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Toxicology in Vitro 19 (2005) 879–886
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Ecotoxicological evaluation of diethanolamine using a battery of microbiotests

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Received 16 April 2005; accepted 17 June 2005
Available online 10 August 2005

Abstract

In order to investigate the potential ecotoxicity of diethanolamine (DEA), a battery of model systems was developed. DEA is widely used as a chemical intermediate and as a surface-active agent in cosmetic formulations, pharmaceuticals and agricultural products. DEA was studied using ecotoxicological model systems, representing four trophic levels, with several bioindicators evaluated at different exposure time periods. The battery included bioluminescence inhibition of the bacterium *Vibrio fischeri*, growth inhibition of the alga *Chlorella vulgaris* and immobilization of the cladoceran *Daphnia magna*. Cell morphology, total protein content, neutral red uptake, MTS metabolization, lysosomal function, succinate dehydrogenase activity, G6PDH activity, metallothionein levels and EROD activity were studied in the hepatoma fish cell line PLHC-1, derived from *Poeciliopsis lucida*. The systems most sensitive to DEA were both *D. magna* and *V. fischeri*, followed by *C. vulgaris* and the fish cell line PLHC-1. The most prominent morphological effect observed in PLHC-1 cultures exposed to DEA was the induction of a marked steatosis, followed by death at high concentrations, in some cases by apoptosis. The main biochemical modification was a nearly three-fold increase in metallothionein levels, followed by the stimulations of lysosomal function and succinate dehydrogenase and G6PDH activities. Judging by the EC_{50} values in the assay systems, DEA is not expected to produce acute toxic effects in the aquatic biota. However, chronic and synergistic effects with other chemicals cannot be excluded.

Keywords: Diethanolamine; In vitro; Ecotoxicology; Cytotoxicity; Contamination; Alternatives

1. Introduction

Model systems and bioassays are currently used in experimental ecotoxicology and environmental toxicology to provide information for risk assessment and registration of chemicals. The most promising alternatives in ecotoxicological evaluation involve the use of lower organisms with limited sentience and those which are not protected by legislation controlling animal experiments (i.e. bacteria, fungi, algae, plants and invertebrates; vertebrates used at the early stages of development from fish, amphibians, reptiles and birds to mammals; and, particularly, the employment of in vitro methods using material from these organisms) (Balls, 1998; Repetto et al., 2003). In environmental toxicology, in vitro systems are expected not only to allow for extrapolation from in vitro to in vivo toxic effects, but

Abbreviations: DEA, Diethanolamine; EC_{50}, Mean effective concentration; EROD, Ethoxyresorufin-*O*-deethylase; G6PDH, Glucose-6-phosphate dehydrogenase; LC_{50}, Mean lethal concentration; MTS, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)- 2H-tetrazolium, inner salt.

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doi:10.1016/j.tiv.2005.06.035

also to provide information on biological responses at the supra-individual and ecological levels. Furthermore, the use of in vitro methods in environmental testing, particularly those employing fish cell cultures, is an area of expanding possibilities in the ecotoxicological evaluation of mixtures, for controlling chemicals, emissions, effluents and hazardous wastes (Repetto et al., 2003; Castaño et al., 2003).

Diethanolamine (DEA) is a viscous liquid widely used as a chemical intermediate, as an anticorrosion agent in metalworking fluids and as a surface-active agent in cosmetic formulations, pharmaceuticals and agricultural products. The International Agency for Research on Cancer has included DEA in group 3: not classifiable regarding its carcinogenicity to humans (IARC, 2000). The US National Toxicology Program completed a study in 1998 that found an association between the increase of liver and kidney tumours in F344/N rats and B6C3F1 mice and the application of DEA. To date, there is a lack of available information about the toxic effects of DEA in the aquatic environment, thus it is important to investigate its potential ecotoxicity.

A battery of model systems was developed in order to investigate the potential ecotoxicity of DEA. The battery of ecotoxicological assays, representing four trophic levels, included bioluminescence inhibition of the bacterium *Vibrio fischeri* (decomposer), growth inhibition of the alga *Chlorella vulgaris* (first producer) and immobilization of the cladoceran *Daphnia magna* (first consumer). Although daphnia and alga tests are accepted in most environmental legislations, including OECD Guidelines for the testing of chemicals, the vibrio test has only been adopted by some regulations for the characterization of hazardous wastes. Cell morphology, total protein content, neutral red uptake, MTS metabolization, lysosomal function, succinate dehydrogenase activity, G6PDH activity, metallothionein levels and EROD activity were studied in the permanent fish cell line PLHC-1, derived from a hepatocellular carcinoma of the topminnow *Poeciliopsis lucida* (second consumer).

2. Materials and methods

2.1. Toxicant exposure

A range of different concentrations of exposure solutions of DEA (Sigma®) was prepared before use in different culture media, according to the appropriate assay, and sterilized by filtration through a 0.22 μm (Millipore®) filter. After replacing the medium with the exposure solutions, the systems were incubated for the adequate exposure time period.

2.2. Model systems

2.2.1. V. fischeri

Bioluminescence inhibition in the marine bacterium *V. fischeri* was evaluated according to Cordina et al. (1993) by using freeze-dried bacteria incubated at 15 °C from Microtox® test (Microbics Corp. Carlsbad, USA).

2.2.2. C. vulgaris

Growth inhibition of the alga *C. vulgaris var viridis* was evaluated in 96-well culture plates seeded with 200 μl/well of a 1,000,000 cells/ml algae culture in exponential growth phase in Bold's Basal Medium, using constant agitation and a temperature of 22 °C, under a water-saturated sterile atmosphere containing 5% CO_2 and a cold light source of 8000 lux. Absorbency at 450 nm was read on a Multiscan RC plate reader (Labsystem, Helsinki, Finland). As quality criteria the control cultures had to grow at least 10-fold in 48 h (Ramos et al., 1996).

2.2.3. D. magna

D. magna clone A (donated by Dr Muñoz-Reoyo, CISA, Spain) was maintained at 20 °C and fed with *C. vulgaris*. Acute toxicity immobilization tests were performed in standard reference water according to OECD Guideline 202 (2004) in replicate groups of 10 neonates in 25 ml, in 70 ml polystyrene flasks (Costar, Cambridge, MA, USA).

2.2.4. PLHC-1 cells

The permanent fish cell line PLHC-1, derived from an adult female *P. lucida*, a topminnow from the Sonoran desert (ATCC® # CRL-2406), was grown at 30 °C in a humidified incubator containing 5% CO_2 and propagated in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% foetal bovine serum (Gibco™), L-glutamine (BioWhitaker), sodium pyruvate (BioWhitaker) and non-essential amino acids (BioWhitaker). PLHC-1 cells in exponential growth phase were plated at a density of 450,000 cells/well in 96-well tissue culture plates (Costar). After 24 h at 30 °C, the cultures received 0.2 ml medium containing the test chemical and were incubated for a further 24, 48 or 72 h. Total cellular protein content was quantified in situ, using Coomassie brilliant blue G-250 (Bradford, 1976) in the same 96-well tissue-culture plates in which exposure originally took place (Repetto et al., 2001; Jos et al., 2003). Absorbency at 620 nm was read on a Multiscan RC plate reader (Labsystem, Helsinki, Finland). Neutral red uptake was evaluated according to Babich and Borenfreund (1987), and lysosomal function in relative form to protein content (Repetto and Sanz, 1993). The MTS tetrazolium reduction assay was performed according to a procedure based on Baltrop et al. (1991). The MTS tetrazolium