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Organochlorine Pesticides in Gonad, Brain, and Blood of Mice in Two Agricultural Areas of Sinaloa

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Abstract The adverse effect of pesticides on non-target wildlife and human health is a primary concern in the world, but in Mexico, we do not know which wildlife species are at the greatest risk. The aim of this study was to determine organochlorine pesticides in mice of two agricultural fields in Sinaloa, Culiacan and Guasave. Procedures of extraction, analysis, and quantification were followed according to the modified EPA 8081b method. In three mouse tissues (gonad, brain, and blood), γBHC and decachlorobiphenyl with a frequency higher than 50% and endosulfan sulfate with 43% were observed. The wildlife fauna living in agricultural areas are at great risk due to: (1) diversity of the chemicals used for pest control, like mice, and (2) variety of organochlorine pesticides in direct or indirect contact with non-target organisms, affecting the health of animals and humans (toxic effects and accumulation).

Keywords Pesticides pollution · γBHC · Mammals · *Mus musculus* · Mice

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Agricultural pesticides constitute a potential risk to mammals (Jin et al. 2010). Exposure of small mammals to agricultural pesticides is given by pesticide application on their feeding areas and by the chemicals used to protect crops (Liu et al. 2014). Pesticides pollution is a great problem in the world, because mixtures of pesticides are used to control and protect crops against adverse pests, such as weed, insect and rodent populations (McCoy et al. 2008; Reyes et al. 1999; USEPA 2016).

These chemicals are cataloged as environmental contaminants due to their high prevalence in the ecosystem and are associated with adverse health effects in non-target organisms (Colborn et al. 1993). Research has shown that some of these chemicals have the ability to act as endocrine disruptors in humans and wildlife (Baker et al. 2009), because they build up in the fatty tissues of animals (Vos et al. 2000; Waliszewski et al. 2012). This is the reason why organochlorine pesticides (OCP) have been prohibited and restricted in Mexico since 1991; however they continue to be identified in areas of intensive farming.

Fields of Sinaloa use pesticides to protect their crops, however, the unsuited treatment of residues increases the risk of exposure of organisms living in these sites. House mice are common and abundant in agricultural fields worldwide, because it is the species most widely distributed globally (Álvarez-Romero and Medellín 2005; Ishizuka et al. 2008). *Mus musculus* could be used as key organisms for environmental monitoring (Ieradi et al. 1998). Due to the uncontrolled use of OCP, non-target wildlife is at risk (Chang et al. 2011), owing to the exposure of more than one pesticide by applications of tank mixes or co-formulations, sequential applications to the same crop, or by wildlife traveling between treated fields (Thompson 1996).

Mammals living in agricultural areas are at great risk of being damaged, not only because of the chemicals used for

their control, but also because they are in contact with other toxic chemicals that might exert negative effects on their health. In Sinaloa, very little information exists about the effect of OCP on wildlife and it is not known which species are at the greatest risk of harm. The objective was to determine the presence of OCP in gonad, blood, and brain tissues of mice in two agricultural fields in Sinaloa.

Materials and Methods

During June and August 2013, 13 mice were collected at two agricultural areas in Sinaloa, at coordinates 25° 25′ 27.63″ N and 108° 38′ 23.28″ W and 24° 43′ 34.33″ N and 107° 31′ 12.08″ W. Mice were captured with Sherman traps (15 × 9 × 23 cm) with bait made of mixed tuna, oats, and vanilla, following the method described by Torres-Perez et al. (2004) and samples were manipulated according to Mills and Childs (1998).

Anesthetized mice were measured, weighed, sexed, and euthanized through decapitation. Blood was obtained from the jugular vein using a BD Vacutainer® with EDTA. Gonad and brain tissues were obtained and stored in sterile Whirl-Pak® bags (9.5 × 18 cm). All collected tissues were stored at −30°C until further analysis in the laboratory. Extraction of OCP, detection and quantification of analytes were made according to the method described by USEPA (2007) modified 8081b method. Reactive and solvent chemicals used were commercial grade pesticides. Pesticides were extracted through maceration of gonadal and brain tissue with hexane and anhydrous sodium sulfate. The blood sample was centrifuged at 3500 rpm for 10 min. The supernatant was placed into an Eppendorf tube and supplemented with a 1:5 saponification solution (5.6 g NaOH with 94.4 mL of ethanol), placed in a water bath (55 ± 2°C for 10 min). Hexane was added and centrifuged at 3500 rpm for 10 min. The recovered hexane was purified with a clean-up column (fiberglass wool, alumina, florisil, silica gel, and anhydrous sodium sulfate, at a proportion of 2:1:1:1:3, respectively). The extracts were concentrated and completely dried in a hood, and then dissolved in 2 mL of isooctane.

Quantification of OCP was performed in a gas chromatograph XL (PerkinElmer®) with ^{63}Ni-ECD (electron capture detector). The analytical chromatographic column was an Elite-CLP2 capillary (PerkinElmer®). The programmed conditions were: oven 120°C (hold 1 min) to 240°C at 4°C/min; EDC at 300°C; injector 260°C; 2 µL of samples

Table 1 Limits of detection, average of recovery, average recovery of fortification samples, and coefficient variation

Organochlorine pesticides	Linearity (r2)	Detection limit (µg/kg)	Amount recovery[a] (X ± S.D.) (%)	Variation coefficient (%)
Tetrachloro-m-xylene	0.981	0.04	76.81 ± 50.36	65.57
α-BHC	0.982	0.03	82.69 ± 51.65	62.47
β-BHC	0.995	0.03	75.25 ± 45.18	60.05
γ-BHC	0.998	0.04	84.34 ± 58.51	69.38
δ-BHC	0.971	0.03	71.49 ± 49.25	68.90
Heptaclor	0.995	0.03	71.80 ± 46.90	65.32
Aldrin	0.998	0.02	74.84 ± 40.12	53.61
Heptaclor epoxide	0.996	0.02	69.79 ± 49.20	70.50
γ-chlordane	0.991	0.03	76.16 ± 48.21	63.30
α-chlordane	0.997	0.04	74.36 ± 57.66	77.55
Endosulfan I	0.996	0.02	81.37 ± 47.03	57.80
DDE	0.997	0.04	75.41 ± 52.84	70.07
Dieldrin	0.996	0.02	71.24 ± 44.63	62.65
Endrin	0.988	0.01	79.31 ± 46.82	59.04
DDD	0.993	0.02	76.01 ± 44.64	58.73
Endosulfan II	0.985	0.01	102.11 ± 90.63	88.76
DDT	0.988	0.01	82.04 ± 50.01	60.95
Endrin aldehide	0.991	0.01	76.79 ± 42.05	54.77
Methoxychlor	0.977	0.01	97.70 ± 82.57	84.52
Endosulfan sulfate	0.982	0.01	112.59 ± 120.75	107.25
Endrin ketone	0.977	0.007	134.41 ± 185.94	138.35
Decachlorobiphenyl	0.962	0.03	87.68 ± 58.98	67.28

**Percentage of average recovery with fortification sampling of 0.1 µl OCP standard

[a]Linearity with six concentration level of 0.025 to 0.8 µl of OCP standard

injected; split-splitless on; attenuation 16; nitrogen gas carrier at 8.7 psi; nitrogen make-up gas at 30 mL/min. Quality controls (Table 1) were standard pesticide 8081, standard mix, and pesticides surrogate spike mix (SUPELCO® Part number: CRM46845 and CRM48460, respectively).

Results were analyzed using the Statistix 8® software; descriptive statistics were obtained (Henriksen et al. 2011) and due to the small sample size in this study, non-parametric analyses were used with Kruskal–Wallis tests ($p < 0.05$). The relative density was calculated for small mammals as the number of captures/number of total tramps x 100 (Torres-Perez et al. 2004).

Results and Discussion

Thirteen adult and young organisms were captured. Relative density was of 38.8% and 33.3% in Guasave and Culiacan, respectively. Other studies reported 2.5% (Torres-Perez et al. 2004) and 2.19% (Gallina et al. 2008) of relative density of mice in agricultural areas. Samples were obtained from the blood (mean 2.61 mL ± 1.41), brain (mean 0.19 g ± 0.09), and gonads (mean 0.37 g ± 0.16); 18 OCP analytes were detected, the frequency (in percentage) and mean concentration of each analyte are shown in Table 2 and for each tissue in Table 3.

Data reveal that some OCP are present in small mice living in agricultural areas, which confirms that some pesticides that have been restricted for years for agricultural use still exist in the ecosystem of Sinaloa. Even though we did not evaluate the damage by OCP in the studied mice, it is likely that these pesticides can be found also in higher mammals, threatening the health of wildlife species and humans, due to direct or indirect contact with chemicals used in agricultural areas or the presence of communities near the dispersion area of the chemical compounds. Although comparisons of results among analytes in samples and means do not reveal statistically significant differences, the order of magnitude and number of analytes were in the sequence gonad > brain > blood.

The blood is circulating the whole time, carrying fatty acids to the whole body; perhaps this is why it showed the lowest concentration and fewer analytes. In this sense, the blood is serving as an OCP transport to other tissues, such as the gonads and brain. Both tissues, brain and gonads, are bathed constantly in recirculating blood, allowing the OCP to enter the tissues and interact with the fatty acids. The presence of these analytes can be due to their affinity to the lipids present in the samples, agreeing with Waliszewski et al. (2012), i.e., that the OCP accumulate in lipid-rich tissues, such as the adipose tissue and blood serum. The brain is constituted by a diverse variety of complex lipids, at approximately 10% of a wet-weight basis (Jumpsen and Clandinin 1995); in mice, the gonadal fat pad corresponds to approximately 12% by weight of fat (Eisen 2005). However, the question is whether the interaction of analytes with the lipids is selective? Do analytes have some affinity for certain types of lipids? Or are they not selective? Or does their high affinity to interact with any lipid provide the ability to remain in the tissues?

Table 2 Frequency percentage and mean concentration ± SD of pesticide residues in all samples

Organochlorine pesticides	% Frequency (n = 39)	Concentration ng/g mean ± SD	(n)[a]
Tetrachloro-m-xylene	20.51	1.50 ± 3.86	8
γ-BHC	**53.84**	0.08 ± 0.10	**21**
δ-BHC	2.56	0.01	1
Heptachlor	5.13	0.29 ± 0.11	2
Aldrin	10.26	0.46 ± 0.51	4
Heptachlor epoxide	10.26	0.38 ± 0.68	4
α-Chlordane	25.64	0.24 ± 0.16	10
Endosulfan I	7.69	0.85 ± 1.43	3
DDE	2.56	1.61	1
Dieldrin	5.13	0.31 ± 0.42	2
Endrin	5.13	2.94 ± 3.75	2
DDD	12.82	0.40 ± 0.24	5
DDT	10.26	0.76 ± 1.61	4
Endrin aldehyde	5.13	0.17 ± 0.23	2
Methoxychlor	7.69	0.54 ± 0.72	3
Endosulfan sulfate	**43.59**	0.53 ± 0.50	**17**
Endrin ketone	10.26	0.39 ± 0.43	4
Decachlorobiphenyl	**64.10**	0.38 ± 0.64	**25**
Σ		**11.83 ± 14.56**	

[a]Number of samples. The words and numbers in bold indicate the three highest values

Table 3 shows that, in blood samples, 12 OCP were detected, γ-BHC was the one with the highest percentage of frequency; this pesticide was present in the highest count of samples. However, the analyte with the highest mean concentration was dieldrin; the lowest concentration was of endosulfan I. In the brain sample, seven analytes were detected, the pesticide with the highest frequency and highest mean concentration registered was endosulfan sulfate, and was present in the highest amount of samples. The lowest mean concentration registered corresponded to γ-BHC. In the gonad samples, 16 analytes were detected; in this tissue, decachlorobiphenyl was present with the highest percentage of frequency, and it was found in the highest count of samples. Endrin was present at the highest mean concentration; the lowest mean concentration was of δ-BHC. Perhaps one of the implications of the presence of these OCP in gonadal tissues could be a disturbance in reproduction, since they have been linked to reproductive harm.

Table 3 Percentage frequency and mean concentration ± SD of pesticide residues for the three tissues

Organochlorine pesticides	Brain			Blood			Gonads		
	%F[a]	Concentration ng/g		%F	Concentration ng/g		%F	Concentration ng/g	
	(n = 13)	Mean ± SD	n[b]	(n = 13)	Mean ± SD	n	(n = 13)	Mean ± SD	n
Tetrachloro-m-xylene	7.69	0.28	1	23.0	0.01 ± 0.009	3	30.76	2.91 ± 5.426	4
γ-BHC	69.2	**0.11** ± 0.056	9	**69.2**	0.009 ± 0.005	**9**	23.07	0.18 ± 0.204	3
δ-BHC	ND			ND			7.69	**0.012**	1
Heptachlor	ND			ND			15.38	0.28 ± 0.115	2
Aldrin	ND			7.69	0.016	1	23.07	0.61 ± 0.515	3
Heptachlor epoxide	ND			7.69	0.016	1	23.07	0.49 ± 0.785	3
α-Chlordane	53.8	0.16 ± 0.115	7	ND			23.07	0.42 ± 0.088	3
Endosulfan I	ND			7.69	**0.005**	1	15.38	1.26 ± 1.739	2
DDE	ND			ND			7.69	1.60	1
Dieldrin	ND			15.38	**0.31** ± 0.41	2	ND		
Endrin	7.69	0.28	1	ND			7.69	**5.59**	1
DDD	ND			ND			38.46	0.39 ± 0.24	5
DDT	ND			15.38	0.11 ± 0.07	2	15.38	1.41 ± 1.52	2
Endrin aldehyde	7.69	0.33	1	7.69	0.009	1	ND		
Methoxychlor	ND			7.69	0.12	1	ND		
Endosulfan sulfate	**84.6**	**0.73** ± 0.51	**11**	30.76	0.09 ± 0.04	4	15.38	0.30 ± 0.27	2
Endrin ketone	ND			15.38	0.09 ± 0.05	2	15.38	0.69 ± 0.45	2
Decachlorobiphenyl	61.5	0.19 ± 0.22	8	61.53	0.07 ± 0.09	8	**69.23**	0.81 ± 0.91	**9**

ND Value not detected, is below detection limits. Bold fonts indicate the highest and lowest values

[a]Percentage of frequency

[b]Number of samples

Another concern is that these compounds might be transmitted through copulation to the progeny. Thus, not only is the mother transmitting pesticides to the progeny through breast-feeding, but also the father is contributing to the presence of these chemicals in fetuses.

Of the 18 pesticides detected in the samples, decachlorobiphenyl was present with a frequency of 64.10%, followed by γ-BHC with 53.84% and endosulfan sulfate with 43.59%. Decachlorobiphenyl was present in 25 samples of 39; in 8 samples each in brain and blood, and in 9 gonad samples. This pesticide is frequently found in the environment, generally distributed in a variety of systems, such as the atmosphere, water, and food. The analyte is part of the polychlorinated biphenyl family (known as PCB-209); because of their lipophilic/hydrophobic nature, they accumulate in the adipose tissue and are difficult to metabolize and remove, causing long-term deleterious effects, such as neurotoxicity, carcinogenesis, and reproductive alterations (Alonso et al. 2008). The presence of decachlorobiphenyl in the tissues of mice could be due to its extensive use in electronic equipment, aside from the physical and chemicals characteristics of this pesticide (Koshioka et al. 1987). PCBs have the capacity to remain unchanged in the environment, for this reason the Stockholm Convention list defines as persistent organic chemicals those that remain more than 2 months in water or 6 months in soil (ONU 2008). It can be inferred that this decachlorobiphenyl remained in the crops for at least 2 months before its contact with mice.

γ-BHC was present in 21 samples; nine were for each brain and blood and three for gonads. This analyte is known as lindane, it is a volatile analyte, with the ability to be transported long distances leading to the contamination of various unchanged ecosystems (Tripathi et al. 2015). In agriculture, γ-BHC is used to treat the seeds that will be used in planting; in the livestock area, it is used to control insects and arachnids in farm animals (INECC 2015). About 4–7 million tons of lindane and other BHC isomer residues remain worldwide and these residues pose a serious threat to the environment and mammals (Tripathi et al. 2014, 2015). In June 2015, lindane was classified as "carcinogenic to humans" (Loomis et al. 2015). It has been reported that γ-BHC may cause reproductive damage, specifically reducing the reproductive success of small mammals; however, in this study, the gonadotropin levels were not measured, therefore, we cannot determine the existence of reproductive harm in rodents of agricultural areas.

Endosulfan sulfate was present in 17 samples analyzed; the highest detection was in brain samples, followed by gonads and blood. This analyte is a persistent

environmental metabolite of endosulfan, used for insect pests in agriculture. Endosulfan sulfate is also the most frequently detected in sediment, soil, and water; it is the oxide form of endosulfan and exerts a similar toxicity (Carriger et al. 2011). It is the most stable form and common in water samples contaminated with OCP and of long half-life, ranging from 2 to 6 years depending on the environmental condition (Shah et al. 2015; Sin et al. 2015). Because of its extensive use, bioaccumulation, and toxicity in the ecosystem, endosulfan sulfate is included in the Stockholm Convention list of OCP pollutants and is being phased out globally (Du et al. 2015; ONU 2008). Because of its toxicity and little knowledge about its effects on mammals, it is necessary to perform bioassays and research in situ, because it was mostly found in brain samples.

The health of wildlife predators is relevant for the scientific world due to the process of OCP bioaccumulation and bio-magnification through the predator–prey cycle (Wang et al. 1999; You et al. 2004). Due to the excessive use of OCP, wildlife is at risk, mainly those feeding on *Mus musculus* that live in crops. In conclusion, in Sinaloa, knowledge about the hazard caused by OCP on wildlife is scarce. The OCP detected in the three tissues are not employed to control rodent populations, but their presence could be due to having been exposed indirectly to these chemicals. The wildlife that feeds in agricultural areas could be at risk by: (1) the chemicals used for the control of pest; and (2) a variety of the pesticides that get in direct or indirect contact with non-target organisms, capable of damaging the health of other animals and humans. These pesticides bioaccumulate and biomagnify through the food chain, passing from one organism to another through the predator–prey behavior. Monitoring environmental health by sampling rodent populations could be useful for inferring the state of higher organisms. It is not adequate to perform invasive tissue analyses in wildlife predators due to their importance for the ecosystems and their protection status. Thus, the effect caused by exposure to these chemicals in higher organisms remains unknown and risk assessment in them is complicated.

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