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Determinants of Legacy Persistent Organic Pollutant Levels in the European Pond Turtle (*Emys orbicularis*) in the Camargue Wetland, France

Louisiane Burkart,[a] Anthony Olivier,[b] Olivier Lourdais,[c,d] Marion Vittecoq,[b,e] Gabriel Blouin-Demers,[f] Fabrice Alliot,[a] Clément Le Gac,[b] Nicolas Martin,[b] and Aurélie Goutte[a,]*

[a]École Pratique des Hautes Études, PSL Research University, UMR 7619 Sorbonne University, Paris, France
[b]La Tour du Valat, Research Institute for the Conservation of Mediterranean Wetlands, Arles, France
[c]Centre d'études biologiques de Chizé, CNRS, UMR 7372, Villiers en Bois, France
[d]School of Life Sciences, Arizona State University, Tempe, Arizona, USA
[e]MIGEVEC Laboratoire maladies infectieuses & vecteurs: écologie, génétique évolution et contrôle, UMR (CNRS/IRD 224/UM), Montpellier, France
[f]Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

Abstract: Many banned persistent organic pollutants (POPs) remain for decades in the aquatic environment and can have harmful effects on long-lived predators because of their high bioaccumulation and biomagnification potentials. We investigated the occurrence and levels of 18 polychlorinated biphenyls (PCBs) and 16 organochlorine pesticides in European pond turtles ($n = 174$) from April to July 2018 in the Camargue wetland, France. Although the Camargue was highly contaminated in previous decades, plasma occurrence and levels of POPs were very low: we were able to quantify only 3 of the 34 compounds we analyzed in >10% of the turtles. The burdens from POPs did not differ between males and females and were uncorrelated with sampling date and body mass. We observed differences in POP burdens between turtles from the 2 sampling sites. One possible explanation is that the sampling sites were in different agricultural hydraulic systems: plasma occurrence and levels were higher for PCB-52 and hexachlorobenzene in turtles captured in drainage channels and for PCB-153 at the site that receives irrigation. Finally, the occurrence and levels of PCB-153 in turtles increased with age, likely because of bioaccumulation and much higher exposure 20 to 30 yr ago than now. *Environ Toxicol Chem* 2021;40:2261–2268. © 2021 SETAC

Keywords: Reptiles; Polychlorinated biphenyl; Organochlorine pesticides; Delta wetlands

INTRODUCTION

In Europe, the production and use of persistent organic pollutants (POPs) have been prohibited or severely restricted since 2004 through the Stockholm Convention, but POPs still cause several environmental concerns today. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) remain in the environment for decades because of their slow biodegradation and because of improper handling of contaminated wastes. Levels of POPs in animals increase with age (Vives et al. 2005; Binnington and Wania 2014) and trophic level (Goutte et al. 2020). Exposure to POPs causes a wide range of adverse effects, including neurotoxicity, endocrine disruption, immune dysfunction, reproductive impairment, and developmental abnormalities, which may ultimately compromise survival and reproductive output and lead to population declines of wild vertebrates (Goutte et al. 2014, 2015; Salice et al. 2014).

Pollution, especially from industrial and agricultural discharges, is one of the major threats to freshwater ecosystems (Holt 2000; Dudgeon et al. 2006). Wetlands support an extremely rich biodiversity but are among the most transformed and threatened ecosystems of the world (Revenga et al. 2005), experiencing rates of population decline and species extinction far higher than those in forests, grasslands, and coastal ecosystems (Dudgeon et al. 2006). Deltas are often exposed to high water pollution from intensive agriculture (Kuenzer and Renaud 2012) and from discharge of contaminants in upstream waters. The Camargue, in the Rhône River delta, is the largest wetland in France and is of international importance under the Ramsar Convention (Conference of the Contracting

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* Address correspondence to aurelie.goutte@ephe.psl.eu
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Parties 1971); but it is heavily impacted by human activities (Cheiron et al. 2018). The contamination of water bodies by agricultural, industrial, and urban discharges has been well studied over the past decades (Comoretto et al. 2007). In particular, levels of PCBs in sediments of the Rhône River increase from upstream to downstream, reaching 417 µg/kg dry weight (for the sum of 7 PCB congeners: 28, 52, 101, 118, 138, 153, and 180 [Mourier et al. 2014]). Mean flux over the 2011 to 2016 period was 14 kg/yr for PCB-180 in suspended particulate matter at the outlet of the Rhône River (Poulier et al. 2019). Previous studies in the Camargue confirmed that both birds (Berny et al. 2002) and fish (Roche et al. 2002, 2003, 2009b) are exposed to OCPs and PCBs.

Freshwater turtles remain poorly studied in ecotoxicology, although they can provide useful information on local contamination because of their longevity (Campbell and Campbell 2002; El Hassani et al. 2019; Gaus et al. 2019), high trophic level, and low dispersal capacity (Châteauvert et al. 2015; Ming-ch'eng Adams et al. 2016). Moreover, as ectothermic vertebrates, turtles have a lower ability to metabolize pollutants than endothermic mammals and birds (de Solla 2015). The European pond turtle, *Emys orbicularis*, a long-lived (>40–80 yr) opportunistic predator (feeding on fish, amphibians, aquatic insect, gastropods, and crayfish) and scavenger (Ottonello et al. 2005; Ficetola and De Bernardi 2006; Ziane et al. 2020), is facing significant population declines as a result of multiple environmental alterations, including water pollution (Cheylan 1998). The European pond turtle is listed as "near threatened" on the International Union for Conservation of Nature's Red List of threatened species (Tortoise & Freshwater Turtle Specialist Group 1996). Pollution by trace metal elements has been studied in *E. orbicularis* (Namroodi et al. 2017; Guillot et al. 2018; Beau et al. 2019), but data are lacking on burdens of POPs.

We studied contamination levels in 2 populations of *E. orbicularis* in the Natural Reserve of the Tour du Valat in the Camargue, France. Several individuals were of known age owing to a capture–mark–recapture program initiated more than 20 yr ago (Olivier et al. 2010; Ficheux et al. 2014; Arsovski et al. 2018). The hydraulic system consists of irrigation canals originating from the Rhône River and of drainage canals, which receive various environmental contaminants from the Rhône River and agricultural plots, especially rice fields (Chauvelon 1996). In the present study, we assessed recent (2018) levels of OCPs and PCBs in plasma of the European pond turtle ($n = 174$). We also tested the effects of individual traits (mass, sex, and age) on POP burdens. We expected that older and larger individuals should have higher levels of POPs because of bioaccumulation and length of exposure.

Moreover, we compared POP burdens of turtles from 2 populations in different locations in the agricultural hydraulic system (irrigation vs drainage). We expected high levels of OCPs in turtles from the drainage site, as a possible consequence of the remobilization of pesticides from agricultural soils, and high levels of PCBs in turtles from the irrigation channel, as a possible consequence of the historical contamination. We acknowledge the limitations to our inferences about the possible causes of site differences in POP burdens because we had a single site in irrigation canals and a single site in drainage canals. Therefore, we are unable to disentangle site effects from hydrologic effects. Consequently, we only cautiously offer some plausible explanations for site differences.

MATERIAL AND METHODS

Sampling sites and capture

We conducted the study in the Natural Reserve of the Tour du Valat (43°30′N, 4°40′E; Figure 1) in France. We captured European pond turtles ($n = 174$) in canals and marshes from 24 April to 26 July 2018 by hand or with funnel traps (Olivier et al. 2010; Ficheux et al. 2014). We captured turtles at 2 sites: 1) irrigation canals and their associated marshland (site of Esquineau, $n = 126$), and 2) drainage canals of the Fumemorte basin and marshes filled with water by these canals (site of Faïsses, $n = 48$). The sex ratio was the same between the 2 sites (58.3% females at Faïsses and 60.3% females at Esquineau, Pearson's chi-squared test, $p = 0.95$).

Individual turtles were identified as part of a long-term capture–mark–recapture program with unique combinations of shallow notches on the marginal and nuchal scales (Olivier 2002; Olivier et al. 2010). Individuals were weighed and sexed by visual observation of the secondary sexual characteristics. The year of birth can be determined if the first capture occurred within the first 5 yr of life by counting the number of growth streaks (Castanet 1988). The long-term capture–mark–recapture program started in 1997, but a few dozens individuals were marked as adults in 1976. In our data set, 66 of the 174 individuals were of known age (5–26 yr old, corresponding to a first capture between 1997 and 2018), and some were first captured as adults in 1976, thus being considered more than 44 yr old. When turtles were first captured after their first 5 yr of life ($n = 108$), we determined stages of growth based on growth streaks on the plastron (Olivier 2002). By combining known ages and stages of growth, we assigned an age class to each turtle: 4 to 8 yr ($n = 20$), 9 to 13 yr ($n = 46$), 14 to 22 yr ($n = 54$), and 23 to 44+ yr ($n = 54$). Turtles were released on the same day at their capture site.

Blood sampling

Plasma is a good matrix to determine individual burdens of POPs because blood sampling is minimally invasive and because circulating levels of POPs in blood are significantly correlated to concentrations of POPs stored in fat (Keller et al. 2004; Dabrowska et al. 2006) and in liver, kidney, and muscle tissues (Van de Merwe et al. 2010). We collected blood samples (2 mL, always <1% of the turtle body mass) from the dorsal coccygeal vein (Keller et al. 2004; Innis and Knotek 2020) with a Terumo® syringe preimpregnated with heparin to prevent blood clotting during collection and equipped with a 25G needle. We then centrifuged the samples to separate the plasma from the red blood cells. Samples were stored at −18 °C until analysis in the UMR 7619 METIS of Sorbonne Université.

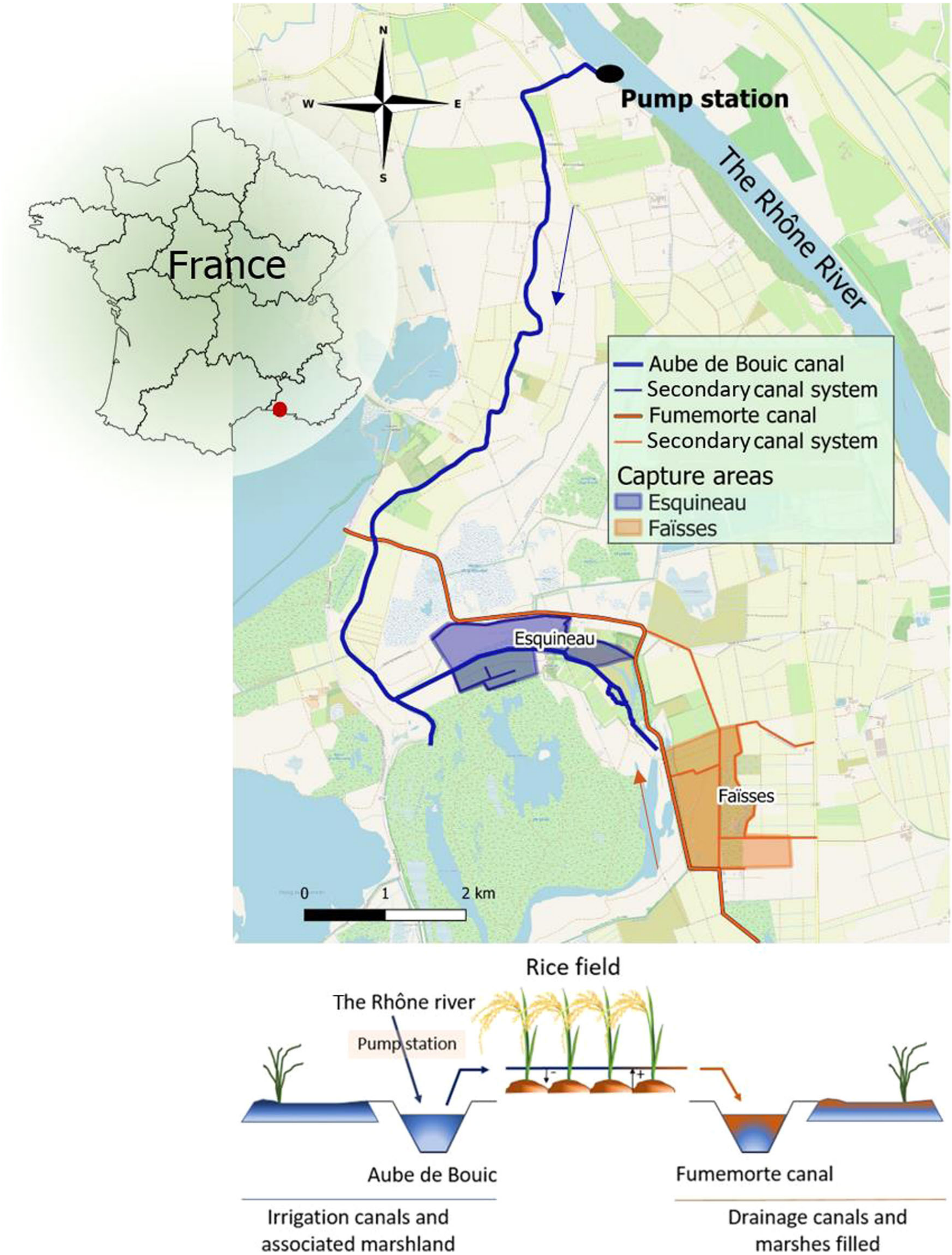

FIGURE 1: Sampling locations for European pond turtles in the Regional Nature Reserve of Tour du Valat, Camargue, France. The 2 capture locations are Esquineau (in blue), irrigated with water pumped from the Rhône River, and Faïsses (in orange), consisting of drainage canals of agricultural parcels.

Chemical analyses

We determined the levels of 17 OCPs (p,p′-dichlorodiphenyltrichloroethane [DDT] and metabolites, p,p′-dichlorodiphenyldichloroethylene [DDE] and p,p′-dichlorodiphenyldichlorethane [DDD]; pentachlorobenzene [PeCB]; hexachlorobenzene [HCB]; pentachloronitrobenzene [quintozene]; 4 isomers of hexachlorocyclohexane [α-, β-, γ- (lindane), δ-HCH]; aldrin; endrin; isodrin; telodrin [isobenzan]; heptachlor; heptachlor epoxide; heptachlor endo-epoxide), 7 marker PCBs (International Union of Pure and Applied Chemistry [IUPAC] nos. 28, 52, 101, 118, 138, 153, and 180), and 12 dioxin-like PCBs (DL-PCBs; IUPAC nos. 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) in the 174 plasma samples.

Samples were processed by solid phase extraction, using a hexane to dichloromethane ratio of 9:1, with a validated protocol (Tapie et al. 2011) that was adapted for plasma samples (see Supplemental Data for a detailed description of the procedure and method validations). PCBs and OCPs were analyzed using an Agilent 7890 A gas chromatograph coupled to a 7000 B triple quadrupole mass spectrometer system (Agilent Technologies). Recovery rates of compounds were assessed on replicate plasma samples of European pond turtles ($n=4$) with spiked solutions (100 ng of each compound). The repeatability of the method was assessed in terms of relative standard deviation of the recovery (Supplemental Data, Table S2). Recovery rates were not satisfactory (<75 or >125%) for isodrin, endrin, heptachlor epoxid, heptachlor-endo-epoxide, p,p′-DDD, PCB-77, and PCB-189 (Supplemental Data, Table S2); and these chemicals were thus not considered further.

Statistical analyses

We performed statistical analyses with R software, Ver 3.3.2 (R Development Core Team 2016). Only quantifiable POPs (i.e., values higher than the limit of quantification [LOQ]) in at least 10% of the samples were included in the statistical analyses. Because age and mass were highly positively correlated ($t=3.65$, $df=64$, $p=0.0005$) and females were significantly heavier than males ($W=474$, $p<0.0001$), effects of body mass, sex, and age were evaluated in separate analyses.

We used generalized linear models with a binomial distribution and a logit link function to test for the effects of sex, site, the interaction sex × site, sampling date, age, and body mass on contaminant occurrence. For each model, we used a backward elimination to progressively remove nonsignificant terms ($p>0.05$). For all analyses, model specification and validation were based on residual analysis.

The effects of sex, sampling site, date, age, and body mass on pollutant concentrations were tested by using all data and applying statistical methods for left-censored data to handle values below the LOQ (Helsel 2005). To do so, group comparisons and linear regressions were performed using Peto-Prentice tests and tobit models, respectively, with the function cendiff of the NADA package and the function tobit of the AER R-package (Shoari and Dubé 2018).

RESULTS

POP levels and occurrence

In 37 of the 174 samples, which represented 10.4% of turtles sampled in Faïsses and 24.4% of turtles sampled in Esquineau, all contaminant levels were below the LOQ. Eleven compounds were never detected at levels above the LOQ in the plasma samples: PeCB, lindane, quintozene, heptachlor, and the DL-PCBs: 118, 105, 114, 126, 156, 157, and 167. The following 7 compounds were detected at levels above the LOQ at least in one sample from Esquineau but not in samples from Faïsses: alpha-HCH, 44′-DDT, and PCBs 28, 101, 123, and 169 (Table 1). Overall, the contaminant concentrations in the plasma samples were very low (Table 1).

Influence of individual traits, sampling site, and date on POP levels and occurrence

Only 3 POPs (HCB, PCB-52, and PCB-153) occurred commonly, being quantified in >10% of individuals (Table 1).

TABLE 1: Concentrations of organochlorine compounds (ng/mL wet wt) in plasma samples of European pond turtles from the 2 sites, Esquineau and Faïsses[a]

Variable	LOQ	Esquineau ($n=126$) Mean ± SD	Max.	*df* (%)	Faïsses ($n=48$) Mean ± SD	Max.	*df* (%)
Organochlorine pesticides							
α-HCH	1.9	0.04 ± 0.29	2.39	2	0.00 ± 0.00	0.00	0
β-HCH	0.9	0.08 ± 0.39	3.22	5	0.02 ± 0.14	0.98	2
δ-HCH	1.3	0.03 ± 0.23	2.19	2	0.08 ± 0.37	2.22	4
HCB	0.2	0.02 ± 0.10	0.77	7	0.07 ± 0.18	1.00	19
Aldrin	1	0.01 ± 0.09	1.03	1	0.02 ± 0.17	1.16	2
Isobenzan	0.6	0.02 ± 0.11	0.79	2	0.17 ± 0.50	2.02	13
4,4′-DDE	1.1	0.04 ± 0.29	2.33	2	0.05 ± 0.34	2.30	2
4,4′-DDT	2.4	0.05 ± 0.41	3.62	2	0.00 ± 0.00	0.00	0
PCBs							
PCB-28	1	0.14 ± 0.53	3.16	8	0.00 ± 0.00	0.00	0
PCB-52	0.2	0.54 ± 1.30	10.18	36	0.73 ± 0.91	3.80	69
PCB-81	0.9	0.02 ± 0.21	2.10	2	0.02 ± 0.17	1.16	2
PCB-101	2.3	0.06 ± 0.48	4.82	2	0.00 ± 0.00	0.00	0
PCB-123	2.6	0.02 ± 0.23	2.61	1	0.00 ± 0.00	0.00	0
PCB-138	1.8	0.23 ± 0.94	7.28	8	0.07 ± 0.27	1.27	6
PCB-153	0.2	0.22 ± 0.34	1.57	46	0.13 ± 0.25	1.03	29
PCB-169	1.4	0.01 ± 0.17	1.86	1	0.00 ± 0.00	0.00	0
PCB-180	0.3	0.02 ± 0.10	0.72	2	0.03 ± 0.14	0.83	4

[a]Means and standard deviation are calculated considering all the data (i.e., levels > limit of quantification [LOQ] and data <LOQ by assigning them zero for value).
HCH = hexachlorocyclohexane; HCB = hexachlorobenzene; 4,4′-DDE = 4,4′-dichlorodiphenyldichloroethylene; 4,4′-DDT = 4,4′-dichlorodiphenyldichlorethane; PCB = polychlorinated biphenyl; SD = standard deviation.

TABLE 2: Results from a generalized linear model with binomial distribution and a logit link function model with persistent organic pollutant levels having an occurrence >10% as dependent variables, as a function of sex, sampling site, and their interaction, sampling date, age, and mass

	PCB-52		PCB-153		HCB	
	χ^2	*p*	χ^2	*p*	χ^2	*p*
Sex	1.699	0.192	0.162	0.688	0.433	0.511
Site	14.347	<0.001	4.488	0.034	4.052	0.044
Date	1.289	0.256	0.951	0.329	3.828	0.050
Sex × site	2.659	0.103	0.285	0.594	0.306	0.580
Age class[a]	4.870	0.182	10.603	0.014	0.840	0.840
Mass[a]	2.119	0.146	0.264	0.608	0.760	0.383

[a]Age class and mass were not tested in the same model because these 2 variables were highly correlated (see *Material and Methods*).
HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.

TABLE 3: Effects of sex, sampling site, age, date, and mass on pollutant levels were tested, using Peto-Prentice tests (group comparisons) and tobit models (linear regressions)

	HCB		PCB-52		PCB-153	
Peto-Prentice tests						
	χ^2	*p*	χ^2	*p*	χ^2	*p*
Sex	0.7	0.403	1.1	0.300	0.6	0.444
Site	5.2	0.022	12.3	<0.001	3.7	0.054
Age class	1.0	0.800	6.7	0.082	9.5	0.023
Tobit models						
	Wald	*p*	Wald	*p*	Wald	*p*
Mass	0.498	0.481	0.086	0.769	1.265	0.261
Date	2.814	0.093	0.191	0.662	0.607	0.436

HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.

Plasma occurrences of PCB-52 and HCB were significantly higher in turtles captured at Faïsses compared with Esquineau, whereas the occurrence of PCB-153 was higher in turtles captured at Esquineau (Tables 1 and 2). The occurrence of HCB slightly increased with sampling date (Table 2). Sex, mass, and age class did not explain POP occurrence in turtles, except for PCB-153 whose detection frequency was higher in older individuals (Table 2).

Levels of HCB and PCB-52 were significantly higher in turtles from Faïsses, whereas PCB-153 levels tended to be higher in turtles from Esquineau (Tables 1 and 3). Moreover, levels of PCB-153 were lower in the youngest turtles (Table 3 and Figure 2). Sex, mass, and date did not explain variation in levels of POPs among turtles (Table 3).

DISCUSSION

The aim of the present study was to characterize legacy levels of POPs (OCPs and PCBs) in the plasma of European pond turtles in the Camargue, France, and to determine whether these levels were a function of individual traits and habitat type. Plasma levels of POPs were low and often below LOQ (0.2–3.1 ng/mL). We found significant differences in burdens of POPs between turtles from the 2 sampling sites, and these differences could be attributed to the hydraulic system (drainage/irrigation), keeping in mind that we lack site replication to conclude firmly an effect of the hydraulic system. The occurrence and levels of PCB-153 were higher in older turtles.

The Rhône River has been historically contaminated, leading to an accumulation of POPs in sediments downstream (Mourier et al. 2014). Previous studies conducted on several animal taxa in the Camargue confirmed high exposure during the last decades, with high concentrations of PCBs in muscles of eels (*Anguilla anguilla*) fished between 1997 and 2000 (Roche et al. 2004) and in eggs of little egrets (*Egretta garzetta*) collected in 1996 (Berny et al. 2002). Levels of POPs were low in the pond turtle in Camargue, which could indicate a decline in exposure to legacy POPs for wild species in the Camargue. Although a longitudinal study of the same species at the same locations would be required to test this hypothesis, decreasing PCB concentrations have been observed in Rhône River sediments in previous decades (Liber et al. 2019), and water analyses conducted by the National Nature Protection Society have not detected PCBs and OCPs in the Fumemorte canal since 2011 (Cheiron 2019). Moreover, organochlorine contamination across food webs tended to diminish through time in the Vaccarès lagoon (Roche et al. 2009b), into which the Fumemorte canal flows. Exotic red swamp crayfish (*Procambarus clarkii*), the main prey of European pond turtles in the Camargue (Ottonello et al. 2005), were not contaminated by POPs in 2019 (i.e., <LOQ, with LOQ ranging 0.9–1.98 ng/g dry wt; A. Goutte, EPHE, PSL Research University, unpublished data).

One of the first studies looking at the plasma concentration of POPs in turtles documented high PCB and OCP levels in snapping turtles (*Chelydra serpentina*) in Ontario, Canada, in 2001 to 2004 (Letcher et al. 2015): plasma concentrations of OCPs (sum of 17 contaminants) ranged from 0.2 to 236 ng/g wet weight, and the most abundant pesticide was p,p′-DDE (mean ± standard error 27 ± 6 ng/g wet wt). In our study,

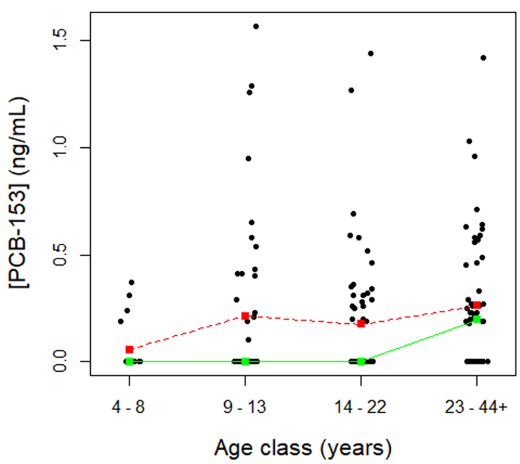

FIGURE 2: Plasma polychlorinated biphenyl 153 levels in European pond turtles increase with age class. Each point represents an individual. Red and green squares correspond to mean and median levels for age class, respectively. Age class was determined based on growth streaks on the plastron (see *Material and Methods*). PCB = polychlorinated biphenyl.

plasma concentrations of POPs (sum of 16 contaminants) were much lower, ranging from 0 to 2 ng/g wet weight, and p,p′-DDE levels did not exceed 2.3 ng/g wet weight. Snapping turtles are freshwater turtles with similar feeding habits to those of European pond turtles; snapping turtles also consume plant and animal matter, including aquatic invertebrates, fish, frogs, and reptiles (Ernst et al. 1994). Other studies of turtles documented plasma concentrations of POPs comparable to those in our study; for instance, for PCB-153 and HCB in another freshwater species, the western pond turtle (*Actinemys marmorata*), in Sequoia National Park, USA, in 2012 (Meyer et al. 2016), as well as in marine turtles such as loggerhead sea turtles (*Caretta caretta*) in the eastern Atlantic Ocean in 2011 and 2012 (Bucchia et al. 2015) and in green turtles (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) in Cape Verde in 2009 to 2011 (Camacho et al. 2014).

In terms of occurrence of POPs, our results in European pond turtles were low compared to other studies (Bucchia et al. 2015; Meyer et al. 2016). The occurrences of DL-PCBs in 8% of European pond turtles, as well as p,p′-DDE in 2% of individuals, were much lower than those found in plasma of loggerhead sea turtles, which ranged from 63% in the Atlantic Ocean to 100% in the Adriatic Sea for DL-PCBs and 100% for p,p′-DDE (Bucchia et al. 2015).

Despite low detection frequencies and levels, we found differences in POP burdens between turtles from the 2 sampling sites. Turtles living in the drainage waters of Faïsses exhibited significantly higher occurrence and levels of HCB compared to turtles from Esquineau, likely because of the remobilization of trapped OCPs in soils. On the other hand, the occurrence and levels of PCB-153 were higher in turtles from Esquineau, a site receiving water from irrigation channels. A previous study has documented higher concentrations of PCBs in the aquatic fauna in irrigation canals compared to the drainage canals downstream of rice fields (Roche et al. 2009a). In contrast to the situation for PCB-153, concentrations of PCB-52 were higher in turtles in Faïsses, which may be due to the latter PCB's lower sedimentation rate associated with its lower molecular weight and greater solubility as a result of a lower level of chlorination (Gong et al. 1998; Alkhatib and Weigand 2002). The processes of deposition and release of PCBs from the irrigation part to the drainage part of the canals need to be further studied, and other populations of pond turtles in the 2 types of hydraulic systems should be studied to confirm this pattern.

The occurrence and levels of PCB-153 were lower in the youngest turtles (4–8 yr old), which could be attributed to a shorter exposure period. This is a recalcitrant chemical with a high hydrophobicity (high octanol–water partition coefficient), high resistance to metabolic transformation, and slow respiratory elimination through air exhalation (high octanol–air partition coefficient). It is also more prone to bioaccumulation than is the case for many other PCB congeners (Kelly et al. 2007), especially in long-lived air-breathing predatory species (Rowe 2008). It is also possible that there is a dietary shift during growth in pond turtles, leading to the consumption of higher–trophic level prey with age; and this diet would be more PCB-contaminated as a result of biomagnification. This hypothesis, however, is not consistent with dietary studies of *E. orbicularis* in Camargue, which instead documented a shift to a more herbivorous diet with age based on prey identification in fecal samples (Ottonello et al. 2005) or no difference in the proportion of plants, invertebrates, and vertebrates in the diet based on metabarcoding (Ducotterd et al. 2020).

We did not detect differences in POP levels between male and female pond turtles, despite males often being more contaminated than females in other turtle species (Guirlet et al. 2010; Bangma et al. 2019; Lambiase et al. 2021) because of a transfer of pollutants from the mother to the eggs through vitellogenesis (Moss et al. 2009; de Solla 2010).

CONCLUSION

The concentrations of POPs were very low in European pond turtles, probably much lower than they were several decades ago in wild vertebrates in Camargue, France. The occurrence and concentrations of POPs may have been influenced by the hydraulic system where the turtles were captured and by turtle age. Further studies are needed to assess the impact of current contaminants on biodiversity, especially for aquatic top predators such as turtles.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5077.

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Ethics Statement—The present study was performed in accordance with laws relative to the capture, transport, and experiments on *E. orbicularis* (DREAL permit CERFA_13616-01), and all procedures were approved by an independent ethical committee (APAFIS 17899-201812022345423 v2).

Disclaimer—The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

Author Contributions Statement—A. Olivier, L. Burkart, C. Le Gac, and N. Martin conducted the fieldwork and collected the blood samples; L. Burkart and F. Alliot conducted the laboratory work to prepare plasma samples for analysis with gas chromatography and tandem mass spectrometry; L. Burkart, O. Lourdais, A. Goutte, A. Olivier, G. Blouin-Demers, and M. Vittecoq conceived and coordinated the study and participated in data analysis and in writing the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (aurelie.goutte@ephe.psl.eu).

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