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Review

Toxicological effects of polychlorinated biphenyls (PCBs) on freshwater turtles in the United States

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HIGHLIGHTS

- PCBs cause increased deformity and mortality rates in juvenile freshwater turtles.
- Toxicological effects are depending on the PCB concertation and configuration.
- The common snapping turtle is more robust than diamondback terrapins and red-eared sliders.
- Freshwater turtles can serve as bioindicators due to strong site fidelity and longevity.

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ABSTRACT

Prediction of vertebrate health effects originating from persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) has remained a challenge for decades thus making the identification of bioindicators difficult. POPs are predominantly present in soil and sediment, where they adhere to particles due to their hydrophobic characteristics. Animals inhabiting soil and sediment can be exposed to PCBs via dermal exposure while others may obtain PCBs through contaminated trophic interaction. Freshwater turtles can serve as bioindicators due to their strong site fidelity, longevity and varied diet. Previous research observed the health effects of PCBs on turtles such as decreased bone mass, changed sexual development and decreased immune responses through studying both contaminated sites along with laboratory experimentation. Higher deformity rates in juveniles, increased mortality and slower growth have also been observed. Toxicological effects of PCBs vary between species of freshwater turtles and depend on the concertation and configuration of PCB congeners. Evaluation of ecotoxicological effects of PCBs in non-endangered turtles could provide important knowledge about the health effects of endangered turtle species thus inform the design of remediation strategies. In this review, the PCB presence in freshwater turtle habitats and the ecotoxicological effects were investigated with the aim of utilizing the health status to identify areas of focus for freshwater turtle conservation.

Contents

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1. Introduction to polychlorinated biphenyls and turtles

As ecological engineers, humans are constantly modifying and shaping their environment to better suit their needs (Jones et al., 1994). Unintended side effects often stem from these changes such as the introduction of chemicals into the environments. Persistent organic pollutants (POPs) are not naturally present in an ecosystem and can induce harmful effects (Bergeron et al., 1994; Danzo, 1997). Polychlorinated biphenyls (PCBs) are a group of POPs that include chemicals such as Aldrin, Heptachlor, Polychlorinated dibenzo-*p*-dioxins and Polychlorinated dibenzofurans as defined by the Stockholm Convention (United Nations Industrial Development OrganizationU, 2015). They have been utilized in industrial processes and products such as coolants, transformer oil and flame retardants in the 1900's until their production was banned in the United States almost 40 years ago (Kimbrough, 1995). Despite the ban, they persist in soil and sediment due to their hydrophobic characteristics and very stable chemical structure characteristics (Kjellerup et al., 2008, 2012). There are existing products and equipment containing PCBs still in use such as paints, adhesives, fire retardants, hydraulic systems and transformers causing risks of contamination for years to come due to resistance to biological, physical and chemical degradation (Gill et al., 1997; Luthy et al., 1997; Glass et al., 2005).

The lipophilic nature of PCBs allows them to persist in the adipose tissues of animals and humans and thus bioaccumulate (Kumar et al., 2014a, 2014b). This can have detrimental effects on long-lived species such as humans, turtles and other amniotes (Bergeron et al., 1994; Hsu et al., 2005). Once PCBs are ingested their association with lipophilic tissue and breastmilk can cause endocrine disruption, cancer, and increased risk of fetal developmental problems (Wolff and Weston, 1997; Cogliano, 1998).In addition, they produce harmful neurological effects in children as well as neuropsychological degradation in the elderly (Safe, 1992; Kannan et al., 1998). The effects of PCBs have been described in a range of animal systems such as striped bass, mussel, mallard, and seal (Hong et al., 1998; Gale et al., 2002; Beckett et al., 2008). In these aquatic systems, bottom feeders and other organisms such as turtles and fish ingest and accumulate PCBs and other xenobiotics, resulting in bio magnification in the food chain. Furthermore, PCBs have been found to cause morphological deformities, when binding to the cytosolic aryl hydrocarbon receptor in vertebrates (Olafsson et al., 1987; Bishop et al., 1998). Longevity, maternal effects and endocrine responses parallel turtles and humans for studying the effects of PCBs in vertebrate systems, which makes turtles an interesting and developmentally relevant bioindicator and model organism.

This review provides an overview of the effects of PCBs in freshwater turtles of North America. This topic is important to examine since parallels can be drawn between turtles and other vertebrates with regards to the consequences of exposure to environmental stressors such as persistent organic pollutants including PCBs. As turtles are an imperiled taxa, understanding all environmental stressors can provide a framework for effective conservation.

2. Effects of PCBs on bone development

One way PCBs manifest their toxic effects on turtles is through decreasing the integrity of the skeletal structure development. Studies have shown that PCB-126 (3,3′,4,4',5-CB) is one of the most toxic towards avians and reptiles (Hong et al., 1998). The effects of PCB-126 in American kestrels (*Falco sparverius*) showed a dose-dependent liver enlargement, coagulative necrosis, increased plasma enzyme activities, decreased bursae and spleen weight as well as decreased bone growth and osteoblast activity (Hoffman et al., 1996, 1998). Not unlike avians, turtles exhibit similar effects, here most tangibly investigated through the development of skeletal structure. Multiple studies have shown that PCB exposure effects juvenile turtle bone development (Ford and Holliday, 2004, 2005; Holliday and Holliday, 2012), where for example the bone mineral density decreased in juvenile *Malaclemys terrapin* exposed to PCB-126 (Ford and Holliday, 2004). In addition, 8-month old turtles that received 10 μg/g PCB-126 by injectionhad significantly smaller carapace lengths ($p < 0.0001$) and mass ($p < 0.0001$) compared to untreated turtles (Holliday et al., 2009; Holliday and Holliday, 2012). PCB-exposed turtle skulls were examined for organic content. Here, specimens had more juvenile characteristics such as larger frontal-parietal fontanelles and more weakly developed nuchal crests than unexposed age-matched individuals (Holliday and Holliday, 2012). Most significantly, PCB-exposed turtles exhibited lower femoral bone density and size with a larger void area compared to unexposed specimens (Holliday and Holliday, 2012). Takentogether, these experiments point to developmental delays due to PCB exposure (Holliday et al., 2009; Holliday and Holliday, 2012). While young unexposed turtles often die, exposure to PCBs may result in even further decreased fitness (Janzen, 1993; Janzen et al., 2000a, 2000b). Additionally, if females exposed to PCB-126 are smaller, they may produce smaller eggs or clutches (Holliday and Holliday, 2012; Holliday et al., 2009; Iverson and Smith, 1993; Rowe, 1994a, b; Spencer, 2002).

Exposure studies provide valuable information on how PCB-126 acts on turtle development and growth (Holliday and Holliday, 2012). It has been suggested that PCB-126 affects the aryl hydrocarbon and osteoblast estrogen receptors as an agonist, which can change bone formation (Bonefeld-Jorgensen et al., 2001; Ford and Holliday, 2004; Holliday and Holliday, 2012). Bone structure deformities can also occur as a stress response, even inducing bone cell death since more effort was spent on stress instead of growth (Holliday and Holliday, 2012). Sub-lethal effects of PCB-126 exposure have been linked to stress responses in other vertebrates such as birds and mammals (Macek, 1969; Nordin et al., 1994; McKenna et al., 1999; Rudiger et al., 2003; Eisenreich et al., 2009; Lattin et al., 2014). Both stress and PCB exposure can decrease the immune function thus further hindering growth and development (Yu et al., 2012; Kumar et al., 2014b) and thereby delay growth through bone marrow thus resulting in negative consequences for turtles.

3. PCB influence on environmentally sex determined species

An increased understanding of the effects of POPs on reptiles is especially important for species which have temperature dependent sex determination. Many common freshwater turtles, such as the painted turtle, red-eared slider, and the common snapping turtle have a temperature sex determining mechanism (TSD) (Bull, 1980). Given that PCBs and PCB mixtures can cause effects at small doses, even trace amounts can impact embryonic development including sex reversal (Bergeron et al., 1994; Crews et al., 1995). PCBs can influence sex in TSD species and evaluation of the chemical effects has remained a major focus of research in the past decades. Many studies hypothesize that eggs incubated at male-producing temperatures develop into female hatchlings when exposed to PCBs (Bull, 1980; Crews et al., 1991, 1995; Bergeron et al., 1994; Willingham and Crews, 1999, 2000; Gale et al., 2002; Matsumoto et al., 2014).Several PCBs (individual congeners and Aroclor mixtures) or PCB-like compounds (hydroxylated PCBs), have been examined for sex changing effects in turtles (Bergeron et al., 1994; Crews et al., 1995; Wibbels and Crews, 1995; Schnars et al., 2011). The effect of hydroxylated metabolites of PCBs,

2′,4′,6′-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol on red-eared slider turtle eggs showed that environmentally relevant concentrations of 100 μg of PCB per egg (9 ppm) had a significant effect on the sex of hatchlings (Bergeron et al., 1994; Crews et al., 1995). Exposure to 2′,4′,6′-trichloro-4-biphenylol caused sex reversal at male-producing temperatures for hatchlings. When 2′,4′,6′-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol were combined and administered at only 10 μg per egg, an increase in hatchlings developing ovaries at male producing temperatures was observed (Bergeron et al., 1994; Crews et al., 1995). Individually, the congeners required a dose 10 times higher to cause a similar effect, implying a synergistic effect between the two compounds (Bergeron et al., 1994). More recently, the direct metabolite of PCB 187, hydroxylated PCB 4-OH-CB187 was found in the plasma of male snapping turtles in areas of contamination in Ontario, Canada (Letcher et al., 2015). However, these more active hydroxylated PCBs do not always reflect actual PCB concentrations within the turtle, as OH-PCBs are dominated by OH-CB187 (82%) and PCB congeners −138 (21%), -153 (19%) and −180 (13%), perhaps due to differences in water solubility (Letcher et al., 2015). Therefore, the tested PCBs in Bergeron et al. (1994) and Crews et al. (1995) may not be reflective of actual PCB loads on turtles in the wild. In contrast, a study by Willingham and Crews (1999) showed that exposure to 0.53 ppm of the PCB mixture Aroclor 1242 resulted in a significantly higher rate of turtle feminization than the positive estradiol control (Willingham and Crews, 1999). As with other studies, the synergistic effects of a PCB mixture produced significant negative effects upon a developing reproductive system. An overview of studies showing the developmental effects on turtles can be seen in Table 1.

In contrast to Willingham and Crews' (1999) Aroclor mixture, some PCBs do not exert a full effect upon sex determination development, resulting in intersex turtles (Bergeron et al., 1994; Wibbels and Crews, 1995; Matsumoto et al., 2014). Individual PCB congeners can cause the development of intersex turtles by influencing the natural TSD process (Crews et al., 2000). The natural occurrence of intersex turtles in pristine environments is unknown but likely low due to reduced survival rates thus the exposure to PCBs and other estrogen-like compounds seems to be a major cause, resulting in intersex rates up to 25% (Wibbels and Crews, 1995). Bergeron et al. (1994) observed that female turtles developed testes, but the Müllerian ducts were still presenting intersex turtles. Some hatchlings displayed ambiguous sexual organ phenotypes such as round, vascularized testes with degenerating oviducts, ovaries with malformations or absence of one or more oviducts (Matsumoto et al., 2014). At female-producing temperatures, oviduct malformations also occurred in the presence of a PCB mixture between 4-hydroxy-2′,4′,6′-trichlorobiphenyl and 4-hydroxy-2′,3′,4′,5′-tetrachlorobiphenyl, showing that exposure to PCBs negatively influence both sexes (Matsumoto et al., 2014).

More recently, gene expression profiles for sex determining genes has been characterized under the influence of hydroxylated PCB congeners (Matsumoto et al., 2014). Through RNA and methylation analysis, the effects of 4-hydroxy-2′,4′,6′-trichlorobiphenyl and 4-hydroxy- 2′,3′,4′,5′-tetrachlorobiphenyl were examined on *Trachemys scripta* embryos. In feminized males, expression patterns of aromatase by *FoxL2*, normally suppressed at male producing temperature, increased during this condition (Matsumoto et al., 2014). Additionally, expression of *Dmrt1* and *Sox9*, typically associated with testicular development, were suppressed during this time (Matsumoto et al., 2014). This is important since these factors are also expressed in humans and play a role in human developmental pathways (De Santa Barbara et al., 1998; Cheng et al., 2006; Duffin et al., 2009). Interestingly, epigenetic DNA methylation in embryos did not change at male producing temperatures under the effects of hydroxylated PCBs (Matsumoto et al., 2014). Therefore, PCBs likely influence gene expression through direct pathways and not epigenetic factors.

Considering these results showing that hydroxylated PCBs were important in determining the sex of turtles, the long-term effects of sex ratio change should be evaluated for turtle populations and diversity. If the ratio of female turtles to male turtles increases compared to historical values due to the presence of POPs, it can have an effect on the local populations' behavior (Janzen, 1994; Morjan, 2003; Stewart and Dutton, 2014). A consequence can be increased intra-species competition or a change in breeding behavior since each breeding male will have an increased impact with the amplified number of females available. Fewer males than expected may mean each male has a greater genetic impact on future generations compared to previous males due to the ability for some to mate with more females. While a sex ratio bias skewed towards females might be possible with the onset of climate change and increased temperatures in addition to environmental contaminants, developmental plasticity and nesting behavior might mitigate this effect (Bull et al., 1982; Mitchell et al., 2013). In addition, females are more likely to be hit by vehicles while crossing roadways, which would cause a skewed male bias (Reid and Peery, 2014). More studies are needed to fully understand the effect of POPs including PCBs on the sex-ratio determination of organisms with TSD.

4. Developmental effects *in ovo* and in hatchlings

PCBs can adversely affect ontogenetic development in animals such as rats, mice, and amphibians and turtles are no exception (Li and Hansen, 1996; Qin et al., 2003). A number of studies examined wild common snapping turtles for more than a decade from areas of concern in the northern USA and Canada (Bishop et al., 1991, 1998; Struger et al., 1993; Ashpole et al., 2001, 2004; de Solla et al., 2008). The studies compared congener-specific PCB concentrations in turtle eggs to hatchling deformity rates. Bishop et al. (1998) documented the presence of more than 42 PCB congeners in snapping turtle eggs ranging from 1.1 ng per kg w.w. (Lake Sasajewun) to 2,972 ng per kg (Akwesasne), for PCB-189 and PCB-126, respectively (Bishop et al., 1998). The most common congeners were PCB 37, 81, 77, 126, 169 and 189. Within the Great Lakes region, ten PCB congeners (PCB-49, 60, 66, 70, 99, 105, 118, 141, 151 and 200) were related to the occurrence of deformities in turtles (Bishop et al., 1991, 1998). Specifically, PCB-60 (2,3,4,4′ PCB) and PCB-200 (2,2′,3,3′,4,5,6,6' -PCB) were correlated with increased abnormalities above the reference site abnormality baseline (Bishop et al., 1991, 1998). This was supported by other studies from the Great Lakes area that showed that high concentrations of PCBs often lead to hatching deformities in snapping turtles (Bishop et al., 1991; de Solla et al., 2008). These abnormality rates within the areas of concern were significantly different compared to reference sites (de Solla et al., 2008). Chemicals other than PCBs such as polychlorinated dioxins (PCDDs) and polychlorinated furans (PCDFs) were also detected at elevated levels at these sites (Bishop et al., 1998). Correlations between PCDDs and PCDFs concentrations and abnormality rate were also found (Bishop et al., 1998). A correlation between developmental deformities and sites with substantially higher POP rates exists (Bishop et al., 1991).

In the hatchlings, the most common abnormality was a deformed tail (45%) followed by one additional/missing scute in the carapace (32%). Other deformities in order of commonality included one missing/additional claw, limb deformities, grossly unabsorbed yolk sac, recessed lower jaws or incomplete development of the carapace (Bishop et al., 1998). Some of these deformities interrupted the turtle development so severely that death

Table 1
Overview of laboratory exposure studies and evaluation of field samples showing a wide array of developmental effects on freshwater turtles.

Chemical	Formula	Concentration	Species	Effect	Environment	Reference
PCB-126	3,3′,4,4′,5-CB	NA	*Malaclemys terrapin*	Reduced bone density. Increased density of epipterygoid, prootic and facial skeleton.	Laboratory	Ford and Holliday, 2005
PCB-126	3,3′,4,4′,5-CB	0 – 30 ppt	*Malaclemys terrapin*	Reduced carapace length and mass. Lower hematocrit and CO_2 output.	Laboratory	Holliday et al., 2009
PCB-126	3,3′,4,4′,5-CB	20μg/g	*Malaclemys terrapin*	Developmental delay. Low femoral bone density and size. Reduced carapace length.	Laboratory	Holliday and Holliday, 2012
PCB-126	3,3′,4,4′,5-CB	0.05–5 μg/g egg	*Trachemys scripta*	Toxin effect on embryos. Chemicalsremained in the yolk. Increased rate of intersex hatchlings.	Laboratory	Gale et al., 2002
Aroclor 1254 and 1260	Mixture of PCB congeners	0–10 ppm	*Chelydra serpentina*	Reduced embryosurvival.	Laboratory	Schnars et al., 2011
Compounds F and G	2′,4′,6′-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol	10 ppm 10–190μg/ egg	*Trachemys scripta*	Feminization of males at male-producing incubation temperatures. Synergy of Compounds F and G.	Laboratory	Bergeron et al., 1994
Aroclor 1242	Mixture of CB congeners	0.53 ppm	*Trachemys scripta*	Feminization of males at male-producing incubation temperatures. Synergy of Compounds F and G.	Laboratory	Willingham and Crews, 1999
Aroclor 1242	Mixture of PCB congeners	0.53 μM 0.0848 ng/g egg	*Trachemys scripta*	Sex ratio shift towards female dominance. Synergy of chemical mixtures. Armoatase brain activity affected.	Laboratory	Willingham and Crews, 2000
PCB-F PCB-G	4-hydroxy-2; ,4′,6′-trichlorobiphenyl 4-hydroxy-2′,3′,4′,5′-tetrachlorobiphenyl	100–200μL per egg	*Trachemys scripta*	Sex ratio shift towards female dominance. Increased rate of intersex hatchlings. Activation of ovarian markers. Suppression of testicular markers.	Laboratory	Matsumoto et al., 2014
PCB-31, 28, 52, 60, 66, 70, 87, 95, 99, 101, 105, 110, 138, 118, 151, 153, 170, 174, 180, 182, 187, 190, 194	Multiple	2850–3320 ng/g wet weight	*Chelydra serpentina*	Higher rate of unhatched eggs. Higher rate of deformities.	Field samples	Bishop et al., 1991
PCB-55, 66, 74, 90, 105, 128, 130, 131, 135, 137, 138, 141, 144, 146, 147, 151, 153, 156, 157, 158, 170, 171, 172, 174, 177, 178, 180, 182, 183, 187, 194, 195, 196, 200, 201, 203	Multiple	0.057 –4.76 mg/kg wet weight	*Chelydra serpentina*	Increased concentrations in eggs.	Field samples	Struger et al., 1993
CB -28, 31, 42, 44, 47, 49, 52, 60, 64, 66, 70, 74, 87, 97, 99, 101, 105, 110, 118, 128, 129, 137, 138, 141, 146, 149, 151, 153, 158, 170, 171, 172, 174, 180, 182, 183, 185, 194, 200, 201, 203, 206	Multiple	1.1–2972 ng/ kg wet weight	*Chelydra serpentina*	Increased rate of hatchling deformities.	Field samples	Bishop et al., 1998
34 PCB congeners	Multiple	0.1 ng/g	*Chelydra serpentina*	Decreased hatching rate. Increased rate of deformities.	Field samples	de Solla et al., 2008

occurred. Hatchlings with head and toe deformities were more likely to infer other deformities, unlike hatchlings with only scute deformities (de Solla et al., 2008). In addition, a decrease in immune function was suspected which can further weaken individuals (Rice and Schlenk, 1995; Keller et al., 2006; Yu et al., 2012). While a decreased immune function may not be as prohibitive as extreme deformities, it can still have an effect upon turtles and causing reduced long-term survival. Furthermore, cognitive changes caused by PCB exposure can affect turtle behavior and survival (Crews et al., 1995; Willingham and Crews, 2000). Exposure to PCBs significantly increased the aromatase levels in the brain of the red-eared slider during development, which could affect selection against these feminized males (Willingham and Crews, 2000; Matsumoto et al., 2014).

These results, taken together with the sex-determination results suggest that PCBs can influence the phenotypes of certain turtle

species. Turtles from Lake Ontario had the highest abundance of deformities and abnormality rates and these correlated with measured PCB concentrations (Bishop et al., 1991, 1998). A majority of the observed deformities were non-lethal such as kinked tails and additional scutes in the adult population (Ernst, 2001; Velo-Anton et al., 2011). As turtles are a long-lived species that rely on multiple reproduction efforts to replace a reproducing adult, the decrease in juvenile recruitment may eventually be detrimental if continued over a long period of time (Heppell et al., 1995).

5. Turtle population and ecosystem effects

While studying the effects of PCB exposure at an individual level, the toxicological effects of PCBs on the overall ecosystem may be overlooked. The turtles are influenced by abiotic, human, and ecosystem interactions while constant POP exposure might directly or indirectly add to the reasons for the population decline (Gibbons et al., 2000). Through trophic interactions turtles can ingest PCBs which then become bioavailable to embryos via maternal transfer, but this can also cause increased external uptake, since PCBs have been detected throughout the entire turtle lifecycle. Thus turtles are an example of organisms that can be used as a bioindicator to exemplify important environmental effects throughout the ecosystem (see Fig. 1).

Exposure to PCBs can directly decrease the health of individual turtles. Increased predation on weakened PCB-exposed hatchlings and adults can cripple a population as turtles rely on multiple nesting seasons to replenish a population. It was estimated that an increase in adult mortality of 0.1% each year would reduce a snapping turtle population by 50% in 20 years (Congdon et al., 1994). A smaller female body size due to PCB-induced growth delays and reduced sizes of hatchlings can have detrimental consequences for individual fecundity since smaller sized hatchlings have reduced survival rates (Janzen, 1993; Rowe, 1994b; Beckett et al., 2008; Holliday and Holliday, 2012). In addition, severe PCB-induced abnormalities further reduce the cohorts due to weakened individuals dying directly or more easily becoming prey due to indirect effects. Increased abnormality rates might also be indicative of internal abnormalities and decreased immune function which can become sub-lethal stressors for individual turtles (Bishop et al., 1998; Eisenreich et al., 2009; Yu et al., 2012). The effect of minor abnormalities has yet to be examined in population studies. Taken together, exposure to PCBs adds an additional challenge to an already long list of factors causing reduced health and decline of turtles.

The toxicological effects of PCBs extends to the entire ecosystem. While it is hard to predict change based on a few studies given the diversity of freshwater turtle species in North America, microcosm experiments have documented the importance of turtles on an ecosystem. A study by Lindsay et al. (2013) described ponds with and without the introduction of turtles and observed that turtles significantly increased pH, conductivity, sediment accumulation, leaf litter decomposition rates, and increased the invertebrate biodiversity (Lindsay et al., 2013). The activity of turtles also increased the biogeochemical cycling rates within the turtle-inhabited pond, providing an environment which then supported more species thus increasing the overall biodiversity (Lindsay et al., 2013). As turtles are mobile, they have the potential to carry PCBs from one site to another through ingesting contaminated nutrients and becoming prey in a different location (Zhang et al., 2001; Baker et al., 2009). Eggs with contaminated yolk may be ingested by predators. These are all important biogeochemical cycling processes promoted by turtles. Far-reaching effects of this bioaccumulation may influence snapping turtle fisheries and consumption; to this end warnings against fat, liver, and eggs are issued (Ontario Ministry of the Environment and Climate Change, 2015).

6. Conclusion and future perspectives

There is a lack of long-term studies with rigorous follow-up documenting the effects of PCBs on turtles at individual levels. As long-lived animals, a 20 year picture would be the minimum needed to obtain a wide-ranging understanding of how PCB exposure impacts the environment and this the turtle life cycle. While studies show that adult turtles contain PCBs and other contaminants (Dabrowska et al., 2006; Kelly et al., 2008), there is a significant gap in the understanding of the long term consequences

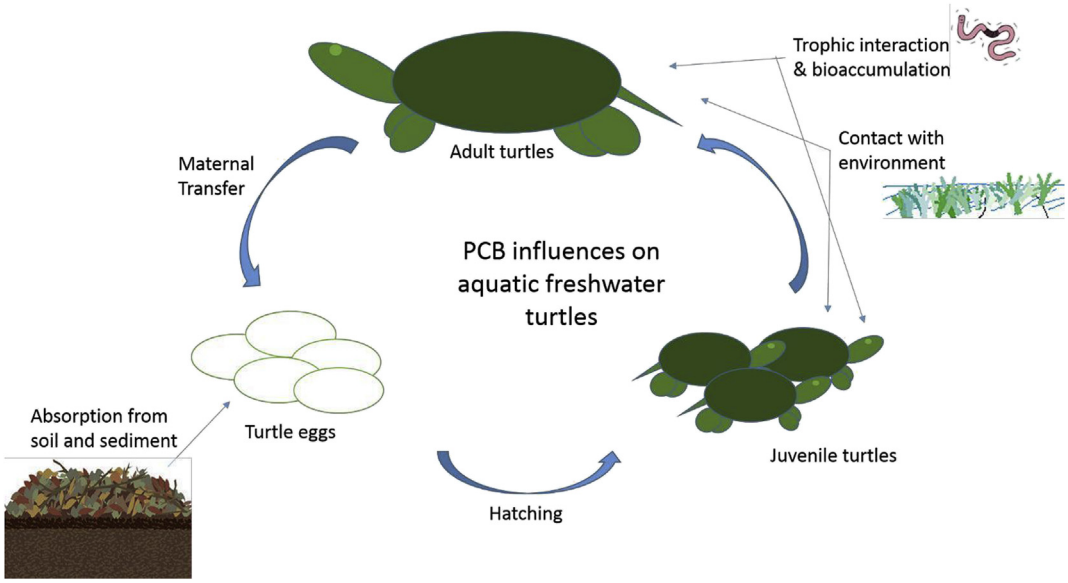

Fig. 1. Juvenile and adult turtles acquire PCBs through trophic interactions and via contact with the environment. PCBs can be stored in the liver and passed on mainly through yolk via maternal transfer. Eggs may absorb PCBs from soil and sediment.

that this can have on turtle functionality and survival (Keller et al., 2004; Dabrowska et al., 2006). The few available long-term studies describing effects of PCB exposure show that sub-lethal effects have implications for behavior and long-term survival despite PCBs not causing immediate death (Ford and Holliday, 2005; Eisenreich et al., 2009; Holliday et al., 2009; Holliday and Holliday, 2012). Longer-term studies of turtles as bioindicators are needed to examine the toxicological effects that PCBs can have on vertebrates throughout their lifecycle. Turtles may experience bioaccumulation of PCBs at a steadily rate throughout their lifetime causing increased PCB concentration in the liver or show traces of PCBs concentrated in egg yolks depending on the external environment that the turtles are exposed to. This information is important in order to understand at which rate PCB bioaccumulation happens, at which rate biodegradation occurs in turtles and where, if even more hazardous degradation products are produced and thus if parallels can be drawn to the human PCB exposure pathways.

Environmental estrogens affect turtles by changing their sex, which subsequently can have an effect on their behavior. For example, males and females of *Trachemys scripta* have different roles in their mating rituals (Jackson and Davis, 1972), but it is possible that PCB-affected turtles can behave differently if their sexes and hormones are influenced during development. In *Chelydra serpentina*, behavioral patterns such as male-male combat is affected by their body size, which can be influenced by stunted growth or hormone imbalances caused by PCB exposure (Brown and Brooks, 1993; Crews et al., 2000; Bonefeld-Jorgensen et al., 2001). Overall, future research must examine to what degree PCBs affect already declining turtle populations, and put this into context with correlations to habitat destruction and trophic interactions (Gibbons et al., 2000).

Turtle longevity makes this organism a good candidate for studying the long-term effects of environmental toxicology. By exploring the effects that PCBs and other POPs play in turtle development and throughout their life cycle, effects on humans and other mammalian population can be evaluated (Valenzuela, 2009; Shaffer et al., 2013). Priority must be given to research on how POPs interact with the environment and how they affect not only individual organisms, but also the overall ecosystem. In this way, future impacts can be predicted and effective remediation solutions can be developed for other contaminants in order to prevent detrimental effects to turtles and other organisms in the environment.

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