2009

# Sublethal Exposure To Two Alkylphenolic Compounds and Their Influence on Development, Growth and Reproductive Behavior of Crayfish

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# SUBLETHAL EXPOSURE TO TWO ALKYLPHENOLIC COMPOUNDS AND THEIR INFLUENCE ON DEVELOPMENT, GROWTH AND REPRODUCTIVE BEHAVIOR OF CRAYFISH

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**ABSTRACT**:

Invertebrate animals make up the greater part of the world's biological diversity and are present in about all habitats. Their survival is fundamental to the maintenance of life and because of their ubiquitous distribution they are often used as biological indicators for pollution. Large numbers of invertebrate species are under severe threat of extinction, or are already extinct due to the extreme transformations of habitats due to human activities or exposure to various chemical pollutants. Crayfish are one such important invertebrate that is affected by chemical pollutants such as pesticide/herbicide runoff and industrial waste effluents. Crayfish are keystone species in most aquatic systems. Crayfish are often keystone species because they are an important resource for many other animals and can directly alter species diversity and abundance. Crayfish are also raised for human food consumption in the aquaculture industry. For these reasons, crayfish are important organisms to study and better understand the effects of pollution on their daily routines and ultimate survival. Alkylphenols are a group of pollutants often concentrated by organisms (bioaccumulation) such as crayfish, fish, and birds, leading to contamination in their internal organs between ten and several

thousand times greater than in the surrounding environment. These chemicals are used in various laboratory detergents and in some pesticide formulations, which makes them very common pollutants in aquatic systems. They have a number of adverse effects in fish and likely have similar harmful impacts for crayfish and consequently Michigan's aquatic systems. We examined the effect of sublethal exposure to two alkylphenol pollutants (nonlyphenol and octylphenol) on crayfish development, growth, reproductive behavior, and success finding food. We found numerous significant impacts on crayfish when exposed to alkylphenols.

## INTRODUCTION:

Fish and aquatic invertebrates are exposed to various levels of contamination throughout the year, which can cause physical damage to different areas of the body of an animal, such as the sex organs. Because the olfactory organs of aquatic organisms are directly exposed to the environment, chemical pollutants likely impair the ability to perceive and respond appropriately to chemical stimuli (Klaprat et al., 1992). Fish have the potential to recover from damage to the olfactory receptor cells because they have high regenerative rates, whereas crayfish only replace the olfactory receptor cells during the molting process (Zeni et al, 1995; Harrison et al., 2001). It is unclear if crayfish olfactory cells contain enzymes capable of breaking down toxic substances, but the enzyme cytochrome P450 has been found in the crayfish hepatopancreas, where breakdown of toxic substances is know to occur (James and Boyle, 1998). This enzyme is also found in mammalian olfactory mucosa and assists in metabolizing contaminants that come in contact with the olfactory mucosa (Reed et al., 1989).

Recent studies have shown that sublethal (nominal) concentrations of pollutants have detrimental effects on several aspects of fish behavior (Saucier et al. 1991). For example, Chinook salmon exposed to sublethal levels of the pesticide diazinon exhibit decreased responses to predatory odors (Scholz et al., 2000). Diazinon, at several sublethal concentrations, also interfered in reproduction of Atlantic salmon by disrupting olfactory priming of the male by female urine (Moore and Waring, 1996). Crayfish are ideal animals for studying the effect of pollutants on olfaction because information concerning many aspects of their environment is acquired through olfactory organs. Crayfish use chemical signals to search for food, mates, determine social status of other crayfish, and to locate and avoid potential predators (Moore and Grills, 1999: Bergman et al., 2003). The olfactory organs of crayfish are vulnerable to damage from environmental pollutants, so decisions crayfish make based on chemical stimuli could be severely impacted (Steele et al. 1992). Behavior of crayfish could be altered by contaminants masking odor signals, by inhibiting the ability of receptor cells to detect signals, or by altering an animal's behavioral response to chemical signals. Masking is a process by which the toxin influences the ability of the animal to distinguish signals from the background noise of the environment.

Alkylphenols, such as nonylphenol and octylphenol, are used industrially in antioxidant formulations for plastic and rubber polymer manufacturing, and are similar in structure to a known endocrine disruptor, bisphenol A. Alkylphenols are used extensively as industrial detergents, such as those used for wool washing and metal finishing. They can also be used in various laboratory detergents and in some pesticide formulations. In fact, in the United States, they are used in many liquid clothes detergents, so contamination of water supplies can from a myriad of sources. Alkylphenols do not break down effectively in sewage treatment plants or in the environment. They tend to loose some of their attached ethoxylate groups quite easily, which prevents them as acting as detergents. However this usually leaves the alkylphenols largely intact, which then persist for long time periods. Alkylphenols usually accumulate where there is inadequate oxygen, e.g. in sediments where crayfish live, and persist in rivers and sewage effluents (Di Corcia et al., 1998). In spite of the potential detrimental aspects of alkylphenols to the environment, they are often not tested for at water treatment plants or in natural environments. When they have been measured, measurable concentrations of alkylphenols have been reported around 15–76 µg/l in some recorded rivers (Blackburn et al., 1999) and even higher concentrations up to 125.58 µg/g in lobsters (Biggers and Laufer, 2004). These two numbers taken together indicate that alkylphenol pollution is a real problem and furthermore that bioaccumulation of the compounds occurs in crustaceans.

Alkylphenols may interfere with normal olfactory behavior in crayfish. We proposed to examine the effects of acute exposure to nonylphenol and octylphenol on the response to food and mating odor cues by the freshwater crayfish *Orconectes propinquus*. We will also examined their developmental progression and overall growth rates when exposed to sublethal levels of nonylphenol and/or octylphenol. Based on the results of our study, we have demonstrated that alkylphenols have on various detrimental influences on the survival of the species.

## MATERIALS AND METHODS:

The crayfish *Orconectes propinquus* (Fig. 1) was used for this research, as juveniles and as adults (male and female). This species was chosen because of its relatively high abundance in Michigan's waterways and were collected from tributaries of the Grand River by means of a seine net. Collected females with eggs were isolated in the lab in a container with one liter of water to increase the survival rate of their eggs. Upon hatching the maters were removed and put into a separate tank. Juvenile crayfish were then fed and separated if tanks became overcrowded. Captured male crayfish were kept in isolation as well for their experimental treatments.


Figure 1. An adult *Orconectes propinquus* used for the experimental treatments.

Males were placed into isolation tanks with one liter of water, and rocks were placed on the bottom of each tank. Using a pipette (0.2 µL- 2 µL) the two alkylphenols (nonlyphenol and octylphenol), which are known surfactant's were injected into each tank (Fig. 2). The amounts of 0.05 µL, 0.1 µL, and 0.25 µL per liter were used to determine the toxicity of these presumed "inert" ingredients in pesticides (Cox 2003). Also two control groups were used, one with acetone, and the other with de-chlorinated tap water. The reasoning behind the use of acetone was the high viscosity of nonlyphenol, and composition of octylphenol. The acetone decreased the viscosity of nonlyphenol and made normally solid octylphenol a solution. At the beginning of each week, crayfish males were exposed to one of the chronic treatments. Each exposure group contained


Figure 2. Laboratory set up of adult male crayfish.

a group of three crayfish (n= 3) for a total of thirty subjects being used.  Exposures began on June 3rd and still ongoing to collect further data.

Juvenile crayfish were exposed to the alkylphenols using a different method.  We performed acute "flash" exposures of a limited duration to the juveniles to simulate runoff from farmland.  The acute exposure periods were 1 hour, 2 hours, 3 hours, and 4 hours, respectively.  Experimental exposures consisted of 0.1 µL and 0.25 µL (n = 5 for each treatment group).  The juvenile crayfish were also placed in a container holding one liter of water and a small amount of gravel.  Gravel was placed in each container because alkylphenols tend to get trapped and subsist in the substrate for extended time periods.

**RESULTS:**

With an over exposure taking place early in the summer to the female population with eggs, a drastic experimental alteration was needed upon the initial data set analysis. A 100-fold drop was used for exposure changes, since the initial recommend values proved 100% lethal. Levels that were thought to be of the sublethal concentrations as suggested by the EPA and others were actually shown to be harmful to the crayfish. The final amounts of 0.05 µL, 0.1 µL, and 0.25 µL per liter were decided upon. Sublethal exposure proved to be more lethal than what was actually known to the juvenile and adult crayfish. Adult crayfish experienced no deaths at the control level, but there was an accumulation of deaths within the exposed trial groups. At the 0.05 µL nonlyphenol deaths took place at a rate of 3.1%, this rate steadily increased by the amount of alkylphenol in solution. At an exposure of 0.1 µL nonlyphenol the percentages of death increased to 20.8%, however at 0.25 µL nonlyphenol the incidence of death was 10.4%. For octylphenol the incident of death was lacking, for 0.05 µL and 0. 1 µL there were no deaths that occurred, for 0.25 µL octylphenol there was a 4.1% occurrence of death (Fig3.).

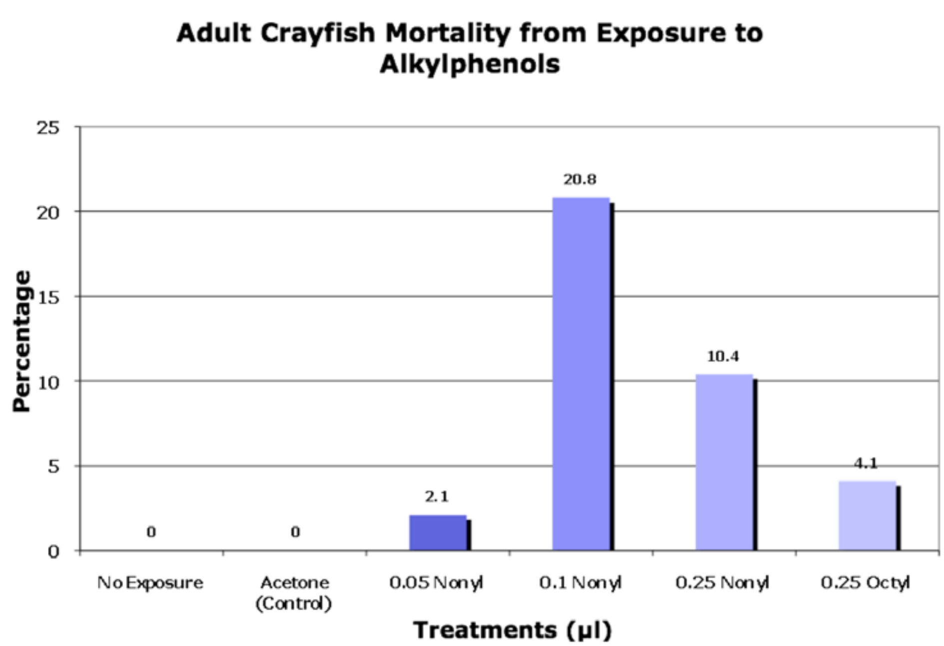


Figure 3. Mortality data for adult crayfish from alkylphenol exposure.

Juvenile deaths were recorded in the same method as the adults were, but the rate of death was much greater.  In all of the exposure groups deaths of the juveniles increased compared to that of the males.  Since nonlyphenol was deemed the more toxic of the two it was used for all of the exposures. During the one hour exposures at 0.1 µL the probability of death was just below 30%.  For the two hour exposure of 0.1 µL the death occurrence was also roughly 30%, three hour exposure of 0.1 µL had a death rate of over 80% and at four hours the rate of death increased to above 85% at the exposure 0.1 µL nonlyphenol.   At an exposure of 0.25 µL there was above a 70% incidence of death at the two hour exposure, three hours had a just above 70% occurrence of death at 0.25 µL, and finally at four hours for 0.25 µL there was above 80% incidence of death occurring in the juveniles (Fig.4).  One-hour exposures of 0.25 µL was not conducted because of the lack of supply of juvenile crayfish.

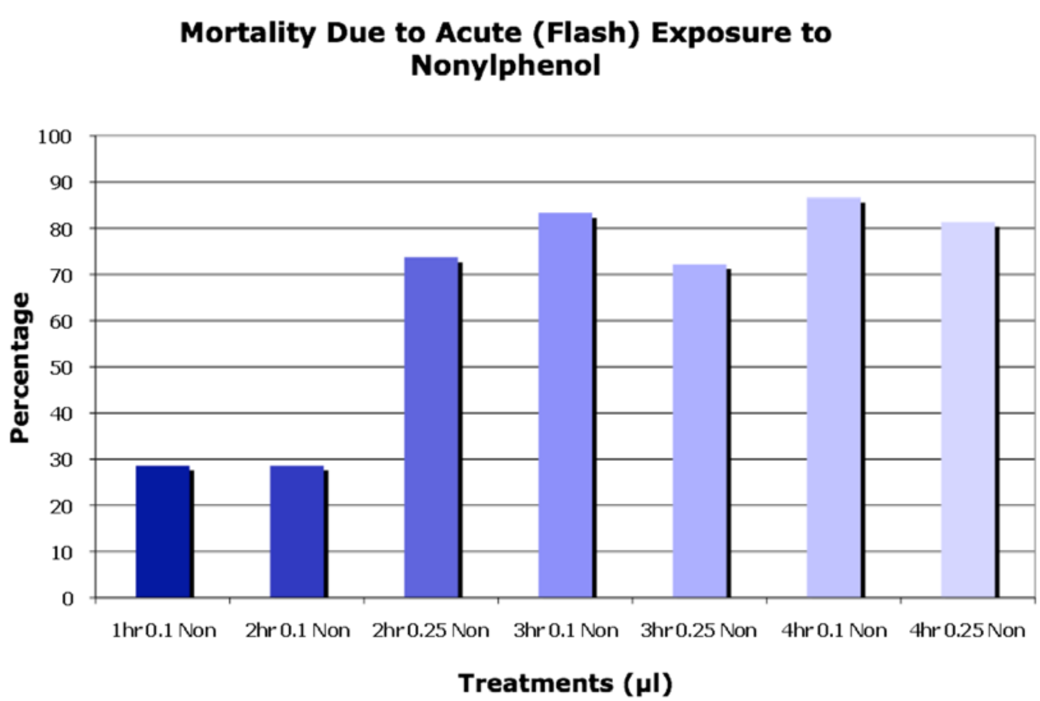


Figure 4. Mortality data for juvenile crayfish from acute nonylphenol exposure.

## DISCUSSION:

In adult crayfish it can be seen there is some resistance to the alkylphenols, this could be primarily due to their overall size and maturity of their physiological makeup. Even with a more developed exoskeleton adult male crayfish were still susceptible to the alkylphenols, thus proving that nonlyphenol and octylphenol, to a lesser extent, pose a threat to the maturation and survival of crayfish. Our data suggest a potential and serious risk to future crayfish populations and consequently food webs. An even greater problem arises because of the immense use of alkylphenols in objects ranging from pesticides, herbicides and even common detergents. With alkylphenols being an "inert" ingredient in those products it causes concern, especially with the recent findings from our study. Alkylphenols have also been linked to many other types of physiological problems ranging from reproductive development, irregular heartbeats, and loss of normal movements (Cox, 2003). It has been seen in other animals other than crayfish. For example, studies on Atlantic salmon showing the detrimental effects, nonlyphenol does on the olfactory priming of males by female urine (Moore and Waring, 1996). Overall it has been seen over the course of this study that nonlyphenol and octylphenol seems to have caused irreversible damage.

Juveniles are constantly under the pressure to grow as quickly as possible to help reduce their predation risk, consequently causing them to molt at a more rapid pace than adult crayfish. The exposure of chemical pollutants can adversely affect the metabolically taxing molting process. This was seen in the rapid incidence of death in the juvenile trials. The increase in exposure length and/or exposure strength resulted in an increase incidence of death per exposure. The death rate among juveniles was likely due to the fragility of their exoskeleton caused by the rapid amount of molting required for growth and the incorporation of the toxin into their tissues.

**FUTURE DIRECTION:**

The lab's and my direction over the course of the upcoming year will further explore the toxic ramifications caused by alkylphenols, specifically nonlyphenol and octylphenol. Crayfish will continue to be exposed to these two compounds in order to find out the depth to which the alkylphenols affect the physiology of the crayfish. Changes in reproductive development will be examined by looking at copulatory styles of the juvenile crayfish. Both internal and external morphological changes will be look upon to see if feminization occurs among males. The affect of alkylphenols on olfactory receptor cells is another problem occurring in crayfish. The reason being, a crayfish's olfactory system is critical for their survival. Damaged olfactory systems will decrease mating and feeding capabilities. To test this hypothesis a food or pheromone scent will be placed downstream of an exposed crayfish and reactions will be noted.

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