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Evaluation of toxicity and genotoxicity of 2-chlorophenol on bacteria, fish and human cells

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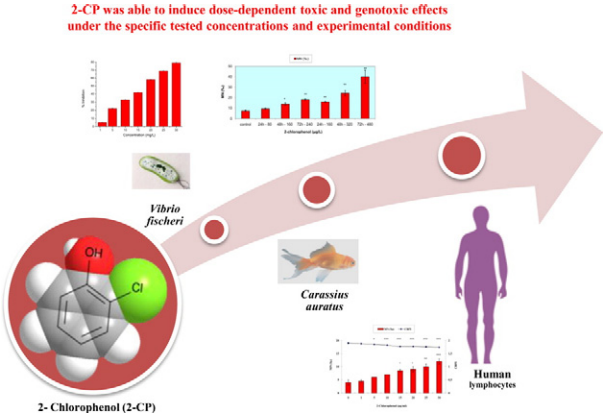
HIGHLIGHTS

- The toxic and genotoxic effects of 2-Chlorophenol were investigated.
- The genotoxic evaluation included the micronucleus test in fish and human cells.
- 2-Chlorophenol was able to induce dose-dependent toxic and genotoxic effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to the extensive use of chlorophenols (CPs) in anthropogenic activities, 2-Chlorophenol (2-CP), among other CPs, can enter aquatic ecosystems and can be harmful to a variety of organisms, including bacteria, fish and humans, that are exposed directly and/or indirectly to such contaminated environments. Based on the existing knowledge and in order to move a step forward, the purpose of this study is to investigate the toxic and mainly the genotoxic effects of 2-CP using a combination of bioassays. The tests include the marine bacterium *Vibrio fischeri* and micronuclei induction in the erythrocytes of *Carassius auratus* as well as in cultured human lymphocytes. The results obtained reveal that 2-CP is able to induce dose-dependent toxic and genotoxic effects on the selected tested concentrations under the specific experimental conditions.

1. Introduction

Numerous anthropogenic chemicals with potential harmful effects to organisms and the ecosystem, are released directly into the

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environment from industrial, domestic and agricultural sources. Once introduced into aqueous systems, pollutants might move up the food chain, causing unexpected effects at higher trophic levels and even in humans (Hickey et al., 2006; Igbinosa et al., 2013). Representatively, high exposure to various pollutants has been related to toxic effects that result in population decline of fish stocks around the world (Shear, 2006).

Chlorophenols (CPs) constitute an important class of aquatic pollutants that are present in aquatic ecosystems at concentration levels of ng/L and mg/L (Euro Chlor, 2002; Pera-Titus et al., 2004; Igbinosa et al., 2013; Antonopoulou et al., 2015). Due to their potential toxic effects to aquatic organisms and humans, several of them have been recognized as priority pollutants, by the US Environmental Protection Agency (US EPA) and European regulatory authorities (EU, 2007; Igbinosa et al., 2013; Antonopoulou et al., 2015). 2-Chlorophenol (2-CP), a low chlorine-substituted phenol and the precursor of the higher-substituted CPs, has been introduced into the environment *via* anthropogenic activities since it is mainly used as intermediate in the synthesis of the higher chlorinated congeners, certain dyes and pesticides (Cernakowa and Zemanovicowa, 1998; ATSDR, 1999; Michałowicz and Duda, 2007). 2-CP, as the other CPs, has also been detected in drinking-water as a disinfection by-product generated by the chlorination of phenols, as by-product of the reaction of hypochlorite with phenolic acids or as degradation product of phenoxy herbicides (WHO, 1989, 2003). The US EPA recommended that the exposure level of drinking water concentrations of 2-CP should not be more than 2.0 mg/L for longer term exposure and 0.04 mg/L for a lifetime exposure for a 70 kg adult (ATSDR, 1999).

Because of the widespread use of large volumes of CPs in modern industrial society (Krijgsheld and Van der Gen, 1986) and their simple formation as transformation products during biotic and abiotic processes, there is an increasing need for pertinent information on 2-CP implications in both aquatic organisms and humans.

Up to now, the available literature data on the ecotoxicity of 2-CP to aquatic organisms, has included mainly its deleterious effects on fish species (*Pimephales promelas*, *Poecilia reticulata*, *Oncorhynchus mykiss*, *Lepomis macrochirus*, *Leuciscus idus melanotus*, *Carassius auratus*, *Platichthys flesus*, *Solea solea*), aquatic invertebrates (*Daphnia magna*, *Daphnia pulex*, *Crangon sptemspinosa*), algae (*Scenedesmus subspicatus*, *Selenastrum carpicornutum*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Pseudokirchneriella subcapita*, *Dunaliella tertiolecta*) (Euro Chlor, 2002; Rijksinstituut Voor Volksgezondheid En Milieu–National Institute of Public Health and the Environment, 2001; Ertürk and Saçan, 2012), bioluminescent bacteria (*Bulkohoderia* species Rasc 2, *Pseudomonas fluorescens*, *Vibrio fischeri*) (Ribo and Kaiser, 1983; Kaiser and McKinnon, 1994; Sixt et al., 1995, Cronin and Schultz, 1996; Cronin and Schultz, 1997; Boyd et al., 2001, Jennings et al., 2001, Aruoja et al., 2011) and ciliated protozoan *Tetrahymena pyriformis* (Cronin and Schultz, 1996).

Although some reports have been conducted revealing mainly LC_{50} (median lethal concentration) and EC_{50} (effective concentration of the tested chemical at which 50% of the tested effect is observed) values of the tested organisms, there are a few studies regarding the potential DNA damages provoked by 2-CP to different organisms.

Since a single bioassay will never provide a full picture of the potential effects of anthropogenic chemicals in organisms and the environment, a minimum ecotoxicological *in vitro* test battery should include, among others, bacteria, fish cell lines, or cells isolated from fish and other species (Isooma and Lilius, 1995; Bierkens et al., 1998; Repetto et al., 2001).

Based on the existing knowledge and in order to move a step forward, the purpose of this study is to investigate the toxic and mainly the genotoxic effects of 2-CP using a combination of bioassays.

The genotoxicity tests include micronuclei induction in the erythrocytes of *Carassius auratus* as well as in cultured human lymphocytes.

The erythrocyte micronucleus test has been used with different fish species to monitor aquatic pollutants displaying mutagenic features in developed countries (De Flora et al., 1993; Saotome and Hayashi, 2003; Pantaleao et al., 2006). Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application of fish used as a test model, is to determine the distribution and effects of chemical contaminants in the aquatic environment and to assess the genotoxicity of water in the field and in the laboratory (Al-Sabti and Metcalfe, 1995).

In addition, cytotoxic and genotoxic effects were investigated in human lymphocytes by the use of the cytokinesis block micronucleus (CBMN) assay, widely performed for assessing the risk of different types of chemical substances and their ability to cause genetic damage and carcinogenetic processes in humans (Bonassi et al., 2011, OECD, 2014). Kirsch-Volders et al. (2011) stated that the implementation of *in vitro* micronucleus assays in the test battery for hazard and risk assessment of potential mutagens/carcinogens is fully justified.

To fully investigate the toxic effects of 2-CP to three different trophic levels, the marine bacterium *Vibrio fischeri* representing prokaryotic microorganisms was used.

The *Vibrio fischeri* bioassay is one of the most applied acute tests for the evaluation of toxicity of a great number of organic and inorganic contaminants being a standard (eco)toxicological bioassay in Europe (DIN EN ISO 11348) (Ma et al., 2014), due to its experimental simplicity, sensitivity, reproducibility and short exposure time (Kapusta and Stańczyk, 2015). In this method, the light emission is closely related to cellular metabolism, reflecting the metabolic status of the bacteria. When the luminescent bacteria are exposed to toxic substances the light intensity decreases rapidly. The reduction in light is attributable to the toxic effect of the tested substance (Gellert, 2000; Ma et al., 2014).

2. Materials and methods

2.1. Chemicals

2-Chlorophenol (2-CP) with ≥99% purity was supplied by Sigma–Aldrich (CAS No. 95–57-8). Cytochalasin-B (Cyt-B) was purchased from Sigma (St. Louis, MO, USA). Ham's F-10 medium, foetal bovine serum and phytohaemaglutinin were commercially supplied (Gibco, UK). HPLC-grade water, analytical grade of $ZnSO_4{}^*7H_2O$ and phenol were obtained by Merck (Darmstadt, Germany).

2.2. Luminescence inhibition assay with marine bacteria Vibrio fischeri

The acute effects of 2-CP on the bioluminescence of *Vibro fischeri* were assessed by monitoring changes in the natural bioluminescence of the bacteria using a Microtox® Model 500 Toxicity Analyzer (AZUR Environmental, 1998). 2-CP aqueous solutions in a medium containing 2% NaCl, toxic-free control solution (negative, 2% NaCl) and positive controls (phenol and $ZnSO_4{}^*7H_2O$) were tested and luminescence was recorded after 5,15, 30, 60 and 90 min of exposure at 15 °C, following the established protocol of the Microtox calculation program. EC_{50} value (concentration that causes a 50% reduction in the bacterial bioluminescence) were determined by means of the standard Microtox Omni software. All samples were analyzed in triplicate and mean values, whose standard deviation never exceeded 1.5%, are quoted as results. The acute ecotoxicity endpoint was determined as the EC_{50} with 95% confidence limits.

2.3. Micronucleus test in erythrocytes of Carassius auratus fish

2.3.1. Experimental animals

A group of 40 goldfish (*Carassius auratus*), were purchased from a local pet shop. Upon arrival, fish were divided into two groups (20 fish per group) and kept in glass aquariums of 125 L capacity each