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Organochlorine Pesticides in Honey and Pollen Samples from Managed Colonies of the Honey Bee *Apis mellifera* Linnaeus and the Stingless Bee *Scaptotrigona mexicana* Guérin from Southern, Mexico

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Abstract

In this paper we show the results of investigating the presence of organochlorine pesticides in honey and pollen samples from managed colonies of the honey bee, *Apis mellifera* L. and of the stingless bee *Scaptotrigona Mexicana* Guérin. We found that 88.44% and 93.33% of honey samples, and 22.22% and 100% of pollen samples of *S. mexicana* and *A. mellifera*, respectively, resulted positive to at least one organochlorine. The most abundant pesticides were DDE, DDT, Endrin and heptaclor. Despite the low foraging range of *S. mexicana* the number of pesticides detected in the honey samples was similar to that of *A. mellifera*. Paradoxically we a found a small number of organochlorines in pollen samples of *S. mexicana*, perhaps indicating a rapid turnover of this material as compared to *A. mellifera*.

Keywords

Pesticides, native bee, persistency, biomonitoring

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Introduction

Bees are essential organisms that contribute enormously in preserving ecosystems and human well-being by both pollinating wild plants and increasing the productivity of crops [1]. Highly social species, like honey bees and stingless bees, also produce highly appreciated substances with well-deserved high value in the market, merely honey, wax and propolis, which represent an important economic input for many countries [2]. Many social bees manage to store several kilograms of such products by means of food recruitment communication: they fly several kilometers (up to 5 km in the case of larger species) from the colony, return safely and inform nestmates the location of sources of nectar, pollen and resins. This way the foraging force of a colony focuses in certain patches, instead of wandering around looking for food [3–6]. Unfortunately, since it is not uncommon for bees to share space with agroecosystems, communication also increases the likelihood of transporting agrochemicals like pesticides to the colony, causing several deleterious effects in bee populations, either chronic or acute [7–10].

Pest control is currently thought to be better approached by an integrated pest management scheme, in which cultural, biological and chemical strategies, if used judiciously, are supposed to represent little threat to non-target organisms like bees [11]. However, the most common practices involve the use of chemicals alone, which might pose serious negative effects upon human and environmental health. Acute poisoning of beneficial arthropods during application of pesticides is of current concern, but long term exposure to minute, sublethal, amounts in resources collected by bees, for example, can cause a slow decline in the populations of these species, by affecting several essential physiological and behavioral functions, like reproduction and learning [12–15]. This has taken research to develop new molecules that degrade quickly in the environment and are more specific. Examples of this are organophosphorus pesticides,

pyrethroids and macrolides [16,17]. However, they are more expensive that older alternatives, like organochlorines which, despite the ban in many parts of the world, they are still under heavy production use in third and developing countries, or they were just recently forbidden, like in Mexico [18].

Organochlorine pesticides (OCs) are characterized by their high chemical stability, low water solubility and high solubility in organic solvents. Such features allow organochlorines to bioaccumulate and biomagnificate in the fatty bodies of animals [19–21]. Among the most persistent OCs we can find: DDT (half lifetime: 10 to 15 years), toxaphene (12 years), endrin (10 years), chlordane (8 years), dieldrin (7 years), aldrin (5 years), heptachlor (4 years) and lindane (2 years) [19,22]. In Mexico, OCs began to be used intensively in the 1940s with the arrival of the Green Revolution [23,24]. In the state of Chiapas, in southeastern Mexico, OCs were used intensively both in agriculture and in public health programs aimed to eradicate populations of *Anopheles* spp, vectors of malaria. However, research showed a set of human health disorders and reduction in fauna associated with OCs exposure, so banning began as early as in the 1970s [25,26], but their effects are still present [25,26]. Chiapas was one of the last states in which DDT was banned in agriculture, but it was used until the beginning of the 21st century, since as mentioned lines above, it was considered a key element in the control of malaria [27,28].

Within Chiapas, in the Soconusco region OCs were used for more than 40 years for pest control, mainly in coffee and cotton crops (Table 1). The area devoted to cotton cultivation grew remarkably, from 518 to 35,227 ha cultivated in 1950 – 1978 [29], supposedly thanks to the implementation of modern agricultural technologies, particularly the use of insecticides. Official records indicate that a mixture of toxaphene and DDT was sprayed in doses of 6-7 L/ha per cycle

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[30]. Catalán [29] reported that the application of toxaphene/DDT was as frequent as 21 times per crop cycle; moreover 1,109,650.5 L/year of insecticides were applied when the cultivated cotton acreage was the largest. The Soconusco region is also known for the high incidence of malaria, so DDT was also used to control mosquitoes, with up to one monthly application of 2 g/m^2 on average [31,32]. It is estimated that 69,545 ton of DDT were used just during the Mexican health campaigns in 1957-2000 [33]. It is also important to recall that the phenologies of the crops, the life cycles of the insect pests and climatic conditions make the application of pesticides variable along time and space. Thus it is not surprising that several studies had reported seasonal and spatial variation in pesticide availability in many types of matrixes. For example, Peruzzo et al. [34] and Ruiz-Toledo et al. [35] reported variation in the concentration of glyphosate in water samples collected in rainy and dry seasons in Argentina and Mexico, respectively; Helm et al. [36] also showed a seasonal trend of polychlorinated naphthalene's (PCNs) with top levels occurring during Winter in Canada, Russia and USA; Eqani et al. [37] also found higher concentrations of organochlorine pesticides (OCPs) and polychlorinated biphenyls in fishes during the winter season in Pakistan and China. This means that bees are also exposed to variable concentrations of pesticides, which, along the availability of food sources, can have variable impacts in the survival of these species. Moreover, the identity of the bee species can imply enormous differences in their chances of survival, since larger species can fly longer distances to forage, and thus avoid contaminated patches, while smaller species are more limited in this regard.

The aim of this study was to study the seasonal and the spatial variation in the levels of organochlorine pesticides present, if any, in honey and pollen of managed colonies of both the honey bee *Apis mellifera* L. and the stingless bee *Scaptotrigona mexicana* Guérin in a highly

fragmented landscape, dominated by mango and soybean. We chose these organisms because they are the most common species reared for pollination and honey production and they have different sizes and thus have different foraging distances.

Materials and methods

Study area

The study was carried out in the city of Tapachula, located in the Soconusco region, Chiapas in southern Mexico, between June 2015 and May 2016. Honey and pollen samples were taken directly from colonies housed in wood boxes of *S. mexicana* and *A. mellifera*. All colonies used in this study were considered healthy after a careful inspection by a certified beekeeper.

Sample collection

Six colonies of each species were selected at random and moved to two locations, three colonies of each species in each location. Each colony was sampled three times: June 2015, November 2015 and May 2016. Pollen samples from honey bee colonies were collected by using pollen traps in the entrance. Next, samples were transferred into a 15 ml Falcon tube for transportation; honey was squeezed from the comb into a 50 ml polyethylene Falcon tube. Honey and pollen samples from *S. mexicana* were obtained from pots inside the colony using a sterilized syringe or a spoon, respectively, and transported in 15 ml Falcon tubes. All samples were placed inside a cool box with ice packs and frozen at -20 °C until extraction. The total number of samples collected was 36 for honey (18 for *A. mellifera* y 18 for *S. mexicana*) and 36 for pollen (18 for *A. mellifera* y 18 for *S. mexicana*).

138 **Organochlorine extraction**

139 Organochlorine residues were extracted according to the methodology by Wiest et al. [38]. Five
140 grams of honey or two grams of pollen were weighed in a 50 ml centrifuge tube, 10 ml of water
141 were added and the mixture was vigorously shaken to dissolve honey. Next, 10 ml of acetonitrile
142 (ACN) and 3 ml of hexane (only for pollen), 4 g of anhydrous MgSO4, 1 g of sodium chloride, 1
143 g of sodium citrate dihydrate and 500 mg of disodium citrate sesquihydrate were added; the tube
144 is immediately shaken by hand, vortexed one minute and then centrifuged for 2 min at 5000 g.
145 Six ml of supernatant were transferred into a 15 ml PSA tube that contained 900 mg of anhydrous
146 MgSO4, 150 mg of PSA bonded silica and 150 mg of C18 bonded silica. Then, this tube was
147 immediately shaken by hand, vortexed for 10 s and centrifuged for 2 min at 5000 g. Finally, 4 ml
148 of the extract were transferred into a 10 ml glass cone-ended centrifuge tube, evaporated until a
149 final volume of 50 µl and kept at −18◦C until analysis.
150
151 **Chemicals**
152 For validation and standardization of the analytical method we used a mixture of standard-grade
153 OCs from Ultra Scientific: aldrin, β-HCH, α-HCH, γ-HCH, δ-HCH, p,p'-DDD, p,p'-DDE, p,p'-
154 DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde,
155 heptachlor, and heptachlor epoxide. The organic solvents used for extraction of OCs were HPLC-
156 grade hexane and dichloromethane from Sigma-Aldrich.
157
158 **Gas chromatography analysis**
159 The extracts were analyzed by gas chromatography using a Perkin Elmer Clarus 500 gas
160 chromatograph, equipped with an electron capture detector, autosampler, and a programmable
161 split/splitless injector. The injection volume of extract was 2 µl in splitless mode. The initial

temperature of the injector was 120 °C, and the speed of the carrier gas (hydrogen) was 48 cm/s. The detector temperature was 350 °C, and the make-up flow was 30 ml/min. An Agilent J&W DB-35MS column (p/n 122-3832) of 30 m length, 0.250 mm inner diameter, and 0.25 µm film thickness was used. The initial oven temperature was 110 °C, which was maintained for 1.4 min, followed by a temperature ramp with increments of 13 °C/min up to 285 °C, holding at 285 °C for 1 min, another ramp of 30 °C/min up to 300 °C, and holding until the end of the routine (3 min). The total time of the analysis was 19.36 min.

Under previous described conditions the residue levels of OCs were quantitatively determined by the external standard method using peak area. The limits of detection and quantification were determined by the least squares regression method on the OC curves, using data generated by nine replicates near the lowest concentration attainable on the calibration curve. The detection limits varied from 0.55 to 1.14 µg/L, and the quantification limits between 1.78 and 3.79 µg/L, depending on the pesticide. Recoveries were determined by spiking with the surrogate standards prior to extraction. Amounts were similar to detected quantities of analytes in the samples. The recoveries of the OCs ranged from 80 to 102%.

Statistical analysis

A descriptive analysis of the levels of OCs was conducted by calculating geometric means, standard deviations and minimum and maximum. Descriptive analysis and graphs were obtained using the R software package.

Results

Overall, we detected 15 out of the 16 organochlorine compounds included in our screening list (Table 2). We found that 22.22% of *S. mexicana* pollen, 88.33% of *S. mexicana* honey, 100% of *A. mellifera* pollen and 94.44% of *A. mellifera* honey samples were positive to at least one organochlorine. Thus there was variation in the amount and identity of the pesticides detected in the different matrixes, colonies, species and dates (Table 3, Figure 1).

For *S. mexicana* the most abundant pesticide was γ-HCH, present in four pollen samples and nine honey samples (22.22% and 50%, respectively); for *A. mellifera*, 72.22% (13 samples) of the pollen samples contained γ-HCH in greater quantity but 83.33% (15 samples) of the honey samples contained heptachlor (Table 2 and Table 4). All *S. mexicana* and *A. mellifera* colonies survived in the study period, and no visible sign of weakening could be observed at the end of the study.

Discussions

In this study we aimed to detect and quantify organochlorine pesticides in honey and pollen samples from two bee species, the honey bee *A. mellifera* and the small-sized stingless bee *S. mexicana*, which have putatively different flight ranges. All samples had at least one organochlorine, but the pollen samples from honey bees had the higher number of pesticides. Slight differences in the number and identity of pesticides between sampling sites and season were found, but no pattern could be discerned.

It is not uncommon to find honey bee products like honey, pollen and wax, from colonies located in agricultural landscapes, contaminated with pesticides [39–41] in countries like Argentina [42],

Bélgica [43], Brazil [44], China [45], Colombia [46], Egypt [47], France [38], Germany [48], Greece [49], India [50], Mexico [51], Poland [52], Portugal and Spain [53], Turkey [54] y USA [41]. However, given the seasonal variation in the agricultural activities related to insecticide application, climatic factors, among others, not all samples show evidence of contact with such chemicals [55]; for instance, Balayiannis and Balayiannis [56] found organophosphorous insecticides in 56% of their honey samples; Mullin et al. [41], Pohorecka et al. [57] and Panseri [58] reported that 60%, 50% and 94% of their samples (wax, pollen, honey and bee workers) had residues of at least two pesticides. We found no clear correlation between date, site and colony and the presence of pesticides; actually we failed to carry out a survey about the strategy that local growers adopt to apply insecticides to control pest, i.e. whether they constantly monitor pest abundance or they carry out preventive measures and apply insecticides regardless pest abundance. Additionally, it is important to consider the location of our colonies in the agricultural landscape, since its closeness to farms determines the likelihood that bee workers find pesticides [59]; this has yet to be investigated.

Spatial and temporal variations in the identity and amount of pesticides have been documented. Chauzat et al. [60] reported that in 2002-2005 in France, there was variation in the concentrations of several pesticides; Pirard et al. [43], Nguyen et al. [61], Mullin et al. [41], García-Chao et al. [62], Bernal et al. [63] and Valdovinos-Flores et al. [51], also found, in different countries, variations in the amount and identity of pesticides in distant localities. The most common chemicals include fungicides, herbicides [64,41,63,65], neonicotinoids [64,60,66,67], organophosphorous [47], organochlorines and pyretroids [18]. Given the reduced use of neonicotinoids in our study area and the short halftime of organophosphorous insecticides we focused on organochlorines.

The concentration of the pesticides in our study also varied between sites, date of sampling, colonies and type of sample. Many studies have also shown a similar pattern. For instance, Mullin et al. [41] reported 121 pesticides (and metabolites) at concentrations ranging 99-204 ppm in pollen, wax and worker samples; Santos de Azebedo [68] found aldrin (2 ng/g), endosulfan (1 - 33 ng/g), endrin aldehyde, heptachlor epoxide, p, p'-DDE and p, p'-DDT (1-94 ng/g) in pollen samples; Panseri et al. [58] found DDT, DDD y DDE in honey samples at 8.8 ng/g, 1.9 ng/g and 15.4±0.3 ng/g, respectively; Malhat et al. [18] documented that a honey sample had γ-HCH at a concentration higher than the permissible upper limit (0.01 mg/kg); in Mexico, Valdovinos-Flores et al. [51] reported samples of honey and wax contaminated with 93 pesticides, including DDT at 0.175 mg/kg. In our study we found endrin (10 mg/kg), p,p′-DDE (2.7 mg/kg) and heptachlor (2.6 mg/kg) to be the most abundant pesticides, even at concentrations higher than reported in other works. Surprisingly the concentrations we found are close to the LD50 calculated for honeybees, but our colonies did not show any clear sign of depopulation or reduced activity [69].

It has been shown that the amount and variety of pesticides in honey bee colonies is closely related to the closeness to the source of contamination and the length of the exposure [64,18,41,58,60,62]. Other important factor is the half-life of the pesticides; organochlorine pesticides have been banned for decades in many countries, including Mexico, but residues are still present. Table 2 shows that DDT, DDE and DDD can be found in the study zone. It is possible to estimate how recent are DDT applications by calculating the ratio DDE/DDT [70]. It is worrying to discover that our data reveals that it is highly likely that DDT is still used despite the ban, so the current policies are not efficient in controlling the production and marketing of organochlorines in this part of Mexico.

Pollen samples from *A. mellifera* had the highest number of pesticides (14 out of 16) with the highest concentrations, so they represent a closer view of the situation regarding OCs in the study area. Chauzat et al. [66] also pointed out that pollen is the best matrix to determine the presence of pesticides. Moreover, other studies have indicated that the high octanol/water partition coefficient (log K_{ow}) of pesticides allows them to be absorbed by pollen more easily than by honey [59]. *Scaptotrigona mexicana* pollen and honey samples only resulted positive to 4 and 9 OCs, respectively. Such difference might be the result of the shorter foraging range of *S. mexicana*; it is estimated that 10000 - 25000 honey bee workers make 10 round trips to collect food, and that the exploring area covers up to 10 km^2 around the colony [44,71–73]. Thus, the likelihood that foragers encounter pesticides is high [74]. In the case of *S. mexicana* it is known that, given its size and the smaller number of workers compared to *A. mellifera*, the foraging range is far shorter than for *A. mellifera* [75], thus reducing the exposure to a lesser degree.

Conclusions

Our data provide a clear indication of the widespread use of OCs in the study area, confirming honey bee and beehive matrixes as appropriate sentinels for bio-environmental monitoring. This could be an effective tool for beekeepers to select production areas in particular for the production of organic honey. In addition, this study revealed, for the first time, that bees in our study area are exposed to OCs pesticides, which represents a risk to both bees and human health. Our study has unexpectedly revealed the presence of a wide spectrum of OC compounds, even though it is not officially used in Chiapas from the 2000's. Efforts should be directed to find out the source of these compounds and to make sure none of them is being smuggled into the country. However, more studies conducted in other parts of Mexico might be necessary in order

281 to determine the actual the prevalence of these pesticides residues, and their impact on the honey
282 industry and the safety of consumers.
283
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288
289 **Author Contributions**
290 Jovani Ruiz-Toledo and Daniel Sánchez initiated the study idea and developed the concept
291 together with Rémy Vandame, Ricardo A. Castro-Chan, Rosa P. Penilla-Navarro and Jaime
292 Gómez. Ricardo A. Castro-Chan contributed to chromatography analysis. Jovani Ruiz-Toledo
293 and Daniel Sánchez drafted the manuscript. All authors contributed to and approved the final
294 version of the manuscript.
295
296 **Conflicts of Interest**
297 The authors declare no conflict of interest.
298
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Tables and figures

Table 1. Organochlorine residues reported in the Soconusco region, Chiapas, México.

Sample	Organochlorine pesticide	Concentration	Reference
Water	DDD	2 µg/L	Hernández-Romero et al. [76]
Sediments of lagoon system	DDE	247 ng/L	Hernández-Romero et al. [76]
	Endosulfan	814 ng/L	
Air	Chlordane	5.8-12 pg/m3	Alegría et al. [28]
	Toxaphene	6.2-229 pg/m3	
	Dieldrin	0.9-11 pg/m3	
	Endosulfan	92-341 pg/m3	
	DDT	239-2360 pg/m3	
Soil	Chlordane	<0.0033- 2.7 ng/g	Wong et al. [77]
	Toxaphene	<LD- 334 ng/g	
	Endosulfan	<LD- 909 ng/g	
	DDT	<LD- 360 ng/g	
Fish	DDT	373.67-1937.90 ng/g lipids	Herrera-Portugal et al.[78]
Cheese	DDT	7.0 – 10.87 ng/g lipids	Herrera-Portugal et al. [79]
	DDD	1.11 – 3.71 ng/g lipids	
	DDE	17.0 – 38.52 ng/g lipids	
Blood plasma	DDT	67.4 µg/L	Barraza-Villareal et al. [80]
		nd – 46.76 µg/L	Herrera-Portugal et al.[31]
		12.08 ± 8.58 µg/L	Herrera-Portugal et al.[81]
		50.2 ng/mL	Pérez-Maldonado et al. [32]
		15.4-17,886.5 ng/g lipid	Trejo-Acevedo et al. [82]
		1596.4 ng/g Lipid	Rivero-Pérez et al. [83]
		6.37-29.66 µg/L	Ruiz-Suárez et al. [84]
	DDE	nd – 68.09 µg/L	Herrera-Portugal et al. [31]
		53.32 ± 35.61 µg/L	Herrera-Portugal et al. [81]
		203.5 µg/L	Barraza-Villareal et al. [80]
		3213.8 ng/g Lipid	Trejo-Acevedo et al.[82]
		15,457 ng/g Lipid	Rivero-Pérez et al. [83]
		1.1-222.6 µg/L	Ruiz-Suárez et al. [84]
	Lindane	351.1-6,153.8 ng/g lipid	Trejo-Acevedo et al.[82]
		1,596.4 ng/g Lipid	Rivero-Pérez et al. [83]
	Lindane	0.77-6.25 µg/L	Ruiz-Suárez et al. [84]
	β-HCH,	2.03-8.74 µg/L	
	Heptachlor	1.74-4.40 µg/L	
	β-endosulfan	0.70-43.90 µg/L	
	Endrin aldehyde	0.51-6.76 µg/L	

*DDD (1,1-dichloro-2,2-bis (p-chlorophenyl)ethane), **DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene)

596 **Table 2.** Concentration of OCs in honey and pollen samples from *A. mellifera* and *S. mexicana*.

Analyte	N	%≥DL[a]	GM[b]	SD	Minimun	Maximun
α-HCH	72	9.72	1.02	4.34	3.74	32.61
γ-HCH	72	36.11	18.99	39.95	5.43	207.15
β-HCH	72	9.72	4.05	13.27	9.72	68.41
Heptachlor	72	44.44	191.78	484.81	5.67	2,570.31
δ-HCH	72	0	nd	nd	nd	nd
Aldrin	72	1.39	0.17	1.49	1.30	12.69
Heptachlor epoxide	72	25.00	25.27	104.54	10.33	699.26
α-Endosulfan	72	12.50	6.13	26.23	4.77	204.27
p,p′-DDE	72	11.11	94.04	420.32	10.61	2,696.98
Dieldrin	72	4.17	1.24	6.57	4.16	47.06
Endrin	72	18.06	348.25	1,485.93	18.05	10,032.14
p,p′-DDD	72	1.39	1.09	9.27	1.38	78.73
β-Endosulfan	72	4.17	0.93	5.78	4.16	46.83
p,p-DDT	72	19.44	30.86	76.58	19.44	440.77
Endrin aldehyde	72	8.33	3.79	13.45	8.33	78.72
Endosulfan sulfate	72	1.39	0.61	5.23	1.38	44.45

597 Organochlorines pesticides concentration in honey and pollen are reported in µg/Kg; [a] % of samples with detectable
598 levels (%≥DL); [b] values reported as geometric mean (GM); (SD) standard deviation; (nd) not detected.

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601 **Table 3.** Number of pesticides detected in each sample in each colony in our study. Each sample
602 was collected in a different date. Colonies 1, 2 and 3 are located in site 1, separated by 27
603 kilometers from site 2 where colonies 3, 4 and 5 are located.

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		Honey		Pollen	
Colony	**Date**	*S. mexicana*	*A. mellifera*	*S. mexicana*	*A. mellifera*
1	01/06/2015	1	3	0	3
	01/11/2015	1	3	2	3
	01/05/2016	1	0	2	1
2	01/06/2015	0	3	2	2
	01/11/2015	0	6	2	1
	01/05/2016	0	4	0	1
3	01/06/2015	1	2	0	2
	01/11/2015	1	3	0	4
	01/05/2016	1	2	0	1
4	01/06/2015	1	4	0	3
	01/11/2015	5	3	0	2
	01/05/2016	5	3	0	1
5	01/06/2015	3	2	0	12
	01/11/2015	3	3	0	4
	01/05/2016	2	2	0	2
6	01/06/2015	2	3	0	5
	01/11/2015	5	4	0	3
	01/05/2016	4	2	0	3

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608 **Table 4.** Concentration of OCs in honey samples of *A. mellifera* (Amell) and *S. mexicana* (Smex)

		%≥DL[a]		GM[b]		SD		Minimum		Maximum	
Analyte	**N**	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***
α-HCH	18	11.11	11.11	0.51	0.48	0.51	0.33	5.46	3.8	7.63	4.79
γ-HCH	18	61.11	50.00	9.95	37.36	9.95	14.29	5.43	8.8	143.14	207.15
β-HCH	18	16.67	22.22	3.83	9.55	3.83	4.71	22.57	26.1	53.30	68.41
Heptachlor	18	83.33	44.44	199.54	106.53	199.54	45.03	24.35	96.4	2,570.32	645.08
δ-HCH	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
Aldrin	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
Heptachlor epoxide	18	38.89	16.67	80.401	3.37	47.27	1.83	13.89	18.1	699.26	21.68
α-Endosulfan	18	33.33	11.11	16.272	6.12	11.32	4.21	4.77	51.0	204.27	59.12
p,p′-DDE	18	22.22	11.11	370.563	3.29	187.05	2.29	885.98	25.1	2,696.98	34.10
Dieldrin	18	11.11	0.00	3.488	nd	2.71	nd	15.72	nd	47.06	nd
Endrin	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
p,p′-DDD	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
β-Endosulfan	18	11.11	0.00	1.12	nd	0.85	nd	5.78	nd	14.4753	nd
p,p-DDT	18	0.00	16.67	nd	44.09	nd	27.62	nd	99.0	nd	440.78
Endrin aldehyde	18	0.00	16.67	nd	6.49	nd	3.58	nd	33.2	nd	47.96
Endosulfan sulfate	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd

609 Organochlorines pesticides concentration in honey reported in µg/Kg; [a] % of samples with detectable levels (%≥DL);

610 [b] values reported as geometric mean (GM); (SD) standard deviation; (nd) not detected.

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616 **Table 5.** Concentration of OCs in pollen samples of *A. mellifera* and *S. mexicana*.

		%≥DL[a]		GM[b]		SD		Minimun		Maximun	
Analyte	**N**	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***	***Amell***	***Smex***
α-HCH	18	16.67	0.00	2.90	4.45	1.93	2.24	5.44	11.8	32.61	32.49
γ-HCH	18	11.11	22.22	2.19	nd	1.81	nd	7.31	nd	32.11	nd
β-HCH	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
Heptachlor	18	38.89	11.11	140.65	1.75	60.95	1.45	35.90	5.7	794.83	25.77
δ-HCH	18	0.00	0.00	nd	nd	nd	nd	nd	nd	nd	nd
Aldrin	18	5.56	0.00	0.71	nd	0.71	nd	12.69	nd	12.69	nd
Heptachlor epoxide	18	44.44	0.00	17.35	nd	7.08	nd	10.33	nd	92.22	nd
α-Endosulfan	18	5.56	0.00	2.16	nd	2.16	nd	38.93	nd	38.93	nd
p,p′-DDE	18	11.11	0.00	2.32	nd	1.79	nd	10.61	nd	31.09	nd
Dieldrin	18	5.56	0.00	1.49	nd	1.49	nd	26.85	nd	26.85	nd
Endrin	18	72.22	0.00	1393.01	nd	653.23	nd	29.83	nd	10,032.14	nd
p,p′-DDD	18	5.56	0.00	4.37	nd	4.37	nd	78.73	nd	78.73	nd
β-Endosulfan	18	5.56	0.00	2.60	nd	2.60	nd	46.83	nd	46.83	nd
p,p-DDT	18	50.00	11.11	74.18	5.20	19.17	3.89	124.87	27.7	219.35	65.96
Endrin aldehyde	18	16.67	0.00	8.67	nd	5.07	nd	34.77	nd	78.62	nd
Endosulfan sulfate	18	5.56	0.00	2.47	nd	2.47	nd	44.45	nd	44.45	nd

617 Concentration in µg/Kg; [a] % of samples with detectable levels (%≥DL); [b] values reported as geometric mean (GM); [1]

618 *A. mellifera* and [2] *S. mexicana;* (SD) standard deviation; (nd) not detected.

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625 **Figure 1.** Concentration of organochlorines in honey and pollen samples from *A. mellifera* and *S.*

626 *mexicana* from June 2015 to May 2016

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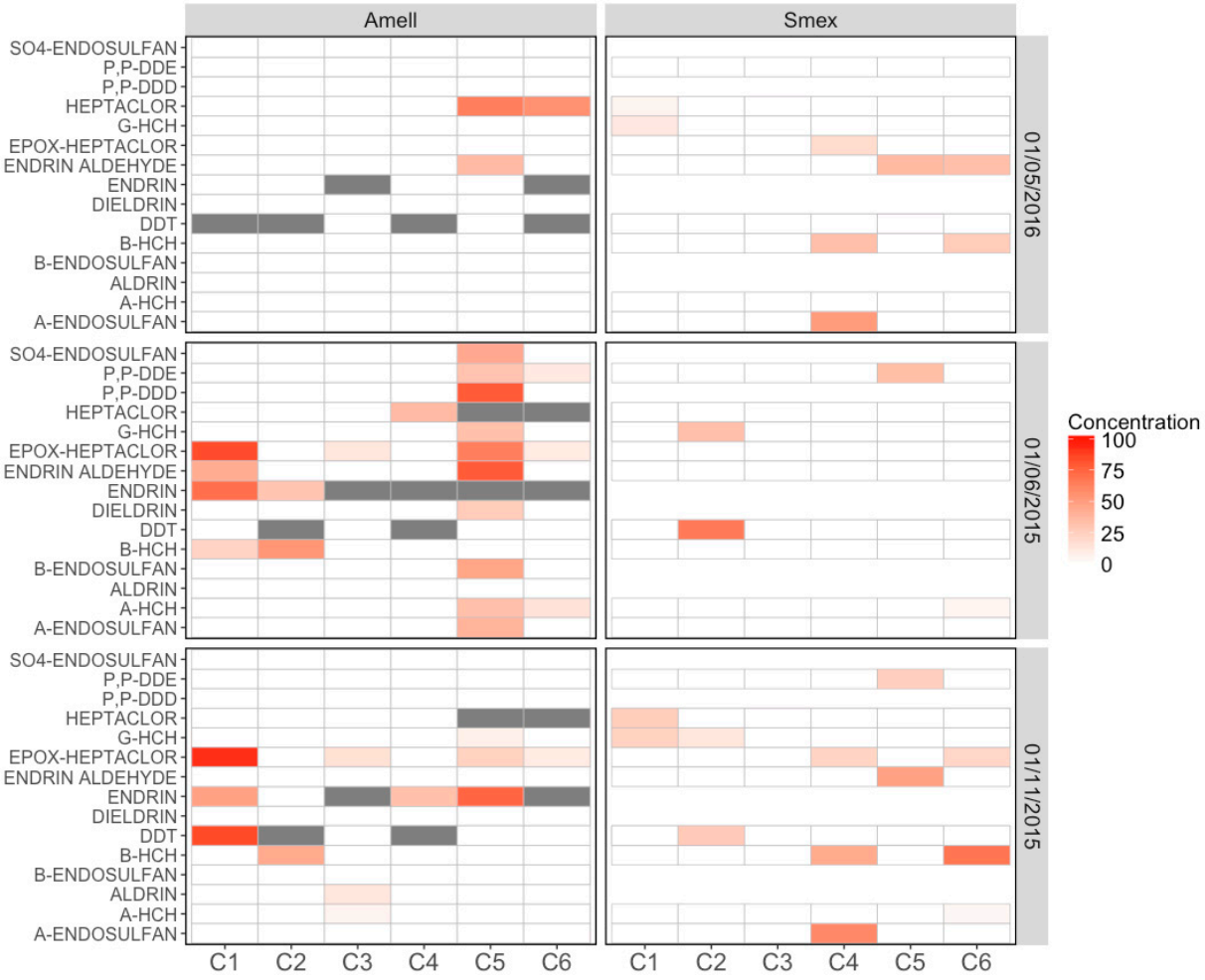

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