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Toxicity evaluation with *Vibrio fischeri* test of organic chemicals used in aquaculture

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Abstract

The evaluation of acute toxicity by *Vibrio fischeri* test for different organic chemicals (antibiotics, pesticides, therapeutants, herbicides) commonly applied in aquaculture and a degradation product of surfactants, 4-nonylphenol, is presented in this work. Simazine, atrazine, emamectin benzoate and leucomalachite green have no toxic effects on *V. fischeri* at the concentration tested (up to 6 mg l^{-1}) which correspond to the maximum water solubility. Ciprofloxacin, terbutryn and deltamethrin, caused inhibition effects of 28%, 22% and 30% at concentrations up to 5 mg l^{-1}. Toxic effects were not observed in the case of flumequine and oxolinic acid at the maximum concentration tested (0.189 mg l^{-1}). According to the toxicity categories established in the EU legislation, ciprofloxacin, terbutryn and deltamethrin could be considered non-harmful for *V. fischeri*. Malachite green and 4-nonylphenol are "very toxic to aquatic organisms" ($EC_{50,30min} = 0.031$ mg l^{-1} and 0.48 mg l^{-1}, respectively). Carbaryl is "toxic to aquatic organisms" (2.4 mg l^{-1}). and glyphosate is harmful to *V. fischeri* ($EC_{50,30min} = 44.2$ mg l^{-1}). The matrix effect was evaluated comparing the toxicity measurements of the target compounds solubilized in seawater and distilled water. Malachite green, 4-nonylphenol and glyphosate, showed higher toxicity in distilled water than in seawater. Carbaryl was more toxic in seawater. All the compounds tested in seawater were not harmful at concentrations of ng l^{-1} (10 and 50). However, 4-nonlylphenol and malachite green may act as toxic compounds in the environment at a low ppb level, since both may be detected in water at this concentration level.

Keywords: Aquaculture; Seawater; Chemicals; Toxicity; Bacteria; *V. fischeri*

1. Introduction

Marine aquaculture is undergoing a rapid expansion, more than all other animal food producing sectors (FAO, 2004; UNEP-MAP, 2004). In fact, intensive fish farming is accompanied by the increasing use of natural resources and chemicals. A wide variety of chemicals such as antibiotics, therapeutants and biocides are routinely applied to enhance production efficiency and animal welfare (GESAMP, 1997). Direct emissions of micro-contaminants in seawater from fish farming facilities may be a cause of the contamination in the marine environment.

The presence of certain classes of chemicals in the marine environment has been documented. At the present, the study of the presence of antibiotics in environmental compartments close to marine fish farms has been mainly focused on sediments (Björklund et al., 1991; Capone et al., 1996; Herwing et al., 1997; Halling-Sørensen et al., 1998; Le and Munekage, 2004). The data related to the detection in water is scarce in spite of the fact the diffusion process of antibiotics from sediments to the water column may occur, such as in the case of oxytetracycline (Smith and Samuelsen, 1996). The studies related with

Abbreviations: APEs, alkylphenol etoxylates; DMSO, dimethyl sulfoxide; EC_{50}, effective concentration; LC–MS, liquid chromatography–mass spectrometry.

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the occurrence of therapeutants in seawater are also uncommon. For instance, water column concentrations of emamectin benzoate applied for the control of parasitic infestations have been estimated using mathematical models (Willis et al., 2005). Regarding the herbicides or fungicides, currently used in aquaculture, were originally developed for their use in the agriculture or as additives for boat anti-fouling paints and the published data of their occurrence in marine waters are mainly related to such activities (Martinez et al., 2001; Konstantinou and Albanis, 2004).

Marine pollution due to chemicals applied in aquaculture activities is not yet well documented. The available information on the occurrence of these compounds in the marine environment indicates their presence at low concentrations (low ng l^{-1} level). However, certain chemicals may show toxic effects on non-target organisms at trace concentrations. Upto now, ecotoxicological data on non-target organisms are available only for a limited number of chemotherapeutants. Toxic effects of antibiotics (i.e. oxolinic acid, erythromycin, flumequine) for algae, bacteria and crustacean have been described in literature (Migliore et al., 1997; Backhaus and Grimme, 1999; Backhaus et al., 2000; Halling-Sørensen, 2000; Wollenberger et al., 2000). For other therapeutants, widely used in aquaculture such as emamectin benzoate and malachite green, several toxicity studies have been carried out using various aquatic organisms (Willis and Ling, 2003). In particular, malachite green has been tested on a wide range of different species of fish and certain mammals (Srivastava et al., 2004). There is also available toxicity data for pesticides or herbicides related to the effects on fish (Bradbury and Coats, 1989; Köprücü and Aydim, 2004; Milam et al., 2005), crustaceans (Rawash et al., 1975; Pereira and Rostad, 1990; Davies et al., 1994; Milam et al., 2005; Tsui et al., 2005), bacteria (Arufe et al., 2004a,b), algae (Fairchild et al., 1997; Carder and Hoagland, 1998; Tang et al., 1998) and macrophytes (Lytle and Lytle, 1998).

The toxicity evaluation of chemicals on organisms of different trophic level represents a useful tool to define the toxicological profile. To the best of our knowledge there is scarce data of the toxicity of therapeutants and other organic chemicals commonly used in aquaculture for the marine bacteria *Vibrio fischeri.* In this study, the acute toxicity of a selected group of organic chemicals used in Mediterranean marine aquaculture (UNEP-MAP, 2004) has been determined for *V. fischeri*. The compounds studied are antibiotics (ciprofloxacin, enrofloxacin, erythromycin, oxolinic acid and flumequine), therapeutants (malachite green and emamectin benzoate), herbicides (atrazine, simazine, glyphosate, cypermethrin and deltamethrin), pesticides (carbaryl and terbutryn). The toxicity of leucomalachite green, the reduced form of malachite green, used as an indicator of contamination due to malachite green, has been determined (Srivastava et al., 2004). In addition, 4-nonylphenol, an alkylphenol ethoxylate (APEs), which may be detected in water as consequence of degradation processes of surfactants has also been included in this study. This is a compound included as pollutant by the Water FrameWork Directive (EC, 2000), to be monitored in aquatic compartments, and its detection in surrounding areas of fish farm cage systems, can be used as a parameter indicative of other contamination sources.

In this study, acute toxicity of the target chemicals is classified according EU-Directive which establishes toxicity categories for aquatic organisms (EC, 1993, 1996) based on the values of effective concentration (EC_{50}). Matrix effects have been assessed in both distilled and marine water. Acute toxicity of the target chemicals have also been tested at trace levels (10 and 50 ng l^{-1}), as possible concentrations of these compounds which may be detected in the marine environment.

2. Experimental

2.1. Chemicals

The following chemicals were selected for this study: erythromycin (purity 96%), carbaryl (analytical standard grade), deltamethrin (analytical standard grade), malachite green (80%), leucomalachite green (97%), atrazine (95%), simazine (95%), glyphosate (95%), terbutryn (analytical standard grade), cypermethrin (95%) and 4-nonylphenol (89%) were purchased from Sigma–Aldrich (Saint Quentin, Fallaviers, France). Ciprofloxacin (98%), enrofloxacin (98%), oxolinic acid (97%), flumequine (analytical standard grade) and emamectin benzoate (81.0%) were supplied by Dr. Ehrenstorfer (Ausburg, Germany).

Stock solutions of pesticides were prepared in distilled water for testing acute toxic effects by *V. fischeri* bioassay. Aqueous solutions of most compounds were prepared using 2% (v/v) methanol. Solutions of atrazine and deltamethrin in water were prepared at 2–3% (v/v) of dimethyl sulfoxide (DMSO). Solutions of glyphosate were prepared in distilled water. Methanol analytical grade and DMSO were purchased from Merck (Darmstadt, Germany).

Previously to the development of toxicity tests, solutions of methanol and DMSO in distilled water were analyzed to check the absence of toxic effects for *V. fischeri*. The aqueous solutions of methanol and DMSO prepared within the percentage of 2–3%, did not show toxic effects. To determine EC_{50} of the target compounds, serial dilutions in distilled water and in seawater were done. For the solutions prepared in distilled water, the osmolality was adjusted to 2% NaCl for the optimal performance of this bioassay.

2.2. *V. fischeri* bioluminescence assay

Toxicity was evaluated using the bioassay based on the inhibition of the luminescence emitted by the bacteria *V. fischeri*. Light emitted from bacteria is a result of the interaction of the enzyme luciferase, reduced flavin, and a long-chain aldehyde in the presence of oxygen. The metabolic energy generated in this pathway is converted to