BACKGROUND

In 2001, the EPA began restricting the use of organophosphate pesticides in bug sprays and strips to protect the health of humans and other mammals. Their main replacement has been another class of highly toxic pesticides known as pyrethroids.

The pyrethroid molecule is basically a chemically stabilized version of the natural pyrethin molecule found in mums (yes, the flowers). In its modified form, it is more persistent in the environment, more hydrophobic (it will stick to sediment) and more toxic.

Vertebrates, including humans, have enzymes that can break down low doses of pyrethroids but they tend to have endocrine-disrupting effects. Both the pesticides and the compounds produced during their metabolism can potently mimic sex hormones.

Fishes are particularly sensitive to endocrine disrupting compounds (EDCs), due to their size and continuous exposure via contaminated water. Fish exposed to EDCs may display a variety of physiological and behavioral abnormalities, including poor sperm quality, skewed sex ratios and possibly population declines. The advent of new technologies has made it possible to document endocrine-disrupting effects at the parts-per-trillion range in sensitive aquatic species. Some studies have shown that low concentrations of EDCs can sometimes be more potent than higher ones.

Besides the obvious implications for aquatic ecosystems, EDCs also pose significant human health concerns. According to scientists, the endocrine system is highly conserved evolutionarily among vertebrates. Pesticides, plasticizers and other EDCs that cause problems for fish will likely be harmful to people at high doses or over longer exposure periods.

PROJECT

The project explored evidence of endocrine disruption in a ubiquitous small fish (*Menidia audens*) collected from Suisun Slough and Denvertor Slough in the Sacramento-San Joaquin Delta. The fish, known colloquially as Mississippi silversides, was introduced to California in the 1960s and has since become an important forage species regionally.

The two field sites were purposefully selected to contrast the effects of urban vs. farm runoff. Suisun Slough receives non-point source pollution from Suisun City and effluent from the City of Fairfield Municipal Wastewater Treatment Facility, while Denvertor Slough is adjacent to a cattle-grazing pasture and is much less polluted with EDCs.

The scientists used cell lines to detect endocrine-disrupting activity in water samples and developed assays to detect endocrine-dependent gene expression and protein synthesis in fish. Histological studies of gonadal tissues were also conducted, as were population-level evaluations of sex ratios and body sizes. The idea was to 1) document the presence of EDCs, 2) document evidence of effects from this exposure and 3) compare endocrine-disruption effects across multiple biological scales.

Laboratory experiments with a closely related fish species *M. beryllina* were carried out to explore whether low concentrations of the pyrethroid pesticides bifenthrin and permethrin would by themselves be able to trigger estrogen-dependent gene expression and protein synthesis in sexually immature juvenile fish. Bifenthrin is the active ingredient in residential termite and ant control products. Permethrin is put in insect repellents and head-lice shampoos and sprayed on cotton, wheat and alfalfa crops. Both are endocrine disruptors, commonly detected in urban runoff.
EDCs (including bifenthrin and permethrin) were detected at both sites. At the ranch site, however, only estrogenic activity was detected. Androgeic and estrogenic activities were detected at the urban site.

Male fish collected from both sites expressed estrogen-dependent genes, normally only produced by females. Males from the ranch site expressed higher levels of these genes, while females at the urban site had reduced expression of female reproductive genes.

Amazingly, EDCs appear to have altered the sex ratio and sizes of fish, with more and larger males at Suisun Slough. The scientists hypothesize that the presence of androgens in the water at the urban site accounts for the skewed sex ratio.

In the laboratory experiments with juvenile fish, bifenthrin induced endocrine disruption in the parts-per-trillion range, frequently measured in the environment. At this exposure, juveniles produced egg proteins that are normally only expressed in adult female fish. The experiments with permethrin were conducted at the parts-per-billion range, slightly above what is currently measured in the field. At these concentrations, juveniles also produced egg proteins.

Interestingly, in the cell lines both pesticides actually showed anti-estrogenic activity, meaning that the pyrethroid insecticides bound to and blocked estrogen receptors, instead of activating them.

Other studies have shown that pyrethroid metabolites can be more estrogenic than the original compound. Scientists theorize that this is what happened with the fish.

According to the 2011 Pulse of the Delta report, most urban runoff in northern California now contains pyrethroids well above concentrations causing toxicity to sensitive aquatic life. Creek sediments are also often contaminated at levels toxic to small benthic invertebrates.

This work presents compelling evidence that the pyrethroid pesticide bifenthrin is estrogenic at very low, environmentally relevant concentrations and that EDCs such as bifenthrin are likely altering fish populations in Suisun Marsh. The results also highlight the need to monitor environmental contamination effects at multiple levels of biological organization to detect the full suite of possible effects.

At the ranch site, for example, male silversides expressed female genes, even though there were no signs of endocrine effects on testes histology, or in altering male and female sex ratios.

At the more polluted urban site, in contrast, the sex ratio was highly skewed to males, and there was a higher incidence of testicular cell death, but female gene expression (i.e., estrogen-dependent protein production) was comparatively low in both males and females. The scientists believe that the population is being masculinized by androgens in the water.

Numerous studies have linked human exposure to EDCs (such as the plasticizer bisphenol-A) to human health issues. Pyrethroids are not completely removed by either secondary or tertiary municipal wastewater treatment facilities in California. Millions of Californians drink water from the Sacramento-San Joaquin Delta and many millions more eat fruits, nuts and vegetables irrigated with water from the same area. More studies are needed to evaluate the aggregate toxic affect of EDCs on public and environmental health.