WATER RESEARCH 40 (2006) 267–272

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# Toxicity evaluation of surface water treated with different disinfectants in HepG2 cells

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ARTICLE INFO

*Article history:*
Received 12 April 2005
Received in revised form
26 October 2005
Accepted 29 October 2005
Available online 19 December 2005

*Keywords:*
Water disinfection
Chlorine dioxide
Peracetic acid
Sodium hypochlorite
Oxidative stress
HepG2 cells
Disinfection by-products (DBPs)

ABSTRACT

It is well known that water disinfection through chlorination causes the formation of a mixture of disinfection by-products (DBPs), many of which are genotoxic and carcinogenic.

To demonstrate the formation of such compounds, a pilot water plant supplied with water from Lake Trasimeno was set up at the waterworks of Castiglione del Lago (PG, Italy).

The disinfectants, continuously added to prefiltered lake water flowing into three different basins, were sodium hypochlorite, chlorine dioxide and peracetic acid, an alternative disinfectant used until now for disinfecting waste waters, but not yet studied for a possible use in drinking water treatment.

The aim of this study was to evaluate the formation during the disinfection processes of some toxic compounds that could explain the genotoxic effects of drinking waters.

Differently treated waters were concentrated by solid-phase adsorption on silica $C_{18}$ columns and toxicity was assessed in a line of human hepatoma cells (HepG2), a metabolically competent cellular line very useful for human risk evaluation.

The seasonal variability of the physical–chemical water characteristics (AOX, UV 254 nm, potential formation of THM, pH and temperature) made indispensable experimentation with water samples taken during the various seasons.

Autumn waters cause greater toxicity compared to those of other seasons, in particular dilution of the concentrate at 0.5 l equivalent of disinfected waters with chlorine dioxide and peracetic acid causes a 55% reduction in cellular vitality while the cellular vitality is over 80% with the all other water concentrates. Moreover it is very interesting underline that non-cytotoxic quantities of the autumnal water concentrates cause, after 2 h treatment, a decrease in GSH and a statistically significant increase in oxygen radicals, while after prolonged treatment (24 h) cause a GSH increase, without variations in the oxygen radical content. This phenomenon could be interpreted as the cellular adaptation response to an initial oxidative stress.



## 1. Introduction

Toxicological problems resulting from water disinfection do not only depend on the presence of pollutants in reservoirs, but also on the disinfection processes themselves. Chlorination is one of the main procedures for disinfecting raw water for drinking water purposes, but this process results in the formation of mutagenic/carcinogenic disinfection

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doi:10.1016/j.watres.2005.10.038

by-products (DBPs) deriving from the reaction of the chlorine with organic compounds (humic and fulvic acids) naturally or by pollution present in water (WHO, 1996; Boorman et al., 1999). Chlorinated water is mutagenic in bacteria (Kargalioglu et al., 2002) and induces a genotoxic effect on mammalian cells (Lu et al., 2002; Plewa et al., 2002). Epidemiological studies provide evidence that the consumption of chlorinated drinking water may be associated with an increased incidence of some specific types of human cancer and still-birth risk (Koivusalo et al., 1994; Bull et al., 1995; Nissinen et al., 2002; King et al., 2000).

There is a need to improve disinfection techniques and to widen knowledge about potential toxicity and problems for public health that may derive from the introduction of alternative disinfectants. For example, peracetic acid (PAA, $CH_3$-COOH) already used in the treatment of waste waters (reusing municipal and industrial waste water) (Caretti and Lubello, 2003; Gehr et al., 2003) and in many applications in hospital laboratories and factories (Baldry et al., 1991) deserves to be studied for its application in drinking water disinfection. The disinfecting power of this substance is based on oxidative reactions, but very little is known about the possible risk of the formation of by-products. The only DBP produced with this substance in pilot plants and identified using gas chromatography/mass spectrometry (GC/MS) was carboxylic acid not recognised as mutagenic in bacterial mutagenicity tests (Monarca et al., 2002), even if the plant genotoxicity in vivo tests (*Tradescantia*/micronucleus test and root anaphase aberration assay and micronucleus assay in *Allium cepa*) show positive results (Monarca et al., 2003).

This study was as part of a research programme designed to evaluate the influence of different disinfection treatments on the formation of toxic and genotoxic substances in surface waters used for human consumption with a complex battery of test in vivo (aquatic organisms and plants) and in vitro (human leukocytes, HepG2, Salmonella yeasts) (Monarca et al., 2004; Buschini et al., 2004; Bolognesi et al., 2004).

The research was performed at a drinking water pilot plant fed with lake water that, after sedimentation and granular-activated carbon filtration, was divided into four basins: three used for disinfection treatment with sodium hypochlorite (NaClO), chlorine dioxide ($ClO_2$) and peracetic acid (PAA), respectively and one for untreated water as a control (Monarca et al., 2004).

In particular, this paper will assess the possible damage, due to oxidative substances present or forming in waters obtained from the pilot plant, in a human hepatoma cell line (HepG2), metabolically competent cells that can be an useful tool for the evaluation of human risk assessment.

The lake water samples were collected during three periods of the year to assess the three disinfection treatments in various physical–chemical conditions due to the seasonal differences (Monarca et al., 2004). In fact there are many factors that can modify the DBPs formation: the quality, pH and temperature of the waters (Sadiq and Rodriguez, 2004) plus the concentrations and contact times of the disinfectants themselves (Korn et al., 2002).

## 2. Materials and methods

### 2.1. Water source and treatment

A pilot drinking water plant supplied with water from Lake Trasimeno (Central Italy) was used to compare different disinfection treatments (NaClO, $ClO_2$, PAA). This lake water has a high concentration of total organic carbon (TOC 4–9 mg/l) and bromide (0.6–0.7 mg/l), which are potential DBP's precursors.

The study was conducted over a period of about 1 year in different seasons (October 2000, February 2001 and June 2001).

After sedimentation, filtration and neutralisation, the water was divided into four independent stainless steel basins (contact basins), three used for disinfection treatments and one for raw water as control. The water flow from the contact basins down further four stainless steel basins (exposure basins) for in situ exposure of bioindicators (fish, molluscs and plants). The doses of disinfectants added (mean concentration) were adjusted to produce a disinfectant residual lower than 0.2 mg/l to avoid toxicity on the bioindicators.

### 2.2. Solid-phase extraction of water samples

The concentration of water samples was carried out at a 10–15 ml/min flow rate on trifunctional $C_{18}$ cartridges (10 g Sep-Pak Plus tC18 Environmental Cartridges, Waters Chromatography, Milford, MA, USA) according to the US Environmental Protection Agency 525.2 method. (USEPA, 1994). The extracts, eluted from cartridges with ethyl acetate, dichloromethane and methanol (40 ml of each solvent), were first dried with a rotary evaporator, then redissolved in DMSO and later used for in vitro assays.

### 2.3. Cell cultures and treatments

The human HepG2 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 0.03% glutamine, 0.11 g/l pyruvate, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified atmosphere with 5% $CO_2$ at 37 °C.

The medium was changed every 2–3 days and the tests were carried out on confluent cells 7 days after subculture. The confluent monolayers of the HepG2 cell line were treated for 2 and 24 h with a medium containing the water concentrates at the amounts indicated in the individual figures and dissolved in DMSO. All treated and control samples contained a 0.1% final concentration of DMSO.

### 2.4. Cytotoxicity assay

Neutral red uptake (NRU) on the 96-well microplates was measured using the method of Zhang et al. (1990).

### 2.5. Intracellular-reduced glutathione measurements

The samples for measuring GSH were processed according to the method of Hissin and Hilf (1976). The cultured monolayers