Report as of FY2006 for 2005MN147G: "Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain"

Publications

- Articles in Refereed Scientific Journals:
  - Bistodeau, T.J., L.B. Barber, S.E. Bartell, R.A. Cedie, K.J. Grove, J. Klaustermeier, J.C. Woodard, K.E. Lee and H.L. Schoenfuss. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. Aquatic Toxicology 79: 268-277.
- Other Publications:
  - Julius, M.L., J. Stepanek, O. Tedrow, C. Gamble and H.L. Schoenfuss. Estrogen -receptor independent effects of two ubiquitous environmental estrogens on Melosira varians Agardh, a common component of the aquatic primary producer community. In preparation.

Report Follows

Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain

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Abstract

Among estrogenic endocrine disrupting compounds, alkylphenolic surfactants stand out due to their ubiquitous presence in anthropogenically altered surface waters and their occurrence in complex mixtures. Although the parent compounds (nonylphenol and octylphenol) of most alkylphenol polyethoxylates are orders of magnitude less estrogenic than 17ß-estradiol, they are also found in concentrations orders of magnitude greater then the natural estrogen in many treated wastewater effluents and receiving streams and rivers. In addition, the longer-chained alkylphenol polyethoxylates are altering the bioavailability of nonylphenol and octylphenol, thus potentially facilitating the uptake of the more potent parent compounds by aquatic organisms exposed to the alkylphenol mixtures. Furthermore, the chemical nature of surfactant raises the specter that organisms at different levels of the trophic cascade may experience differential effects that may be estrogen receptor independent (diatoms) or estrogen receptor mediated (daphnia and fathead minnow). As a consequence, we proposed to test the effects of an alkylphenol polyethoxylate mixture, realistic in composition and concentration, on three tiers of an abbreviated aquatic food chain: the primary producer community (diatoms); a primary consumer (*Daphnia magna*); and a secondary consumer (fathead minnow, *Pimephales*

promelas). Once we have documented the effects of alkylphenolic mixtures on each level of this aquatic food-chain, we proposed to test food-chain effects of exposure by feeding exposed diatoms to daphnia or larval fathead minnows. We have completed our diatom and fathead minnow exposures to the alkylphenolic mixture and are in the progress of exposing daphnia magna to alkylphenols. We have also developed and tested food-chain experimental protocols that will allow us to produce food pellets of exposed diatoms that can be fed to unexposed D. magna or larval fathead minnows. Our findings from the single organism mixture exposure experiments indicate a degrading effects of alkylphenols on the primary producer community, especially on diatoms which represent the preferred food source of many larval and fingerling fishes. In addition, we have found that diatoms are more sensitive to alkylphenolic compounds than they are to the potent estrogen 17b-estradiol suggesting that the effects of alkylphenolic compounds might disrupt receptor independent pathways at subsequent tiers of the trophic cascade (Julius et al. In preparation). As a consequence of the diatom exposure, the nutritional value of diatoms for *D. magna* and larval fathead minnows was greatly diminished. We have also established that alkylphenol mixtures have a more potent effect on fathead minnows than the parent compound nonylphenol alone (Bistodeau et al. 2006). We are currently finalizing the food chain exposure experiments and are well underway to complete all objectives stated in the original grant proposal.

Introduction & Research Objectives

Endocrine disrupting compounds have been detected in many anthropogenically altered surface waters in North America (Kolpin et al. 2002, EST 36: 1202-1211), and Europe (Desbrow 1998, EST 32: 1549-1558). Several classes of endocrine disrupting compounds are usually recognized, including natural/synthetic hormones (estrone, estradiol, ethynylestradiol), personal care products (i.e., the antimicrobial soap ingredient Triclosan) and alkylphenolic surfactants. The latter have been found almost ubiquitously in anthropogenically altered surface waters in part because they use is inherently water related. Alkylphenols are a group of compounds used in large quantities as industrial and household surfactants and have been found to be estrogenic (Hemmer et al. 2001, ETC 20:336-343). Alkylphenols are known to bind to the estrogen receptor of mammalian cells and disrupt the homeostasis of the internal milieu of the organism. Environmental estrogens such as alkylphenols are known to disrupt normal endocrine hormone that are central to maturation and reproduction in fishes, and the ubiquitous presence of these biologically active compounds in surface waters should be of environmental and human health concern. To date, alkylphenol studies have focused on 4-nonylphenol, the metabolic product of both aerobic and anaerobic microbial degradation of higher-chained alkylphenols and the US EPA has recently proposed effluent emissions criteria for this compound. However, mixtures of nonylphenol and higher chained alkylphenols are found routinely in effluents and their combined action is entirely unknown. In this study, we propose to examine the effects of alkylphenol mixtures on three tiers of the aquatic food chain: the primary producer community (diatoms), a primary consumer (Daphnia magna), and a vertebrate near the top of the food chain (the fathead minnow). We furthermore will link all three tiers through feeding trials to examine the effects of alkylphenol mixtures on the aquatic food chain. The present study proposes three objectives to

determine the relationship between alkylphenol contamination of surface waters and adverse organismal effects. These are (1) determine the effects of alkylphenol exposure on the reproductive success of three tiers of the aquatic food chain; (2) determine the impacts of alkylphenol mixtures across the food chain; and (3) to test the three assays at a field site known to discharge alkylphenols.

To date we have completed the fish and diatom exposures with alkylphenol mixtures. We have been able to demonstrate that the combined effects of alkylphenols exceeds that of individual alkylphenols in the fathead minnow (Bistodeau et al. 2006) and have also established that diatoms serve as sensitive indicators of biological disruption caused by the presence of alkylphenol mixtures (Julius et al. In preparation). Furthermore, we have established protocols for the Daphnia magna exposure and are currently exposing these organisms as an intermediate level in the trophic cascade. Finally, we have developed an experimental design to link all three tiers of our abbreviated food chain through pelleting large quantities of alkylphenol exposed diatoms for larval fathead minnow feeding. In summary, we are well underway to complete all proposed components of the National Institute of Water Resources funded study.

Methodology & Preliminary Findings

We have completed several rounds of diatom exposures (M. varians) to graded concentrations of 4-nonylphenol (NP) singularly and to mixtures of alkylphenolic compounds (Table 1) including NP, nonylphenol-1-ethoxylate (NP1EO), nonylphenol-2-ethoxylate (NP2EO), nonylphenol-1-carboxylate (NP1EC), and nonylphenol-2-carboxylate (NP2EC). In addition, we exposed diatoms to 17β-estradiol, a compound with known endocrine disrupting activity that served as a reference exposure for this study.

Table 1: Concentrations used in M. varians exposures for 4-nonylphenol (4-NP), 17β-estradiol (E2) and the alkylphenol mixture (AP).

Treatment	Low Exposure Concentration (mg/L)	Medium Exposure Concentration (mg/L)	High Exposure Concentration (mg/L)
17β estradiol	3	30	300
4-nonylphenol	2	20	200
Alkylphenol mixture*	74.5	373	746

Table 2: Alkylphenol compounds detected in the Metropolitan treated wastewater effluent (St. Paul, MN) and their environmental concentrations, used for determining experimental dose values.

Compound	Concentration (mg/L)
NP	2.11
NP1EO	3.536
NP2EO	6.987
NP1EC	25.201
NP2EC	33.618
SUM	**71.5**

For the diatom exposures, monocultures of Melosira varians were grown in sterile WC media, then exposed to pre-determined test chemical concentrations and incubated in diurnal growth chambers with a 12:12 light:dark cycle for a period of ten days. Procedures were as follows:

100 ml of homogenized culture aliquots were added to 900 ml of sterile media and allowed a period > 24 hours to acclimate. Due to its affinity for binding to glass, sterile polystyrene cell tissue rollers were used in the case of the 4-nonylphenol exposures, and 2 liter glass Florence flasks were used for the estradiol trials. Treatments consisted of control, low,

medium and high exposure concentrations. Once treated, samples were taken on day one for cell count and chlorophyll-a analysis. Exposed cultures were then allowed to grow for a period of ten days, after which samples were once again obtained for chlorophyll-a and cell count. The chlorophyll-a content of the cells was measured using a fluorometer and averages for each treatment group were determined in order to quantitatively assess diatom health. Elevated chlorophyll A tissue concentration indicate a stress-effect caused by the exposure. In three exposure experiments using graded series of 17ß-estradiol (experiment 1: 2µg/L, 20µg/L, 200µg/L: experiment 2: 4µg/L, 40µg/L, 400µg/L; experiment 3: 8µg/L, 80µg/L, 800µg/L) treatments at or above 80µg/L consistently found statistically significant (one way ANOVA, $p<0.05$) increases in chlorophyll A: lipid ratio indicating a reduced nutritional value of diatoms for higher levels of the trophic cascade. Clearly these 17ß-estradiol concentrations are well beyond environmentally relevant concentrations and indicate that 17ß-estrdiol does not adversely affect exposed diatoms. In contrast, three exposure experiments using series of alkylphenols (experiment 1: 2µg/L, 20µg/L, 200µg/L: experiment 2: 4µg/L, 40µg/L, 400µg/L; experiment 3: 8µg/L, 80µg/L, 800µg/L) found statistically significant (one-way ANOVA, $p<0.05$) increases in the Chlorophyll A ; lipid ratio at and above 40µg/L. This concentration of total alkylphenols has been exceeded in many treated wastewater effluents and indicates that the primary producer community is likely adversely affected by environmental concentrations of alkylphenols.

In addition to the diatom exposures, we have also completed the fathead minnow exposures to a mixture of several alkylphenolic compounds (NP, NP1EO, NP2EO, NP1EC, NP2EC) in mixture or to nonylphenol alone at the effluent measured concentration (similar to the concentration in the alkylphenol mixture - Table 2). Our results indicate that reproductive competence is impaired in male fathead minnows that were exposed to the mixture for 28 days at

realistic concentrations (Fisher's Exact test; $p<0.05$). In addition, secondary sexual characters and the gonadosomatic index are significantly reduced when compared to control males (Student t-test, $p<0.05$). Even at a mixture concentration representing 50% the mixture concentration measured in the treated effluent, reproductive competence was significantly reduced. In contrast, nonylphenol alone had an excitatory effect on nest holding ability (Fisher's Exact test, $p<0.05$) that is likely the result of a priming effect of the low-concentration estrogenic compound. Detail results of the fathead minnow exposures are published in Bistodeau et al. (2006).

Publications, Presentations, or Published Abstracts:

Publications

Bistodeau, T.J., L.B. Barber, S.E. Bartell, R.A. Cedie, K.J. Grove, J. Klaustermeier, J.C. Woodard, K.E. Lee and H.L. Schoenfuss. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquatic Toxicology* 79: 268-277.

Julius, M.L., Stepanek, J., Tedrow, O., Gamble, C. and H.L. Schoenfuss. Estrogen -receptor independent effects of two ubiquitous environmental estrogens on Melosira varians Agardh, a common component of the aquatic primary producer community. In preparation.

Presentations (indicates student presentation)*

Schoenfuss, HL and TJ Bistodeau. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Gable, C*, A. Gikineh and ML Julius. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Allen, AK*, T Loes and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Grove, KJ*, RA Cediel and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Koch, JK*, M Minger and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Schoenfuss, HL, Bistodeau, TJ 2006 Minnesota Water, Brooklyn Park, MN, October.

Schoenfuss, HL, Bistodeau, TJ, Society for Environmental Toxicology and Chemistry, Montreal, Canada, November 2006.

Student(s) supported by this project:
Kent Grove (MS expected Fall 2007)
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None to date