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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,