemicals, section 2: effects on biotic systems. https://www.oecd-ilibrary.org/environment/test-no-213-honeybees-acute-oral-toxicity-test_9789264070165-en.
OECD, 1998b. Test no. 214: honeybees, acute contact toxicity test. Guidelines for the testing of chemicals, section 2: effects on biotic systems. https://www.oecd-ilibrary.org/environment/test-no-214-honeybees-acute-contact-toxicity-test_9789264070189-en.
Oruc, H.H., Hranitz, J.M., Sorucu, A., Duell, M., Cakmak, I., Aydin, L., Orman, A., 2012. Determination of acute oral toxicity of flumethrin in honey bees. J. Econ. Entomol. 105 (6), 1890–1894. https://doi.org/10.1603/EC12055.
Ostiguy, N., Drummond, F.A., Aronstein, K., Eitzer, B., Ellis, J.D., Spivak, M., Sheppard, W.S., 2019. Honey bee exposure to pesticides: a four-year nationwide study. Insects 10 (01), 13. https://doi.org/10.3390/insects10010013.
Perugini, M., Tulini, S.M.R., Zezza, D., Fenucci, S., Conte, A., Amorena, M., 2018. Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013–2015. Sci. Total Environ. 625 (2018), 470–476. https://doi.org/10.1016/j.scitotenv.2017.12.321.
Piechowicz, B., Woś, I., Podbielska, M., Grodzicki, P., 2018. The transfer of active ingredients of insecticides and fungicides from an orchard to beehives. J. Environ. Sci. Health B 53 (1), 18–24. https://doi.org/10.1080/03601234.2017.1369320.
Pilling, E.D., Bromley-Challenor, K.A.C., Walker, C.H., Jepson, P.C., 1995. Mechanism of synergism between the pyrethroid insecticide λ-cyhalothrin and the imidazole fungicide prochloraz, in the honeybee (*Apis mellifera* L.). Pestic. Biochem. Physiol. 51 (1), 1–11. https://doi.org/10.1006/pest.1995.1001.
Ravoet, J., Reybroeck, W., de Graaf, D.C., 2015. Pesticides for apicultural and/or agricultural application found in Belgian honey bee wax combs. Bull. Environ. Contam. Toxicol. 94, 543–548. https://doi.org/10.1007/s00128-015-1511-y.
Rembold, H., Dietz, A., 1966. Biologically active substances in royal jelly. Vitam. Hormones 23, 359–382. https://doi.org/10.1016/S0083-6729(08)60385-4.
Rembold, H., Lackner, B., 1981. Rearing of honeybee larvae in vitro: effect of yeast extract on queen differentiation. J. Apic. Res. 20 (3), 165–171. https://doi.org/10.1080/00218839.1981.11100492.
Rortais, A., Villemant, C., Gargominy, O., Rome, Q., Haxaire, J., Papachristoforou, A., Arnold, G., 2010. A new enemy of honeybees in Europe: the Asian hornet Vespa velutina. In: Settele, J., Penev, L.D., Georgiev, T.A., Grabaum, R., Grobelnik, V., Hammen, V., Klotz, S., Kotarac, M., Kühn, I. (Eds.), Atlas of Biodiversit Risk. Chapter: 7. Decline of Pollinators and Its Impact. Pensoft Publishers 264p. (ISBN: 9789546424464).
Rortais, A., Arnold, G., Dorne, J.-L., More, S.J., Sperandio, G., Streissl, F., Szentes, C., Verdonck, F., 2017. Risk assessment of pesticides and other stressors in bees: principles, data gaps and perspectives from the European Food Safety Authority. Sci. Total Environ. 587–588 (2017), 524–537. https://doi.org/10.1016/j.scitotenv.2016.09.127.
Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of Varroa destructor. J. Invertebr. Pathol. 103 (2010), S96–S119. https://doi.org/10.1016/j.jip.2009.07.016.
Sánchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees – a risk assessment. PLoS One 9 (4), e94482. https://doi.org/10.1371/journal.pone.0094482.
Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., Desneux, N., 2016. Are bee diseases linked to pesticides? — a brief review. Environ. Int. 89–90 (2016), 7–11. https://doi.org/10.1016/j.envint.2016.01.009.
Schmehl, D.R., Tomé, H.V.V., Mortensen, A.N., Martins, G.F., Ellis, J.D., 2016. Protocol for the in vitro rearing of honey bee (*Apis mellifera* L.) workers. J. Apic. Res. 55 (2), 113–129. https://doi.org/10.1080/00218839.2016.1203530.
SciCom, 2018. Advice 18-2018 of the Scientific Committee of the FASFC Regarding the Risk to Bee Health of Contaminated and Adulterated Beeswax (SciCom 2016/27). Brussels, 14th November 2018. Full text only available in French. http://www.favv-afsca.fgov.be/comitescientifique/avis/2018/_documents/Avis18-2018_SciCom2016-27_residus_cire_santeabeilles.pdf or in Dutch. http://www.favv-afsca.fgov.be/wetenschappelijkcomite/adviezen/2018/_documents/Advies18-2018_SciCom2016-27_residuen_bijenwas_bijengezondheid.pdf.
Serra-Bonvehí, J., Orantes-Bermejo, J., 2010. Acaricides and their residues in Spanish commercial beeswax. Pest Manag. Sci. 66 (11), 1230–1235. https://doi.org/10.1002/ps.1999.
Shi, J., Zhang, R., Pei, Y., Liao, C., Wu, X., 2020. Exposure to acetamiprid influences the development and survival ability of worker bees (*Apis mellifera* L.) from larvae to adults. Environ. Pollut. 266 (2020), 115345. https://doi.org/10.1016/j.envpol.2020.115345.
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.
Simon-Delso, N., San Martin, G., Bruneau, E., Minsart, L.-A., Mouret, C., Hautier, L., 2014. Honeybee colony disorder in crop areas: the role of pesticides and viruses. PLoS One 9 (7), e103073. https://doi.org/10.1371/journal.pone.0103073.
Snodgrass, R.E., 1910. The Anatomy of the Honey Bee. Technical Series, No. 18. United States. Dept. of Agriculture. Bureau of Entomology. Government Printing Office, Washington https://doi.org/10.5962/bhl.title.87234.
Stoner, K.A., Eitzer, B.D., 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. PLoS One 8 (10), e77550. https://doi.org/10.1371/journal.pone.0077550.
Tavares, D.A., Dussaubat, C., Kretzschmar, A., Carvalho, S.M., Silva-Zacarin, E.C.M., Malaspina, O., Bérail, G., Brunet, J.L., Belzunces, L.P., 2017. Exposure of larvae to thiamethoxam affects the survival and physiology of the honey bee at post-embryonic stages. Environ. Pollut. 229, 386–393. https://doi.org/10.1016/j.envpol.2017.05.092.
Thompson, H.M., 2012. Interaction between pesticides and other factors in effects on bees. EFSA Support. Publ. https://doi.org/10.2903/sp.efsa.2012.EN-340 2012:EN-340.
Tomé, H.V.V., Schmehl, D.R., Wedde, A.E., Godoy, R.S.M., Ravaiano, S.V., Guedes, R.N.C., Martins, G.F., Ellis, J.D., 2020. Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. Environ. Pollut. 256 (2020), 113420. https://doi.org/10.1016/j.envpol.2019.113420.
Tong, Z., Duan, J., Wu, Y., Liu, Q., He, Q., Shi, Y., Yu, L., Cao, H., 2018. A survey of multiple pesticide residues in pollen and beebread collected in China. Sci. Total Environ. 640–641 (2018), 1578–1586. https://doi.org/10.1016/j.scitotenv.2018.04.424.
Traynor, K.S., Pettis, J.S., Tarpy, D.R., Mullin, C.A., Frazier, J.L., Frazier, M., vanEngelsdorp, D., 2016. In-hive pesticide exposome: assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States. Sci. Rep. 6, 33207. https://doi.org/10.1038/srep33207.
Vandenberg, J.D., Shimanuki, H., 1987. Technique for rearing worker honeybees in the laboratory. J. Apic. Res. 26 (2), 90–97. https://doi.org/10.1080/00218839.1987.11100743.
Wade, A., Lin, C.-H., Kurkul, C., Regan, E.R., Johnson, R.M., 2019. Combined toxicity of insecticides and fungicides applied to California almond orchards to honey bee larvae and adults. Insects 10 (01), 20. https://doi.org/10.3390/insects10010020.
Wallner, K., 1999. Varroacides and their residues in bee products. Apidologie. 30 (1999), 235–248. https://doi.org/10.1051/apido:19990212.
Wang, Y., Zhu, Y.C., Li, W., 2020. Interaction patterns and combined toxic effects of acetamiprid in combination with seven pesticides on honey bee (*Apis mellifera* L.). Ecotoxicol. Environ. Saf. 190 (2020), 110100. https://doi.org/10.1016/j.ecoenv.2019.110100.

Wilmart, O., Legrève, A., Scippo, M.-L., Reybroeck, W., Urbain, B., de Graaf, D.C., Steurbaut, W., Delahaut, P., Gustin, P., Nguyen, B.K., Saegerman, C., 2016. Residues in beeswax: a health risk for the consumer of honey and beeswax? J. Agric. Food Chem. 64 (44), 8425–8434. https://pubs.acs.org/doi/10.1021/acs.jafc.6b02813.

Winston, M.L., 1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, Mass 281 p. (ISBN:0674074084).

Wright, G.A., Nicolson, S.W., Shafir, S., 2018. Nutritional physiology and ecology of honey bees. Annu. Rev. Entomol. 63 (1), 327–344. https://doi.org/10.1146/annurev-ento-020117-043423.

Wu, J.Y., Smart, M.D., Anelli, C.M., Sheppard, W.S., 2012. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to Nosema (Microsporidia) infection. J. Invertebr. Pathol. 109 (2012), 326–329. https://doi.org/10.1016/j.jip.2012.01.005.

Yao, J., Zhu, Y.C., Adamczyk, J., 2018. Responses of honey bees to lethal and sublethal doses of formulated clothianidin alone and mixtures. J. Econ. Entomol. 111 (4), 1517–1525. https://doi.org/10.1093/jee/toy140.

Zhu, W., Schmehl, D.R., Mullin, C.A., Frazier, J.L., 2014. Four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honey bee larvae. PLoS One 9 (1), e77547. https://doi.org/10.1371/journal.pone.0077547.