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Developmental effects in Japanese medaka (*Oryzias latipes*) exposed to nonylphenol ethoxylates and their degradation products

Gordon Balch *, Chris Metcalfe [1]

Environmental and Resource Studies Program, Trent University, 1600 West Bank Drive, Peterborough, Ont., Canada K9J 7B8

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Abstract

The endocrine modulating potency of five alkylphenol compounds to fish, including nonylphenol (NP), three nonylphenol ethoxylate mixtures (NP1EO, NP4EO, NP9EO) and one nonylphenol ethoxycarboxylate (NP1EC) was assessed using in vivo tests conducted with Japanese medaka (*Oryzias latipes*). Medaka exposed to test materials from 1 day to 100 days post-hatch were monitored for alterations to sex ratios and secondary sex characteristics and development of gonadal intersex (i.e., testis–ova). The treatment with 100 $\mu g\,l^{-1}$ NP (measured 29 $\mu g\,l^{-1}$) induced gonadal intersex in over 80% of exposed males, mixed secondary sex characteristics in over 40% of exposed fish and suppression of the development of papillae on the anal fin of 100% of males. The 30 $\mu g\,l^{-1}$ NP (measured 8.7 $\mu g\,l^{-1}$) treatment induced gonadal intersex in only one of the *22* exposed males and mixed secondary sex characteristics in approximately 20% of the exposed fish. An elevated incidence of fish with mixed secondary sex characteristics and suppression of papillae development was also observed in the treatment with NP1EO at the highest test concentration of 300 $\mu g\,l^{-1}$ (measured 105 $\mu g\,l^{-1}$). There was no evidence of mixed secondary sex characteristics or gonadal intersex in treatments with the remaining test mixtures. This study confirms that NP is an estrogenic compound that could affect gonadal development in fish chronically exposed to concentrations in the range of 10 $\mu g\,l^{-1}$. NP1EO is very weakly estrogenic at concentrations that are an order of magnitude higher than the lowest observed effect concentration for nonylphenol.

Keywords: Endocrine disruptors; Estrogen; Nonylphenol; Medaka; Intersex

1. Introduction

Endocrine disrupting substances present in wastewaters discharged by industries and municipal wastewater treatment plants (WWTPs) include phthalates, bisphenol A and both natural and synthetic estrogen hormones (Desbrow et al., 1998; Larsson et al., 1999; Ternes et al., 1999). However, considerable regulatory attention has been focussed on the estrogenic potential of the biodegradation metabolites of nonylphenol ethoxylate (NPEO) surfactants (Renner, 1997). Alkylphenol polyethoxylate surfactants (APE) have been used for more than 40 years in a variety of industrial processes and in detergents and cleaning products. The majority of

* Corresponding author. Tel.: +1 705 748 1011x7071; fax: +1 705 748 1569.
E-mail addresses: gbalch@trentu.ca (G. Balch), cmetcalfe@trentu.ca (C. Metcalfe).
[1] Tel.: +1 705 748 1011x7272; fax: +1 705 748 1569.

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APE surfactants currently in use in North America are the nonylphenol polyethoxylates (NPEs), but octylphenol ethoxylates (OPEs) are also used for industrial applications. The microbial degradation of these compounds in the environment leads to the formation of compounds that have estrogenic activity; including compounds with two (AP2EO) and one (AP1EO) ethylene oxide groups, as well as the completely de-ethoxylated nonylphenol (NP) and octylphenol (Nimrod and Benson, 1997). Microbial biodegradation of APEOs under oxidative conditions also results in the formation of alkylphenol ether carboxylates, including NP1EC and NP2EC.

The concentrations of the degradation products of nonylphenol ethoxylates in WWTPs have been thoroughly reviewed by Bennie (1999). NP, NP1EO and NP2EO are major nonylphenol ethoxylate degradation products in WWTP effluents, and concentrations of these compounds in final effluents are generally in the range of 10–300 $\mu g\,l^{-1}$ (Bennie, 1999). Concentrations of NP and NPEOs in surface waters are generally below 1 $\mu g\,l^{-1}$ (Bennie, 1999; Ferguson et al., 2000; Kolpin et al., 2002). NP1EC and NP2EC are also major NPE degradation products in sewage effluents. In a survey of the final effluents of several Canadian wastewater treatment plants, concentrations were high as 703 $\mu g\,l^{-1}$ for NP1EC and 565.5 $\mu g\,l^{-1}$ for NP2EC (Lee et al., 1998).

Because of the potential for biological effects in aquatic organisms exposed to the degradation products of NPEs downstream of industrial and municipal discharges, European Union countries have phased out APEO surfactants from use in detergents, cleaners and industrial processes. Environment Canada is currently reviewing regulatory options regarding this class of compounds. The current toxicity data base is generally adequate for evaluating acute toxic effects of the biodegradation products of APEs on aquatic organisms, but the in vivo data on the endocrine modulating effects of these chemicals on aquatic organisms are presently inadequate (Servos, 1999). Reliable data on the lowest observed adverse effect concentrations (LOAECs) and no observed effect concentrations (NOECs) for endocrine modulation in aquatic organisms are required in order to make informed decisions regarding regulatory limits for discharges from industries and STPs.

In this study, we conducted tests to determine the endocrine modulating potency of selected biodegradation products of APEO using an in vivo assay with the Japanese medaka (*Oryzias latipes*), a small aquarium fish that has been used widely to test for endocrine disrupting substances (Gray and Metcalfe, 1997; Metcalfe et al., 1999, 2001; Foran et al., 2002; Kang et al., 2002).

2. Methods

2.1. Fish exposures and assessment

Exposures of Japanese medaka to test compounds were conducted essentially as described previously (Metcalfe et al., 2001). All Japanese medaka tested originated from the brood culture of the golden strain originally purchased from Carolina Biological Supply (Burlington, NC, USA) and held at Trent University for the past ten years. This stock has been periodically augmented with new organisms of the same strain to maintain genetic diversity. Broodstock were reared in dechlorinated city tap water with a pH ranging between 7.4 and 7.8, alkalinity was 60–80 $mg\,l^{-1}$ $CaCO_3$ and hardness was 80–100 $mg\,l^{-1}$ $CaCO_3$. Water temperature was held at 27 ± 1 °C and photoperiod was a standard 16 h light and 8 h dark. Fertilized eggs were collected from this broodstock and raised until hatch as described by Metcalfe et al. (2001). Newly hatched medaka were then placed into glass containers and exposed to the test chemical under the same environmental conditions as used for the broodstock.

Exposure to the fry began within 1 day of hatch and continued for 100 days under static conditions. The test water in individual exposure tanks was renewed every 48 h. Renewal was 100%, with the exception of the first two weeks when 15–20% of the test water was left so that the young fish did not need to be physically handled. Gentle aeration was applied to the tank water so that dissolved oxygen was at or near saturation. Survival and growth were monitored in each treatment after the first and second month of exposure by taking a digital image of the exposure tank and counting the number of fish. After counting, 20 fish were removed, euthanized and total body length and weight was measured to assess growth. Treatments that experienced greater than 20% mortality in the first month of exposure, or exceeded a total cumulative mortality of 30% prior to the termination of exposure were eliminated and not included in the analysis.

A total of five nonylphenol compounds were tested, with each compound assessed at four or five test concentrations. Each treatment was started with 150 fry to ensure at least 50 fish survived to the end of the 100-day exposure period. It was anticipated that natural mortality, particularly within the first few weeks of exposure might approach 20–30%. In addition, 40 fish from each treatment were removed and euthanized for growth measurements. None of the treatments were replicated.

Fifty randomly chosen fish from each treatment were sacrificed with an overdose of tricaine methane sulfonate (MS-222; Fisher Scientific, Toronto, ON) at the end of the 100-day exposure period for assessment purposes. Individual fish were viewed under a dissecting microscope