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Biodegradability, toxicity and mutagenicity of detergents: Integrated experimental evaluations

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ABSTRACT

The widespread use of detergents has raised concern with regard to the environmental pollution caused by their active ingredients, which are biorefractory, toxic and persistent. Since detergents are complex mixtures of different substances, in which synergistic effects may occur, we aimed to assess the mutagenicity of different detergent formulations, taking into account aquatic toxicity and ready biodegradability. We performed a ready biodegradability test (OECD 301 F), *Daphnia magna* and *Vibrio fischeri* toxicity tests, and mutagenicity tests (Salmonella/microsome test, *Allium cepa* test and comet assay). Six detergent formulations were examined, 3 pre-manufacture and 3 commercially available. All detergents presented ready biodegradability. EC_{50} values varied for all products, according to the marker organism used, but were always higher than the more stringent value considered for aquatic toxicity assessment (*V. fischeri* 10–60 mg/L; *D. magna* 25–300 mg/L; *A. cepa* 250–2000 mg/L). None of the detergents caused mutations in bacteria. However, one commercial ecolabelled product induced an increase in micronucleus frequency in *A. cepa* root cells. All pre-manufacture detergents and one commercial one, which gave negative results in the Ames and *A. cepa* tests, induced DNA damage in human leukocytes. A more accurate evaluation of the environmental impact of complex mixtures such as detergents requires a battery of tests to describe degradation, as well as toxicological and mutagenic features.

1. Introduction

Detergents are complex mixtures of surfactants, builders, bleaching agents, enzymes, bleaching agent activators, fillers and other minor additives, such as dispersing agents, fabric softening clay, dye-transfer inhibiting ingredients, optical brighteners and perfumes (Ho Tan Tai, 2000; Pettersson et al., 2000; Stjerndahl and Holmberg, 2005; Yangxin et al., 2008).

Due to their huge consumption, more stringent environmental regulations and increasing awareness of the potential toxicity of detergents to humans and ecosystems have resulted in efforts to develop new classes of surfactants, builders and other constituents with a lower impact in terms of persistence and toxicity and better washing performance, as well as economic convenience. Besides conventional surfactants, often deriving from biological molecules, increasing attention has been also given to the so-called biosurfactants (Banat et al., 2000; Gunjikar et al., 2006; Stjerndahl and Holmberg, 2005; Yakimchuk et al., 2004; Yangxin et al., 2008). In parallel with the development and optimization of new substances, the evaluation of possible risks for consumers, and for human health and the environment in general, has become a crucial topic in science and policy, in accordance with recent European Regulations (EC 648/2004, 2004; EC 907/2006, 2006; EC 1336/2008, 2008 and EC 1272/2008, 2008).

The behaviour of a substance in the environment can be predicted according to its physical and chemical properties; moreover, biodegradability in water, soil and sediments provides important information for assessing its final fate (Glathe and Schermer, 2003; Sanderson et al., 2006; Gómez et al., 2007; Sharvelle et al., 2007). Several standard tests have been proposed and discussed in the scientific literature for evaluating biodegradability – ready,

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inherent, ultimate – (OECD, 1993, 2006; Blok and Struys, 1996; Pagga, 1997; Reuschenbach et al., 2003; Guhl and Steber, 2006; O' Malley, 2006; Boethling and Lynch, 2007; Stasinakis et al., 2008). Suitable tests should be established, based on usage, and hence on the main environmental compartments where a chemical can be found. In the case of detergents, for instance, aquatic environment should be considered, together with wastewater treatment plants. Although all conditions should be applied, aerobiosis is absolutely the most significant, since metabolic pathways are energetically favoured by molecular oxygen and degradation rates are usually higher. Nowadays, OECD 301 A–F ready biodegradability tests are a valuable means for monitoring the extent of biodegradation of chemicals, taking into account abiotic processes such as adsorption and hydrolysis. At present, as far as detergents are concerned, the inadequacy of these protocols for technical surfactant evaluation, based on their particular chemical nature, has been underlined (Richterich and Steber, 2001). In addition, some prescriptions, such as the 10-d window criterion (see Section 2.1), have been modified by legislative documents (Regulation EC 648/2004, 2004).

The aim of this research was to apply different characterization tests on final detergent formulations: according to the above-mentioned regulations, and the Ecolabel assignment criteria, pure chemicals or mixtures of structurally similar chemicals must be considered separately and a global evaluation must be carried out by means of specific calculations (e.g., for the assessment of CDV, Critical Dilution Volume, the limits of which in ecotoxicity impact assessment have recently been highlighted, together with alternative methods, such as USEtox (Van Hoof et al., 2011). Full details are available for several categories and also for specific compounds (Takamatsu et al., 1996; Jensen, 1999; Scott and Jones, 2000; Stjerndahl and Holmberg, 2005; Belanger et al., 2006; Mohan et al., 2006; Sharvelle et al., 2007; Sibila et al., 2008). A more accurate prediction of the eco-compatibility of a detergent, however, should not take only biodegradability into account, because synergistic factors can amplify toxicity, and biodegradation metabolites themselves can also become significant (Pettersson et al., 2000). Furthermore, specific aspects such as mutagenesis and alteration of endocrine systems, become more and more relevant, based on scientific knowledge.

Single components of detergents may exhibit toxic and mutagenic activity to different organisms, even at low concentrations. Quaternary ammonium compounds induce micronuclei in mammalian and plant cells (Ferk et al., 2007). Among the others, García et al., 2001, showed that the estimated concentration of amino-oxide-based surfactants yielding 50% immobilisation of *Daphnia magna* (IC_{50}) ranges from 6 to 45 mg/L, whereas the estimated concentration resulting in a 50% reduction of bacteria luminescence EC_{50} ranges from 0.1 to 11 mg/L; non-ionic surfactants and their degradation products are toxic to marine and fresh-water organisms (Ying, 2006) and have endocrine effects on fish (Jobling and Sumpter, 1993; Purdom et al., 1994; De Weert et al., 2011); sodium citrate induces chromosomal aberrations in *Allium cepa* (Türkoğlu, 2007); sodium perborate and polycarboxylates induce point mutations in bacteria (Kaplan et al., 2004; Seiler, 1989); corrosion suppressants, such as benzotriazole, as well as other additives, are toxic to water organisms, such as bacteria, invertebrates (crustaceans) and fish used as standard aquatic laboratory test organisms (Pillard et al., 2001); the components of some perfumes, e.g., cinnamaldehyde, react with DNA (Stammati et al., 1999), and limonene produces tumours in male rats by a non-DNA-reactive mechanism which is not relevant to humans (IARC, 1999). Very few studies that have considered the toxic effects of final detergent formulates or, more specifically, taken the effects on algae into account, showing the ability of these mixtures to interfere with cellular permeability, affect cell mobility, and inhibit some specific functions, such as photosynthesis or biosynthesis and biodegradation pathways (Aizdaicher and Markina, 2006; Azizullah et al., 2011). Acute toxicity in *D. magna* was also studied (Pettersson et al., 2000) but no information on their mutagenicity is available.

A more accurate assessment of the mutagenicity of a detergent should take into account possible interactions between single chemicals, which might play a crucial role in such complex mixtures. Therefore, it may be useful to test raw detergents rather than single components. Furthermore, some properties, such as mutagenicity and the ability to influence endocrine system activity (''endocrine disruption''), have raised more and more concern in recent years (WHO, 2002', 2004).

This paper reports the results of a battery of biological tests performed on various pre-manufacture (not yet available on the market) and commercial detergents for laundry, dishwashers and washing up.

2. Materials and methods

Ready biodegradability, toxicity and genotoxicity of some detergents in their complete formulation were examined. Three pre-manufacture detergents pending ecolabel certification (A, B and C, for laundry, dishwashers and washing-up, respectively), two ecolabelled commercial detergents (E and F, for dishwashers and laundry, respectively) and a conventional one (D, for dishwashers) were studied.

2.1. Detergents tested

Six detergent formulations, either pre-manufacture or already commercially available, were examined. Their usage, surfactant contents and other constituents, and COD (chemical oxygen demand) concentration are listed in Table 1.

Detailed manufacturer's information on surfactant content was used for new detergents, and consumer information appearing on the packaging or website allowed a rough evaluation for commercial products.

2.2. Ready biodegradability test

Detergents underwent a ready biodegradability test according to OECD 301 F (Manometric Respirometry Test) (OECD, 1993).

The biodegradation test described by OECD 301 F was carried out using the respirometric BOD OxiTop method (APHA, 1998). Activated sludge taken from a municipal waste water treatment plant was used as inoculum. Abiotic sterile control was obtained by dosing NaClO. Glucose and sodium glutamate (1:1 w/w ratio) were added as reference organic compounds. Since OECD 301 F consists of a respirometric test, the "pass level" for ready biodegradability corresponds to a 60% decrease in initial ThOD (theoretical oxygen demand) or initial COD, in cases where the test material was insufficiently defined and ThOD could not be calculated. Measurement of COD (colorimetric method 5220-D) (APHA, 1998) was carried out before and at the end of testing. Raw samples were filtered through 0.45 μm cellulose acetate membranes (model 25CS045AN, Advantec MFS Inc.).

2.3. Toxicity tests

EC_{50} (24 h) tests were conducted with Cladocer crustacean *D. magna* Straus for each detergent studied, according to official Italian standards (APAT–IRSA/CNR, 2003); liquors deriving from the OECD 301 F test underwent the assay for direct evaluation of effluent toxicity after 24 and 48 h (APAT–IRSA/CNR, 2003). In EC_{50} evaluation, preliminary screening tests were carried out on detergent formulation concentrations from 0.1 to 100 g/L, as prescribed, whereas subsequent tests were carried out on dilutions down to a concentration of 1 mg/L. Daphnids (neonates less than 24 h old, obtained by hatching ephippia—Ecotox LDS Srl) were introduced into 50 mL glass vessels (5 and 10 organisms/vessel for preliminary and final tests, respectively), together with samples diluted in a standard mineral solution (pH=7.5–8.5; alkalinity=110–120 mg $CaCO_3$/L; hardness=140–160 mg/L $CaCO_3$/L; 10 mg KCl/L, 192 mg $NaHCO_3$/L, 53 mg $MgSO_4$/L, 183 mg $CaSO_4 \cdot 2H_2O$). Bioassays were conducted under static conditions, measuring dissolved oxygen and pH in each sample at both the start and end of testing. Dosage calculation was based on mass/volume ratio; in the event of liquid formulations, density data were used. EC_{50} and 25% confidence limits were calculated as prescribed by the standard method. In the toxicity evaluation of the liquors after biodegradability tests, crustaceans were introduced directly into the samples. All the experiments were performed in triplicate. Test conditions (temperature: 20 ± 1 °C—kept in a refrigerated thermostat, Ecotox LDS Srl, mod. ECO 96/CRS.A; darkness/irradiation-