US EPA Mid-Continent Ecology Division

Research Project Summary

Toxicity and Accumulation of PFOS in Frogs and Small Fish

Overview

Concern for a potential adverse effect upon the environment and human health from polyfluorinated chemicals (PFCs) greatly increased with the documentation of their world wide distribution. Perfluorooctanesulfonate (PFOS) was of particular interest because it was identified more frequently and at higher concentrations in humans and wildlife than other PFCs. PFOS was used commercially as an anti-stain treatment for fabrics, a high-temperature lubricant, and as an agent for suppression of fuel (gas and oil) fires. It has also been shown to be the end degradation product of other chemicals based on the PFOS structure.

Early rodent studies indicated a potential for developmental, reproductive, and systemic toxicity. However, lack of information on the actual mechanism of toxic action limited extrapolation of rodent data to wildlife species. It was recognized that because of the unique physico-chemical properties of PFCs, traditional models used to predict the chemical behavior of non-polar organic chemicals, such as PCBs, may not apply to polar anionic surfactants such as PFOS.

An initial research effort at MED was then made to identify appropriate biological endpoints that could be used for aquatic animal studies with PFOS. A partial-life-cycle small fish model using the fathead minnow (*Pimephales promelas*) was chosen to assess reproduction and developmental endpoints, and an amphibian model using the Northern Leopard frog (*Rana pipiens*) was chosen to assess development during metamorphosis. Frog eggs were exposed to PFOS concentrations ranging from 0.03 to 10 mg/L until emergence of limbs (16 weeks). At 10 mg/L, animals began to die within 14 days, while survival was not affected at or below 3 mg/L. Time to metamorphosis was increased and growth reduced at 3 mg/L, but not below. Overall, *R. pipiens* did not appear to be exceptionally sensitive to PFOS. Whole-body chemical residues were measured up to 100 ug/g (100 ppm). The fathead minnow study consisted of exposing adult spawning pairs to concentrations of 0.03 to 1.0 mg/L for 21 days. At termination of the adult exposure, newly spawned eggs were maintained in exposure water for an additional 24 days. Egg production for each of the spawning pairs was monitored for 21 days prior to, and during the exposure period. It was observed that 1.0 mg/L was lethal to adults. The 21-day EC-50 for fecundity was 0.23 mg/L. Histological alterations were noted in ovaries of adult females, and adult males showed decreased aromatase activity, and increased plasma ketotestosterone and testosterone concentrations. No significant adverse effects on survival or growth of the developing fry were observed. Liver tissue PFOS concentrations exceeded those reported for field collected fish by 2-3 orders of magnitude.

The concentration of PFOS and a metabolic precursor of PFOS, perfluorooctanesulfonamide (PFOSA), was quantified in the liver of bottlenose dolphins affected by a major mass mortality along the US Atlantic coast during 1987 and 1988. This single epizootic resulted in the loss of over 750 near-shore bottlenose dolphins (>50% of this sub-species).

Suppression of the immune system by polychlorinated chemical contaminants had been proposed as a cause of the deaths. Because PFOS and PFOSA have been shown to also suppress the immune system, the concentration of these chemicals was quantified and compared with previously quantified polychlorinated chemical concentrations. It was observed that the concentration of PFOS in livers was as great, or greater than, the highest concentration of chlorinated chemicals, such as PCBs and DDTs. These data show a much more complex picture of the cause of this massive dolphin mortality, and perhaps more importantly, may have implications for the ongoing high mortality rate of first born bottlenose dolphins.

In addition to the 1987/88 mortality event, a second less severe mortality event occurred in the Gulf of Mexico during 1990. These animals will be examined for PFOS to determine if PFOS could have also been a causative agent in these deaths. This set of samples includes several mother/fetus pairs, which will allow us to determine if PFOS may indeed have a role in the newborn dolphin mortality rate.

Finally, fish from all five of the Great Lakes have been collected during 2001 and 2005 for the purpose of PFOS analysis. The production of PFOS by its primary US manufacturer, The 3M Company, was stopped. These analyses will give us a chance to determine if background concentrations are decreasing. Fish have recently been identified as a primary soure of PFOS in humans.

Key Products

Ankley GT, Kuehl DW, Kahl MD, Jensen KM, Butterworth BC, and Nichols JW. 2004. Partial life-cycle toxicity and bioconcentration modeling of perfluorooctanesulfonate in the northern leopard frog (*Rana pipiens*). Environmental Toxicology and Chemistry 23(11): 2745-2755.

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