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ENVIRONMENTAL ISSUES FOR THE PLANET: SOLUTIONS FROM SCIENCE AND TECHNOLOGY

Detection of organochlorine pesticides in infertile eggs of *crocodylus acutus* from sinaloa

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Abstract
Environmental contaminants endanger human health and non-target organisms such as crocodiles (*Crocodylus acutus*) that live in aquatic bodies surrounding agricultural areas. Due to their intrinsic characteristics, these organisms could be bioaccumulating and transmitting organochlorine pesticides (OCs) to their eggs. The objectives of this study were to determine the OCs in infertile eggs of *C. acutus* from Sinaloa and their correlation with the morphometric characteristics (MC), and to perform a preliminary estimate of the ecological risk due to the presence of pesticides using the PERPEST model. In June 2022, 76 infertile eggs (Ie) were collected: 57 from wild areas (Wa) and 19 from a crocodile farm (CSMf). Determination of OC in Ie was performed according to the USEPA method 8081b, modified. The observed percentages of Ie in Wa were 31.48% and 21.33% in CSMf. Twenty OCs were detected in the Ie, where dieldrin recorded the highest average concentration in Wa (6542.6 ng/g), and endosulfan-II in the CSMf (2172.8 ng/g). Bad negative and positive correlations were observed between OCs and MC, standing out the correlations between endosulfan-II and %Ie (-0.688) in the Wa, Cedritos drain, and between endrin and the weight of Ie (0.786) of the CSMf. The evaluation of the ecological risks of the aquatic environment due to the presence of OCs follow the sequence cyclodienes > aromatic > alicyclic hydrocarbons. A potential risk to the endocrine health of the species *C. acutus* was observed. Crocodiles are excellent biological models for monitoring the effects of OCs.

Keywords Non-target organisms · Sentinel species · Crocodiles · Endocrine disruptors

Introduction

Freshwater ecosystems are impacted by organochlorine (OCs) pesticides that threaten the health of the organisms that inhabit them (Daam et al. 2019; Sharma et al. 2020; Wu et al. 2000b). In Sinaloa, levels exceeding the permitted thresholds of some OCs in water bodies and ecosystems have been reported, reflecting the accumulation of more than six decades of intensive agriculture (García-de la Parra et al. 2012). Furthermore, this region is characterized by intense agricultural activity, which occupies the fifth place nationally in production (Servicio de Información Agroalimentaria y Pesquería 2021); consequently, due to the change in land use, the ecological niche of some organisms overlaps with the agricultural areas of the State, such as that of the American crocodile (*Crocodylus acutus*), the only species of crocodile that inhabits Sinaloa in northwestern Mexico.

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Crocodiles, due to their intrinsic characteristics (longevity, high-trophic state predator, and type of habitat), are more susceptible to the accumulation of OCs in their tissues (Gholamhosseini et al. 2021; Skaare et al. 1991; Wu et al. 2000a, b). In recent years, the crocodile population has decreased and studies indicate that this decrease can be attributed to reproductive deterioration (Wu et al. 2006), it is estimated that the latter could be due to exposure to OCs in the early stages of development (Wu et al. 2000a). OCs can affect wildlife (Poletta et al. 2009) at all stages of life; in addition, they are transferred to the progeny, altering from embryonic development to adulthood (Gholamhosseini et al. 2021). Multiple environmental contaminants, including OCs, have been detected in non-viable crocodile eggs (Pepper et al. 2004). It is estimated that the presence of OCs in the eggs could be due to the transfer of contaminants through the pores (used for gas exchange) or by maternal transfer from OCs-exposed females (Warner et al. 2016; Wu et al. 2000a). A study by Wu et al. (2006) points out that the variability of OCs in nests can be estimated using representative samples of infertile eggs (Ie). The objective of this study was to determine the OCs in Ie of *C. acutus* from Sinaloa and their correlation with the morphometric characteristics (MC), and to perform a preliminary estimate of the ecological risk due to presence of pesticides using the PERPEST model.

Materials and methods

Sampling of *C. acutus* nests was carried out during the month of June 2022 in the municipalities of Culiacan, Navolato, and Angostura, in the states of Sinaloa, Mexico, under the collecting permit SGPA/DGVS/03359/22. Infertility of eggs was determined based on the absence of the opaque band, the presence of the band indicates embryonic development (Ferguson 1985). Seventy-five Ie of *C. acutus* were collected, of which 57 were from wild areas (Wa), and 18 from the crocodile farm (CSMf) as a reference point. The Ie were transferred to the Organic and Inorganic Contamination laboratory of the Environment Department at the IPN-CIIDIR-Sinaloa, where length, width, and weight were measured, and the characteristics of each Ie were described (weight, length, width). The contents of the Ie were homogenized to analyze the OCs. In the Wa, a procedure to locate and count the crocodiles was implemented, which consisted of night tours of the water bodies aboard a boat of 4.9 m total length, equipped with a 100-W lamp. On each tour, crocodiles were counted and their size by class was estimated (Platt and Thorbjarnarson 2000), where: neonates correspond to those less than 30 cm, class I (TI) from 31 to 60 cm, class II (TII) corresponds to individuals from 61 to 120 cm, class III (TII) from 121 to 180 cm, class IV (TIV) are individuals from 181 to 240 cm, class V (TV) are individuals greater than 240 cm, and class VI (TVI) is when only the eyes were observed.

Analysis of OCs

The analysis of OCs in Ie was carried out according to method 8081b (USEPA 2007), modified (due to the high fat content and to reduce the amount of solvents). The method 8081b (USEPA 2007), consists of extraction, concentration, purification, drying, resuspension and determination in a gas chromatograph. The extraction process was carried out in 10 mL of sample adding 15 mL of hexane, 3 g of anhydrous sodium sulfate (ASS) and a 1:1 ratio of saponifying solution (5.6 g NaOH: 94.4 mL ethanol) using a sonicator (Branson 2510 Ultrasonic Cleaner, Mexico) for 30 min/sample. Subsequently, the purification process was carried out with clean-up columns packed with fiberglass wool, silica gel, Florisil®, aluminum oxide and ASS (2:1:1:1:3). The filtered samples were placed in an extraction hood, at room temperature, to dry and subsequently resuspended in 2 mL of isooctane

Table 1 Quality control, detection limits, average percent recovery, average percent recovery of fortified samples, and coefficient of variation

OCs	Linearity (R^2)	LD (ng/g)	%RPE	%CVE
Tetrachloro-m-xylene	0.989	9.784	94.5	121.9
αHCH	0.916	0.079	92.1	135.7
βHCH	0.984	3.808	89.6	178.7
δHCH	0.985	0.580	86.5	170.8
γHCH	0.972	0.016	100.9	126.2
Heptachlor	0.985	1.235	85.8	140.5
Heptachlor epoxide	0.976	0.121	86.3	136.1
αChlordane	0.984	0.346	82.3	148.0
γChlordane	0.977	0.644	85.2	144.8
Aldrin	0.979	0.741	68.1	93.9
Dieldrin	0.971	5.665	67.7	95.8
Endrin	0.986	1.450	69.7	79.1
Endrin aldehyde	0.958	5.191	67.5	89.5
Endrin ketone	0.998	2.786	86.0	124.1
Endosulfan I	0.972	0.038	87.0	114.0
Endosulfan II	0.982	0.027	76.6	100.5
Endosulfan sulfate	0.946	1.474	63.7	88.5
DDT	0.912	0.686	99.7	130.8
DDE	0.972	0.314	90.5	140.6
DDD	0.989	3.192	92.5	143.4
Methoxychlor	0.984	0.551	97.3	173.2
Decachlorobiphenyl	0.953	0.013	82.4	144.1

LD = Limits of detection of organochlorines (ng/g). %RPE = average percent recovery of the standards at concentrations of 2, 4, 8, 16, 32, 64, 120, 250, 500, and 1000 ng/mL of the EPA8081b standard. %CVE = percentage of coefficient of variation of standards

Table 2 Record of nests per coordinates, number of total eggs, infertile eggs, percentage of infertile eggs, kilometers traveled, and number of crocodiles observed per kilometer

Sampling site		#n	Number of eggs		Coordinates GMS		km**	ind/km (obs.)
			Total	Infertile* (%Ie)	N	O		
Wa	Chiricahueto Lagoon -Chiricahueto Drain (CHd)	1	20	4 (50%)	24°35′37.01"	107°29′04.93"	5.74	11.8 (68)
		2	**53**	7 (**60.3%**)	24°35′37.05"	107°29′05.45"		
		3	37	9 (40.5%)	24°35′36.17"	107°29′06.29"		
		4	40	**10** (62.5%)	24°35′35.73"	107°29′06.54"		
		7	50	**10** (24%)	24°35′22.67"	107°29′08.89"		
		9	48	9 (22.9%)	24°35′29.89"	107°29′10.96"		
	El Tular Stream (ETs) -Los Ángulos	6	24	2 (8.3%)	24°39′37.02"	107°46′53.82"	2.29	27.9 (64)
	-Los Sánchez	8	20	2 (15%)	24°40′41.73"	107°46′42.21"	3.16	10.1 (32)
	Los Algodones -Drain Cedritos (Cd)	2	27	4 (11%)	24°50′47.75"	107°45′18.15"	19.64	13.19 (138)
CSMf	Santa María Crocodile Farm -Pond 2	3	32	5 (25%)	25°05′58.65"	108°03′24.72"	0.4	200 (80)
		4	24	3 (16.6%)	25°05′58.01"	108°03′24.72"		
	-Pond 3	6	33	2 (15.1%)	25°05′57.24"	108°03′25.36"		
		7	29	3(10.3%)	25°05′57.03"	108°03′26.64"		
		8	19	1 (10.5%)	25°05′58.56"	108°03′25.82"		
		9	31	4 (29.03%)	25°05′58.51"	108°03′26.03"		

*Wa = wild areas. CSMf = Santa María crocodile farm. #n = Number of nests sampled. *This is the number of infertile eggs used in this research. **Kilometers traveled in the sampling. ind/km = individuals per kilometer. The highest results are shown in bold*

(2,2,4-trimethylpentane). To determine OCs, a gas chromatograph was used (PerkinElmer® XL, Waltham, MA, US) provided with Ni^{63} electron capture detector, and the DB-5 chromatographic column (Agilent® Santa Clara, CA, US) with the following programming: 2 µL injection quantity, 250 °C injection temperature, 330 °C detector

Table 3 Correlations between OCs, MC, number of total eggs, and %Ie in the 75 Ie

OCs	Weight (g)	Length (cm)	Width (cm)	Total Eggs	%Ie*
αHCH	-0.044	0.027	-0.211	-0.138	*-0.336*
βHCH	*-0.257*	-0.159	*-0.255*	-0.178	*-0.325*
δHCH	*-0.255*	0.001	*-0.243*	-0.131	*-0.300*
γHCH	*-0.386*	-0.109	*-0.523*	***-0.470***	*-0.328*
Heptachlor	*-0.313*	-0.212	*-0.474*	-0.253	*-0.456*
Heptachlor epoxide	*-0.429*	-0.141	*-0.401*	*-0.252*	***-0.528***
αChlordane	-0.160	-0.010	*-0.384*	-0.153	*-0.391*
γChlordane	-0.280	***-0.509***	-0.396	0.035	*-0.441*
Aldrin	*-0.316*	-0.053	*-0.274*	*-0.293*	*-0.297*
Dieldrin	*0.277*	*0.253*	*0.348*	0.166	0.066
Endrin	-0.222	0.025	*-0.392*	-0.240	*-0.414*
Endrin aldehyde	***-0.436***	-0.061	*-0.492*	*-0.322*	*-0.490*
Endrin ketone	*-0.341*	-0.166	*-0.318*	*-0.268*	*-0.319*
Endosulfan I	*-0.338*	-0.098	*-0.490*	-0.196	*-0.437*
Endosulfan II	*-0.408*	-0.067	-0.346	-0.240	-0.336
Endosulfan sulfate	*-0.266*	-0.085	*-0.314*	*-0.288*	*-0.326*
DDT	*-0.286*	-0.036	*-0.424*	-0.262	*-0.468*
DDE	-0.367	-0.193	-0.511	-0.661	-0.441
DDD	*-0.431*	-0.176	***-0.580***	*-0.444*	*-0.495*
Methoxychlor	*-0.431*	*-0.306*	*-0.440*	*-0.460*	*-0.319*

[*]This is the percentage of infertile eggs used in this research. Numbers in italic are significant differences (Spearman $\rho < 0.05$). Numbers in bold indicate the highest significant correlations per variables

Table 4 Correlations between OCs, MC, number of total eggs and %Ie per Wa and CSMf sites

	Wild Areas					**Crocodile Santa Maria Farm**				
OCs	Weight	Length	Width	Total Eggs	%Ie*	Weight	Length	Width	Total Eggs	%Ie*
αHCH	-0.070	0.052	-0.242	-0.202	*-0.404*	0.571	0.220	-0.012	-0.012	0.108
βHCH	-0.219	-0.207	-0.077	-0.082	-0.272	0.105	0.458	-0.412	-0.237	-0.004
δHCH	-0.116	0.142	-0.142	-0.031	*-0.285*	-0.343	-0.140	0.127	0.233	0.040
γHCH	-0.113	0.095	*-0.312*	*-0.304*	*-0.301*	0.074	0.211	-0.182	-0.039	0.455
Heptachlor	-0.056	-0.077	-0.309	-0.135	*-0.463*	*-0.566*	-0.342	-0.139	0.062	0.483
Heptachlor epoxide	*-0.326*	-0.050	*-0.302*	-0.136	***-0.556***	-0.222	0.127	-0.282	-0.184	0.126
αChlordane	0.074	0.204	-0.246	0.068	*-0.408*	-0.643	-0.252	-0.506	-0.381	0.331
γChlordane	-0.280	***-0.509***	-0.396	0.035	*-0.441*					
Aldrin	***-0.349***	-0.032	*-0.341*	-	*-0.483*	-0.125	-0.016	-0.097	0.252	*0.486*
Dieldrin	0.089	0.116	0.119	-0.062	-0.078	*-0.594*	0.007		-0.086	0.187
Endrin	-0.087	0.185	-0.264	-0.087	*-0.399*	***0.786***	0.505	-0.144	-0.281	-0.505
Endrin aldehyde	*-0.275*	0.052	*-0.354*	-0.107	*-0.452*	0.024	0.351	-0.394	0.022	0.082
Endrin ketone	-0.260	-0.095	-0.222	-0.171	*-0.392*	-0.341	-0.027	-0.230	0.015	*0.628*
Endosulfan I	-0.107	0.053	*-0.326*	0.041	*-0.432*	-0.248	0.055	-0.282	0.079	0.424
Endosulfan II	-0.389	0.126	-0.289	-0.097	*-0.469*	0.314	-0.147	0.696	0.324	0.559
Endosulfan sulfate	-0.125	0.140	-0.186	-0.197	*-0.387*	-0.201	-0.333	-0.053	0.358	***0.601***
DDT	-0.216	0.024	***-0.384***	-0.216	*-0.474*	-0.300	-0.200	-0.103	0.289	0.289
DDE	0.321	-0.270	0.075	-0.259	-0.111					
DDD	-0.142	0.086	-0.241	-0.158	-0.318	0.276	0.242	-0.224	-0.374	0.063
Methoxychlor	-0.187	-0.063	-0.221	*-0.297*	-0.285	-0.296	***-0.564***	0.186	0.467	*0.526*

[*]This is the percentage of infertile eggs used in this research. Numbers in italic are significant differences (Spearman $\rho < 0.05$). Numbers in bold indicate the highest significant correlations per variables

temperature, attenuation 8, nitrogen carrier gas (5 psi), nitrogen make-up gas (30 mL/sec); ramp: start 80 °C for 0.5 s, rise rate 15 °C per min up to 230 °C, rise rate 8 °C per min up to 270 °C. The 8081b and surrogate standards were used (SUPELCO® Part number: CRM46845 and CRM48460, respectively, Merck KGaA, Darmstadt, Germany) at the concentrations of 2, 4, 8, 16, 32, 64, 120, 250, 500, and 1000 ng/mL with were used for the calibration curve, considering R2 = 0.9 as optimal.

The concentration of OCs was quantified according to the method 8081b indicated by USEPA (2007). The calibration factor (CF) was calculated for each analyte per point of the calibration curve, following the formula:

$$CF = \frac{\text{Area of standar analyte of calibration curve}}{\text{Concentration(innanograms)of standar analyte of calibration curve}}$$

Subsequently, the average calibration factor ($\overline{CF}$) was calculated, dividing the sum of CF by the number of points on the calibration curve, following the formula:

$$\overline{CF} = \frac{\sum_{i=1}^{n} CF}{(\mathrm{n})}$$

To calculate the concentration of each analyte detected in the sample, the following formula was considered:

$$\text{Concentration } (ng/L) = \frac{(\mathrm{A}_x)(\mathrm{V}_t)(\mathrm{D})}{(\overline{CF})(\mathrm{V}_i)(\mathrm{V}_s)}$$

where: Ax, is area of the analyte peak in the sample; Vt, is final total volume of the extract (in μL); D, is dilution factor;$\overline{CF}$, is average CF; Vi, is injected volume (in μL); Vs, is initial sample amount (in mL or g). The result is expressed in μg/kg or ng/g for solid samples, and for aqueous samples in μg/L or ng/mL.

Quality control

Quality control was carried out as described in Table 1. Where 1 mL of standard 8081b (SUPELCO® Part number: CRM46845) at the concentration of 1000 ng/mL was added to six randomly selected Ie samples, to evaluate the recoverability of the method and the extraction procedure.

Table 5 Correlations between OCs, MC, number of total eggs and %Ie of the CHd

OCs	**Chiricahueto Drain**				
	Weight	Length	Width	Total Eggs	%Ie*
αHCH	-0.020	-0.041	-0.162	-0.157	*-0.361*
βHCH	-0.197	-0.163	-0.078	-0.100	-0.287
δHCH	-0.082	0.141	-0.165	-0.019	*-0.349*
γHCH	0.133	0.158	-0.074	-0.071	-0.043
Heptachlor	0.246	-0.025	-0.079	0.015	-0.265
Heptachlor epoxide	-0.251	0.009	-0.279	-0.092	*-0.605*
αChlordane	0.252	0.243	-0.148	0.248	*-0.362*
γChlordane	-0.281	***-0.508***	*-0.476*	-0.002	*-0.523*
Aldrin	-0.219	-0.061	-0.150	-0.192	*-0.363*
Dieldrin	0.073	0.106	0.059	-0.138	-0.177
Endrin	0.123	0.230	-0.164	0.136	*-0.414*
Endrin aldehyde	-0.206	0.082	*-0.328*	0.020	*-0.457*
Endrin ketone	-0.191	-0.073	-0.154	-0.065	*-0.356*
Endosulfan I	-0.049	0.070	***-0.350***	0.051	*-0.492*
Endosulfan II	-0.493	-0.119	-0.326	0.029	***-0.688***
Endosulfan sulfate	-0.043	0.125	-0.070	-0.096	*-0.352*
DDT	-0.103	0.044	*-0.326*	-0.131	*-0.468*
DDE	0.321	-0.270	0.075	-0.259	-0.111
DDD	-0.078	-0.049	-0.169	-0.096	-0.307
Methoxychlor	-0.053	-0.088	-0.026	-0.113	-0.115

This is the percentage of infertile eggs used in this research. Numbers in italic are significant differences (Spearman $\rho < 0.05$). Numbers in bold indicate the highest significant correlations by variables

Statistical analysis

To analyze the results, Microsoft Excel was used to perform descriptive statistics (averages, standard deviation and percentage of frequency), Statistix v8 and Statistica v8 to determine the normality test (non-parametric data), Kruskal–Wallis ($p < 0.05$), and Spearman correlation ($\rho < 0.05$) where the correlations considered were: 0 as null, from 0.01 to 0.10 as negligible, 0.11 to 0.39 as weak, 0.40 to 0.69 as moderate, 0.70 to 0.89 as strong, and 0.90 to 0.99 as very strong, and 1 as perfect (Schober et al. 2018). The PERPEST model was used to evaluate the ecological risks of the aquatic environment of crocodile hatchlings exposed to OCs (grouped into alicyclic hydrocarbons, cyclodienes and aromatics), in which eight aquatic groups are considered: algae and macroalgae, community metabolism, fish, insects, macrocrustaceans, microcrustaceans, rotifers, and other macro-invertebrates (Van den Brink et al. 2002), based on the bibliography and previously integrated into the PERPEST software (https://www.pesticidemodels.eu/perpest/home). The observed responses of PERPEST in the aquatic ecosystem are categorized from 0 or 5, according to the degree of risk, as: 0) no effects; 1) no effects have been demonstrated, without clear causality; 2) slight effects; 3) clear short-term effects, lasting less than eight weeks; 4) clear effects have been demonstrated, without demonstrating recovery; 5) clear long-term effects, lasting more than eight weeks, negative effects reported. For the evaluation of ecological risks, the OC analytes were grouped into alicyclic hydrocarbons (HCH's), cyclodiene hydrocarbons (endosulfans, chlordanes, drines and heptachlors), and aromatic hydrocarbons (DDT's and methoxychlor).

Result

In relation to the MC, the 75 Ie of *C. acutus* weighed in average 82 g, with a length and width of 7.2 and 4.6 cm, respectively. The Wa's Ie had an average weight of 86.1 g, with a length and width of 7.2 and 4.7 cm, respectively; whereas those of the CSMf average weighed 70.4 g in average, with a length and width of 7 and 4.2 cm, respectively. The highest percentage of Ie was recorded in nest 2 of the Chiricahueto drain (CHd), while, the lowest percentage was presented in "Los Ángulo" site of the "El Tular" stream (ETs) (Table 2).

OCs concentrations and correlations

Regarding the significant correlations between the OCs and the MCs, it was generally (Table 3) observed that the correlations were from weak to moderate negative and positive; while, in the Wa (Table 4) were from weak to strong negative. Per sampling site, in Wa, no significant correlations were observed between the MC of the Ie and the OCs of the ETs and Cd, however, in the CHd (Table 5), significant correlations were observed from weak to moderate negative. In the CSMf, it was observed that the significant correlations per variable ranged from moderate to strong positive and negative (Table 4).

Twenty OCs were detected in the 75 Ie (Table 6), where the highest average concentration recorded was for dieldrin. Significant statistical differences were observed between dieldrin and γHCH, dieldrin and endosulfan I, dieldrin and heptachlor, endosulfan I and γHCH, and endosulfan I and heptachlor. It was found that the correlations observed between the OCs analytes detected in all the Ie of *C. acutus* ranged from weak to strong positive; the correlation between endrin and endosulfan II stands out (supplementary material).

Table 6 Average concentration and frequency of OCs detected in the 75 Ie of C. acutus

OCs	Average ng/g (n)	± SD	Minimum	Maximum	%F	K-W
Alicyclic hydrocarbons						
αHCH	724.9 (52)	1267.2	31.0	8869.8	69.3	ab
βHCH	516.1 (67)	736.9	6.8	4694.3	89.3	ab
δHCH	452.1 (70)	398.5	10.9	2462.0	93.3	ab
γHCH	202.3 (69)	430.4	0.8	2790.4	92.0	c*
Cyclodiene hydrocarbons						
Heptachlor	191.6 (45)	368.4	0.2	2125.0	60.0	c*
Heptachlor epoxide	561.0 (70)	593.6	36.3	4299.9	93.3	ab
αChlordane	290.1 (43)	325.5	18.4	1514.3	57.3	bc
γChlordane	621.8 (23)	1277.3	36.3	5947.0	30.7	abc
Aldrin	401.1 (73)	1402.5	8.9	11,952.6	97.3	cd
Dieldrin	**5471.9** (64)	6956.9	184.9	27,859.7	85.3	a*
Endrin	597.6 (38)	704.5	3.9	3026.8	50.7	b
Endrin aldehyde	1171.8 (72)	1523.8	3.3	8194.1	96.0	b
Endrin ketone	1066.3 (74)	2085.6	3.0	12,522.5	**98.7**	b
Endosulfan I	269.8 (65)	531.1	14.1	3899.8	86.7	bcd*
Endosulfan II	1278.6 (25)	1558.1	88.9	6654.9	33.3	ab
Endosulfan sulfate	734.4 (74)	1023.9	10.0	6792.5	**98.7**	b
Aromatic hydrocarbons						
DDT	467.7 (48)	521.2	49.5	3332.4	64.0	abc
DDE	387.6 (9)	226.3	137.6	831.6	12.0	ab
DDD	704.6 (54)	1324.1	4.4	6896.7	72.0	ab
Methoxychlor	736.5 (61)	699.4	13.0	2428.5	81.3	ab

%F is the frequency percentage. K-W is the Kruskal–Wallis analysis ($p < 0.05$). The highest results in average concentration and frequency are shown in bold

In the 57 Ie of the Wa, 20 OCs were detected (Table 7), of which dieldrin presented the highest concentration. Significant statistical differences were observed between dieldrin and γHCH, and dieldrin and heptachlor. In the 57 Ie of the Wa, significant correlations were observed ranging from weak to strong positive, both, between γchlordane with DDT, and endosulfan II with endosulfan sulfate (supplementary material).

Dieldrin showed a higher average concentration in CHd and ETs, and endrin ketone in Cd (Table 8). Significant statistical differences were observed among γHCH, DDT, and dieldrin at the CHd site, and between γHCH and dieldrin at the ETs site. Of the sites sampled in Wa, significant correlations were observed only in the CHd with ranges from weak to strong positive, standing out the correlation between DDT and γchlordane (supplementary material).

In CSMf, 19 OCs were observed (Table 9), the highest average concentration was recorded for endosulfan II. γChlordane was not detected in the Ie samples. No significant statistical differences were observed. Significant correlations were observed in the 18 Ie from the CSMf among the OC analytes, which ranged from bad to excellent positive and negative, standing out the correlations of aldrin with DDT and dieldrin with DDT (supplementary material).

Results of analysis with the PERPEST model

The concentrations observed for the analytes per OCs group in both the Wa and CSMf were analyzed with the PERPEST model. Continuous exposure of Ie was considered for an average of 81.6 days. Alicyclic hydrocarbons (Fig. 1) affected negatively macrocrustaceans to a greater extent (with clear effects without recovery after eight weeks), followed by microcrustaceans and the insect group, in the sequence CSMf > ETs > Cd > CHd.

It was observed that cyclodiene hydrocarbons (Fig. 2) generated a negative effect on the groups of macrocrustaceans, insects, and fish of the Wa (CHd, ETs y Cd); however,

Table 7 Average concentration and frequency of OCs detected in the 57 infertile C. acutus eggs from Wa

OCs	Average ng/g (n)	± SD	Minimum	Maximum	%F	K-W
Alicyclic hydrocarbons						
αHCH	729.4 (44)	1357.0	31.0	8869.8	77.2	a
βHCH	388.9 (50)	512.2	6.8	3114.6	87.7	ab
δHCH	427.1 (55)	432.8	10.9	2462.0	96.4	ab
γHCH	141.1 (52)	418.6	0.8	2790.4	91.2	c*
Cyclodiene hydrocarbons						
Heptachlor	169.8 (32)	421.6	0.2	2125.0	56.1	c*
Heptachlor epoxide	505.5 (56)	611.6	40.8	4299.9	98.2	a
αChlordane	243.6 (35)	289.2	18.4	1514.3	61.4	b
γChlordane	621.8 (23)	1277.3	36.3	5947.0	40.3	ab
Aldrin	441.4 (56)	1595.3	8.9	11,952.6	98.2	b
Dieldrin	**6542.6** (52)	7311.7	184.9	27,859.7	91.2	a*
Endrin	437.8 (31)	588.3	3.9	3026.8	54.3	b
Endrin aldehyde	939.2 (56)	1414.4	3.3	8194.1	98.2	a
Endrin ketone	1086.0 (57)	2342.0	3.0	12,522.5	**100**	a
Endosulfan I	209.2 (51)	549.2	14.1	3899.8	89.4	b
Endosulfan II	996.2 (19)	1193.3	88.9	4392.3	33.3	a
Endosulfan sulfate	631.5 (57)	1011.9	74.4	6792.5	**100**	a
Aromatic hydrocarbons						
DDT	452.8 (43)	541.1	49.5	3332.4	75.4	ab
DDE	296.8 (7)	140.3	137.6	481.0	12.2	ab
DDD	205.1 (38)	163.6	4.4	727.1	66.7	b
Methoxychlor	559.1 (46)	633.9	13.0	2428.5	80.7	a

*%F is the frequency percentage. *indicates significant difference (Kruskal–Wallis, $p < 0.05$). The highest results in average concentration and frequency are shown in bold*

in the CSMf microcrustaceans were more negatively affected than insects and fish, regarding microcrustaceans.

Aromatic hydrocarbons (Fig. 3) show clear negative effects mainly in the macrocrustacean group with the sequence CSMf = Cd > ETs = CHd, followed by the insect group (sequence CSMf > ETs = CHd > Cd), the fish group (in the sequence Cd > CSMf > ETs = CHd) and the microcrustacean group (with the sequence ETs = CHd > Cd = CSMf).

Discussion

The presence of OCs in the Ie of *C. acutus* evidences the presence of these environmental contaminants in the habitat of non-target free-living organisms, which generates concern due to the capacity the OCs have to alter the endocrine and reproductive system. The determined OCs concentrations in the Ie, in general, followed the order of magnitude sequence per site Cd > CSMf > ETs > CHd. The eggs found in the Cd reveal it as the most contaminated area by OCs, with an average 35% higher concentration of OCs than in the CSMf. The Cd receives the water from the wastewater treatment plant of the city of Culiacán, which travels approximately 50 km to the bay of Santa María, in the "Los Algodones" estuary, Sinaloa (Salvador et al. 2018). In addition to this, runoff from agricultural areas surrounding Wa could influence the presence of OCs in this aquatic body.

Transfer of OCs into the eggs

In the present work, clutches of 19 to 53 *C. acutus* eggs are reported, of which 1 to 10 Ie per clutch were used. Wu et al. (2006) report clutches of 17 to 35 eggs of *C. moreletii*, and Wu et al. (2000a) used 1 to 6 eggs per clutch of *C. moreletii*. The size of the clutch is a function of the size, age and maturity of the reproductive animals; it is considered that organisms with greater maturity, age and larger size produce larger clutches than young organisms (Khosa et al. 2012). The highest average percentage of Ie was recorded

Table 8 Average concentration and frequency of OCs detected in infertile C. acutus eggs for each site sampled in Wa

OCs	Chiricahueto Drain (*n* = *49*)			"El Tular" Stream (*n* = *4*)			Cedritos drain (*n* = *4*)		
	Average ng/g (n)	± SD	%F	Average ng/g (n)	± SD	%F	Average ng/g (n)	± SD	%F
Alicyclic hydrocarbons									
αHCH	723.5	1417.7	81.6	631.8	493.3	75	1258.8		25
βHCH	363.1	507.2	87.8	416.1	615.9	100	722.9	523.7	75
δHCH	402.8	354.5	95.9	312.6	106.7	100	827.0	1092.9	100
γHCH	56.9*	82.8	89.8	152.7*	124.4	100	1055.0	1283.7	100
Cyclodiene hydrocarbons									
Heptachlor	89.6	222.8	53.1	65.0	39.7	50	743.8	959.4	100
Heptachlor epoxide	491.6	634.1	100.0	490.0	388.2	100	754.7	557.5	75
αChlordane	192.1	169.7	63.3	352.5	351.8	75	1514.4		25
γChlordane	645.6	1302.1	44.9	98.9		25			
Aldrin	197.9	274.9	98.0	459.7	424.3	100	3346.0	5752.9	100
Dieldrin	**6823.6***	7559.9	93.9	**6058.9***	5423.7	100	1048.6	1221.4	50
Endrin	345.6	371.8	49.0	350.8	234.3	100	1292.1	1519.4	75
Endrin aldehyde	876.5	1408.4	100.0	580.8	394.3	100	2441.6	1852.9	75
Endrin ketone	946.6	2063.0	100.0	460.0	341.4	100	**3419.8**	5106.5	100
Endosulfan I	204.2	567.5	93.9	86.6	41.3	100	928.7		25
Endosulfan II	1049.2	1331.4	30.6	636.2	693.8	50	958.6	170.2	50
Endosulfan sulfate	454.3	435.9	100.0	472.2	397.0	100	2961.0	2852.4	100
Aromatic hydrocarbons									
DDT	428.8*	550.2	75.5	395.2	237.0	100	1013.6	753.9	50
DDE	296.8	140.3	14.3						
DDD	190.6	146.4	65.3	192.0	123.5	100	464.3	371.7	50
Methoxychlor	491.6	620.3	81.6	921.5	526.6	100	1184.5	867.8	50

*%F is the frequency percentage. *indicates statistical difference (Kruskal–Wallis, $p < 0.05$). Bold letters indicate the highest average concentrations*

in CHd, whereas the lowest percentage was observed in the Cd. The detection of OCs in the Ie could be due: 1) to maternal transfer from exposed females, due to the mobilization of OCs from the fat deposits of the females to the developing follicles during vitellogenesis, or, 2) the transfer of OCs through the pores of the eggs, which are used for gas exchange (Warner et al. 2016; Wu et al. 2000a), nutrient transport and waste storage during embryonic development (Pepper et al. 2004). Crocodile eggs are at risk of predation by different species that invade nests (Combrink et al. 2016; Magnusson 1982), and the young are at risk of predation by opportunistic predators such as raccoons (*Procyon lotor*), ants (Pepper et al. 2004), Nile monitor lizard (*Varanus niloticus*), and the swamp mongoose (*Atilax paludinosus*) (Combrink et al. 2016), among others; therefore, non-invasively determining the OCs load in Ie helps to determine trophic transfer through food webs, and in turn, estimate the risk due to OC-exposure in crocodiles and other species (Sherwin et al. 2016).

Environmental evaluation with the PERPEST model

Although the highest concentrations of OCs were recorded in Wa, the effects of the ecological risks of the aquatic environment estimated by the PERPEST model are greater in the CSMf because of the presence of contaminants of the alicyclics and aromatics groups; however, in the Wa the cyclodienes showed greater ecological risks. The ecological risks of the aquatic environment where baby crocodiles live could put the health of the species at risk by consuming a contaminated diet. It is known that, at a younger age, crocodiles feed mainly on insects, arachnids, and amphibians, and as they grow in size their diet expands to include crustaceans, mollusks, fish, reptiles, birds and mammals (Casas-Andreu and Barrios-Quiroz 2003; Cott 1961).

Crustaceans (macro and microcrustaceans) are one of the most consumed food groups among crocodiles in sizes from 0.5 to 4.5 m (classification size ranging from TI to TV) (Cott

Table 9 Average concentration and frequency of OCs detected in the 18 infertile eggs of C. acutus from the CSMf

OCs	Average ng/g (n)	± SD	Minimum	Maximum	%F	K-W
Alicyclic hydrocarbons						
αHCH	700.1 (8)	622.2	58.9	1542.6	44.4	ab
βHCH	890.2 (17)	1112.3	65.8	4694.2	**94.4**	ab
δHCH	543.5 (15)	220.4	161.5	836.7	83.3	ab
γHCH	389.5 (17)	423.3	5.8	1663.7	**94.4**	bc
Cyclodiene hydrocarbons						
Heptachlor	245.2 (13)	184.3	1.5	748.9	72.2	b
Heptachlor epoxide	782.8 (14)	470.9	36.3	1816.0	77.8	ab
αChlordane	493.5 (8)	414.2	91.4	1114.1	44.4	ab
γChlordane	nd	nd	nd	nd	nd	nd
Aldrin	268.1 (17)	281.5	13.1	964.8	**94.4**	c
Dieldrin	832.2 (12)	648.9	328.6	2646.7	66.7	abc
Endrin	1305.1 (7)	783.9	205.3	2424.2	38.9	ab
Endrin aldehyde	1986.1 (16)	1657.1	334.4	5917.6	88.9	ab
Endrin ketone	1000.2 (17)	801.0	161.5	2785.9	**94.4**	ab
Endosulfan I	490.5 (14)	402.0	15.6	1128.4	77.8	bc
Endosulfan II	**2172.8** (6)	2294.2	657.9	6654.9	33.3	ab
Endosulfan sulfate	1079.6 (17)	1017.3	10.0	4598.8	**94.4**	ab
Aromatic hydrocarbons						
DDT	595.7 (5)	308.1	293.0	973.5	27.8	ab
DDE	705.7 (2)	177.9	579.9	831.6	11.1	ab
DDD	1890.8 (16)	1998.9	256.7	6896.7	88.9	ab
Methoxychlor	1280.5 (15)	619.8	305.2	2029.5	83.3	a

%F is the frequency percentage. K-W is the Kruskal–Wallis analysis ($p < 0.05$). nd is not detected. The highest results in average concentration and frequency are shown in bold

1961); according to the results of PERPEST, the group of macrocrustaceans presents a greater risk from OCs in the sequence cyclodiene > aromatic > alicyclic hydrocarbons. Of the cyclodiene hydrocarbons, dieldrin presented the highest average concentration and significant differences, in Wa and specifically in the CHd. The maximum concentration of dieldrin, in the present study, which places our results two orders of magnitude above the values observed by Hall et al. (1979), in *C. acutus* eggs, and by the work of Skaare et al. (1991) in *Crocodylus niloticus* eggs (0.03 mg/kg of dieldrin, or 30 ng/g). According to Kohno (2021), dieldrin is one of the OCs that have been associated with alterations in small genitalia and low plasma testosterone concentration in *Alligator mississippiensis* crocodiles, therefore, dieldrin is considered an endocrine disruptor. This agrochemical, derived from the analyte aldrin, is within the group called the "dirty dozen" (Jorgenson 2001) belonging to persistent organic pollutants, which are of global concern due to their widespread presence in the environment (Prabhu and Lakshmipraba 2022).

Relevance of the crocodile as a biological model and the use of the PERPEST model

Crocodiles are recognized as excellent biological models for monitoring the effects of OCs, both at the population and individual level, which is why they are considered sentinel species of local environmental pollution (Grant et al. 2013; Latorre et al. 2013; Somaweera et al. 2020). In addition, they live in contaminated ecosystems that could be generating a negative impact on the endocrine health of the *C. acutus* population. However, there is a lack of blood reference values, both hematological and biochemical. Crocodiles are important sentinels with which to understand the consequences of endocrine disruption in wildlife (Tavalieri et al. 2020).

With the PERPEST model, approximate environmental risks can be predicted in aquatic environments, which are compared with the program's base case, allowing to estimate the probabilities of both direct and indirect toxic effects (Cervantes 2021). The use of models such as PERPEST in developing countries is useful due to the limited information

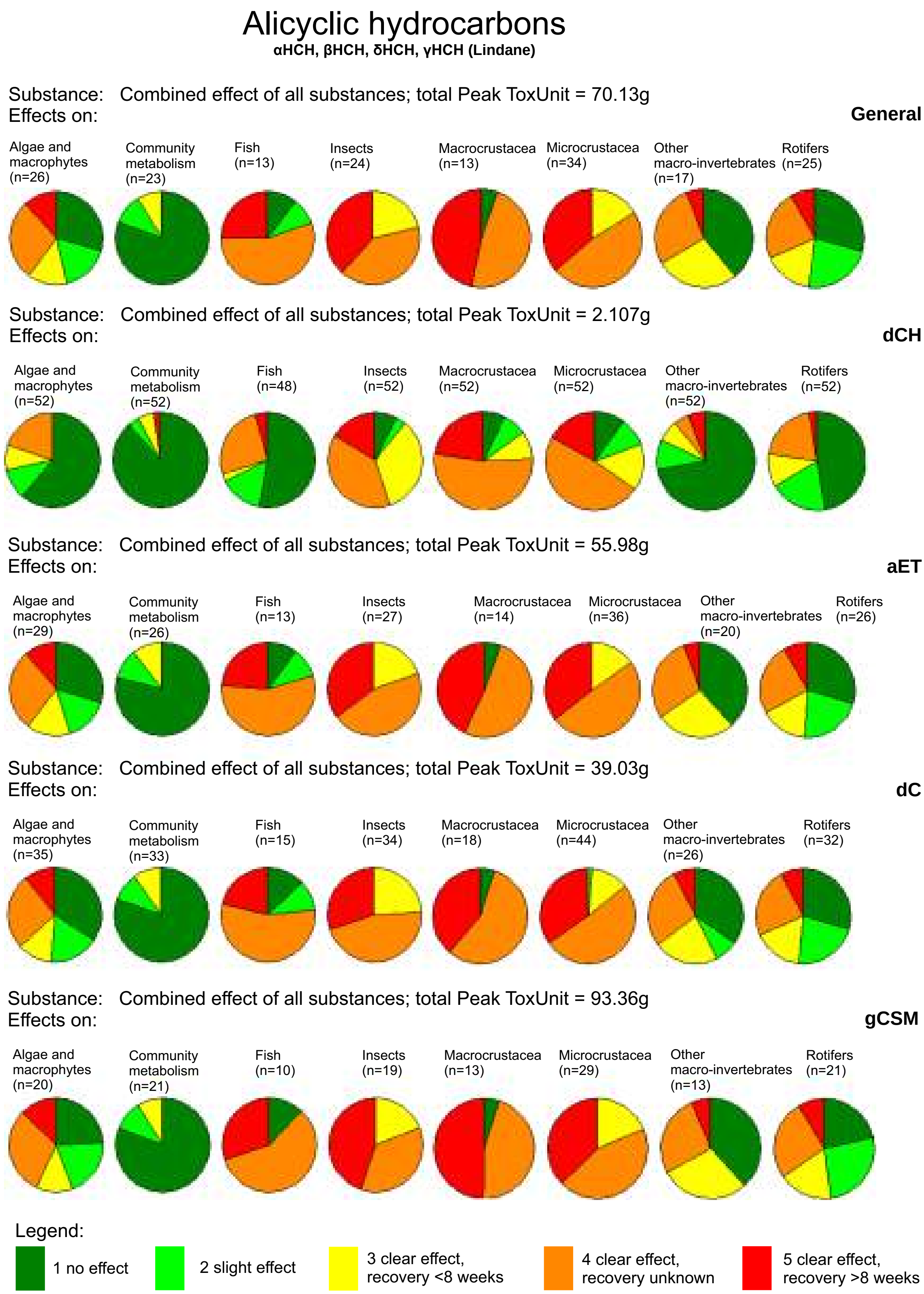

Fig. 1 Results of the PERPEST model of the evaluation of the ecological risks of the aquatic environment exposed to the group of alicyclic hydrocarbons in general, and in the areas dCH, aET, dC and gCSM

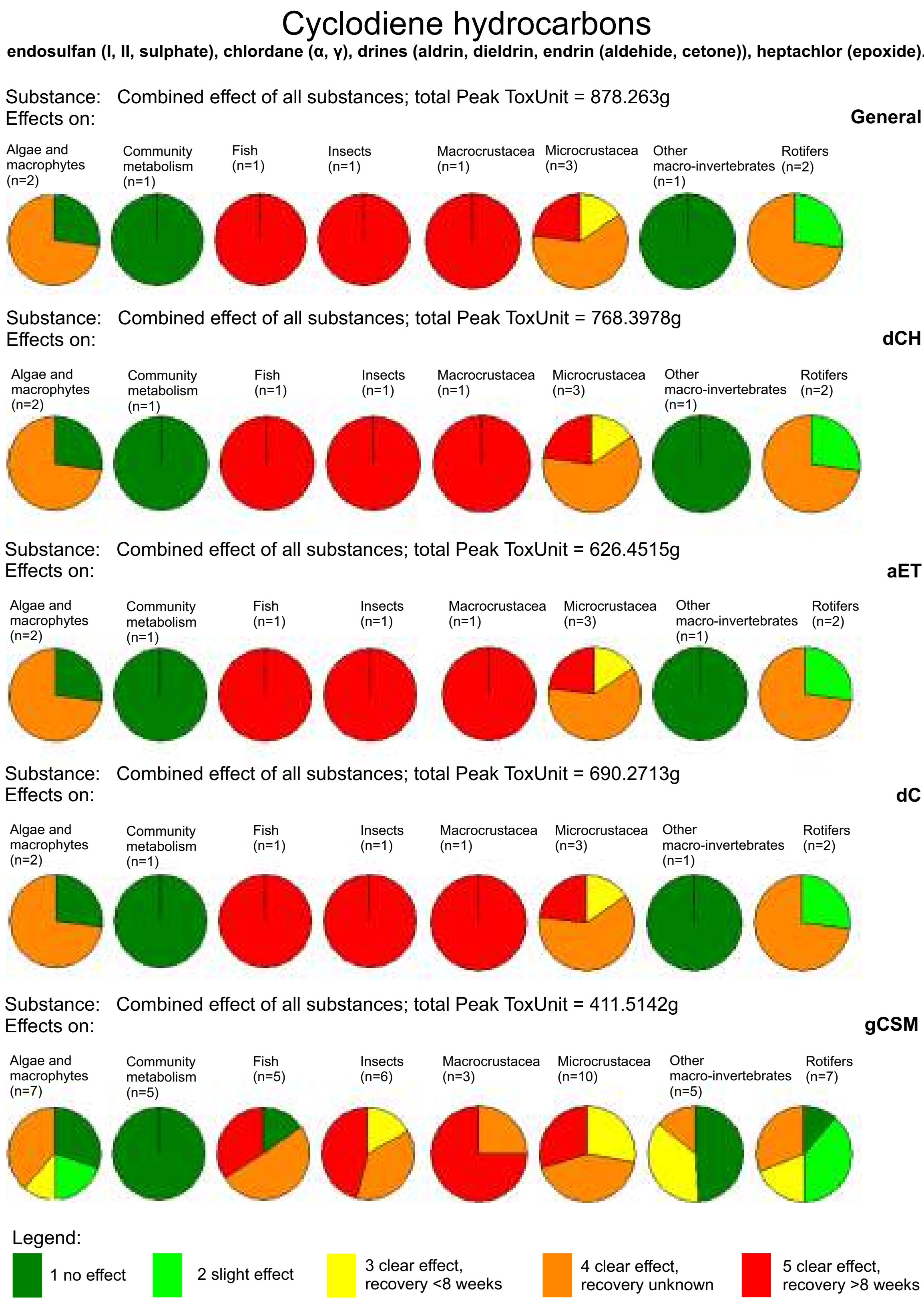

Fig. 2 Results of the PERPEST model of the evaluation of the ecological risks of the aquatic environment exposed to the group of cyclodiene hydrocarbons in general, and in the areas dCH, aET, dC and gCSM

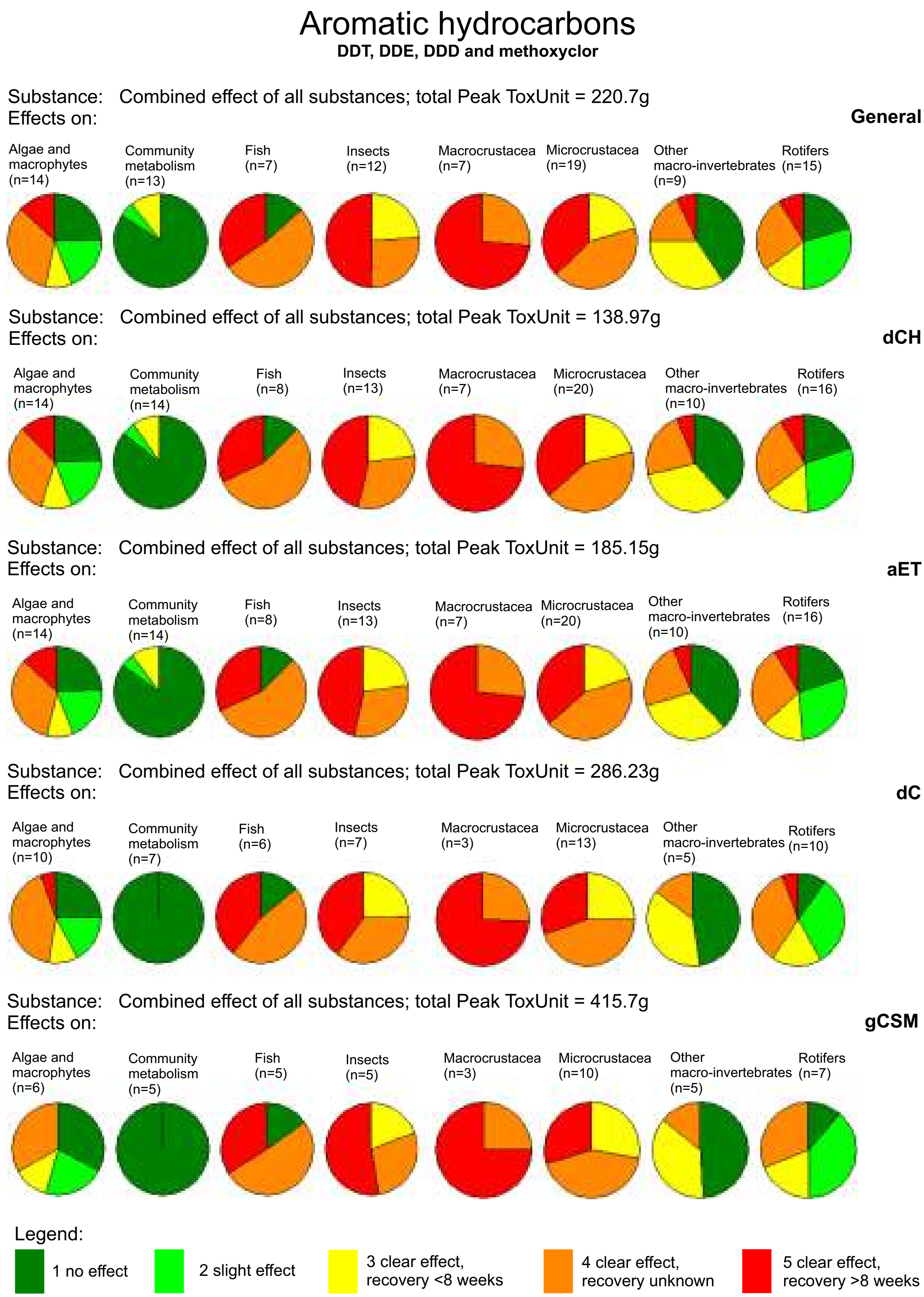

Fig. 3 Results of the PERPEST model of the evaluation of the ecological risks of the aquatic environment exposed to the group of aromatic hydrocarbons in general, and in the dCH, aET, dC and gCSM areas

on environmental risks of non-target OC species in aquatic environments (Ansara-Ross et al. 2008). The PERPEST model is very suitable for carrying out risk assessments of pesticide mixtures, because it takes into account direct and indirect effects, as well as interactions between species (Rämö et al. 2018); however, the data collected from the experiments used by the model, although providing more realistic results (Van Den Brink et al. 2006), is still few in some cases, such as crocodiles, making risk management difficult. Eco-epidemiological and toxicological approaches may provide the best model for predicting ecosystem health (Tavalieri et al. 2020). It is important to generate additional data from laboratory evaluations, bioassays, micro- or mesocosm experiments, chemical monitoring and biomonitoring, in order to refine the risks on potential effects and exposure in free-living non-target species (Ansara-Ross et al. 2008).

Conclusion

These types of studies are a priority to estimate globalized pollution and the need to contribute to solve the public and environmental health problem. Crocodiles are non-target organisms of OCs contaminants, which due to their size, longevity and predatory nature, are excellent bioindicators of exposure to environmental contaminants. The ecological risk assessment of the aquatic environment revealed that wild areas are high-risk sites for the health of crocodiles, especially for hatchlings that will feed on small, potentially contaminated organisms, placing the quality of endocrine and reproductive health of the species *C. acutus* at risk.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection by Ernestina Pérez-González, Ivan-Patricio Quintero-Borquez, César-Paul Ley-Quiñónez, Víctor-Manuel Salomón-Soto, and analyses were performed by Ernestina Pérez-González, María Nancy Herrera-Moreno; Ivan-Patricio Quintero-Borquez, Arturo Polanco-Torres, and Héctor-Abelardo González-Ocampo. The first draft of the manuscript was written by Ernestina Pérez-González and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The data that support the findings of this study are available on request from the corresponding author, [EPG].

Declarations

Ethical Approval Not applicable for that specific section.

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