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SHORT COMMUNICATION

Screening of estrogen-like activity of mineral water stored in PET bottles

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Abstract

Bottled mineral water consumption is steadily rising in the World. Italy is the largest natural mineral water consumer in Western Europe, about 200 L per capita per annum. Recently, research has concentrated upon emerging toxicological problems such as the presence in drinking water of substances which interfere with the function of the endocrine system; defined as endocrine disruptors (EDs). The aim of this study was to assess the risk of exposure of the population to chemicals with estrogen-like activity through mineral water consumption by monitoring the presence of estrogenic compounds in mineral water bottled in polyethylene terephthalate (PET). A solid phase extraction (SPE) with C18 cartridges was carried out. The estrogenic activity of the extracts was assayed using a yeast assay expressing the human estrogen receptor α (hERα). This preliminary study shows that more than 90% of the water samples did not exhibit any appreciable estrogenic activity. The highest estrogenic activity detected in water extracts was equivalent to the activity induced by 23.1 ng/L of the natural hormone 17β-estradiol. Some mineral water samples showed toxicity on yeast cells.

Keywords: Natural mineral water; Estrogenic activity; Yeast assay

Introduction

Recently a significant increase in mineral water consumption has been recorded. We believe this is for reasons of safety and health (it is widely believed that mineral water is more hygienic and better from a nutritional point of view than tap water), and because people prefer its taste. Indeed, disinfecting techniques and the release of materials from network pipelines may introduce an unpleasant taste. Italy ranks first in mineral water consumption (about 200 L per capita per annum) not only in Europe, but also in other parts of the world, and mass consumption is by people who can be more vulnerable to damage (new mothers, babies, teenagers).

Currently, 80% of mineral water is sold in plastic containers, normally polyethylene terephthalate (PET). Glass bottles (24%) are primarily destined for restaurants or biological feeding, while the cardboard packaging (Tetra Pak®) seems destined to come off the market or to be confined to commercial (1.5%) niches.

Studies carried out to date, in order to assess the adverse effects in humans for exposure to low doses of chemicals through water consumption, have mainly considered carcinogenic and mutagenic risks, but recent research has discovered an emerging aspect in environmental toxicology and in food safety, related to the presence in drinking water of substances that possess biological activity on the reproductive system (Wenzel et al., 2003).

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It is widely believed that the decline of the reproductive functions in males, the increasing incidence of breast cancer in young women, and alterations of the thyroid function and neurobehavioural diseases observed in the population of different countries may be, partly, attributable to exposure to several hormone-like environmental chemicals, particularly during the intrauterine development or in critical periods in development.

Recent studies have focused attention on the migration of chemicals from plastic containers to the water. Among these are plasticizers – additives used to impart flexibility and handling properties to several kinds of plastics. Di(2-ethylexyl)phthalate (DEHP) is a widely used plasticizer in the world for polyvinyl chloride products but it is also present in PET (Balafas et al., 1999; Bošnir et al., 2007; Harris et al., 1997). Despite PET being a material characterized by elevated chemical inactivity, various studies point out that different storage conditions (such as exposure to sunlight and high temperatures and the duration of the same) can contribute to migration of chemicals from the bottles to the water (Dabrowska et al., 2003; Evandri et al., 2000; Nawrocki et al., 2002; Westerhoff et al., 2008).

The release of substances with hormone-like activity from packaging material to water may represent a risk factor for humans to develop chronic pathologies following long-term exposure to low doses of chemicals. Recently, it has been shown that the DEHP alters the genic expression in rats and that, at appropriate concentrations, it alters the development of the central nervous system in the fetus (Latini et al., 2006; Liu et al., 2005).

Moreover, it is important to consider that the action of chemical contaminants on the endocrine system generally takes place at lower doses than those necessary to induce acute toxic effects. Some authors have shown that arsenic, an ED that can be found in water for human consumption, both in tap water and mineral water, acts on altering the hormonal function of the glucocorticoid system at a concentration notably lower than normally necessary to cause toxic effects (Kaltreider et al., 2001).

In this work the overall estrogenicity of organic extracts of nine commercial brands (brand ID #) of Italian bottled natural mineral water was evaluated. A recombinant yeast-based in vitro assay (YES) was used to test for the ability of the extracts to bind the human estrogen receptor α.

Materials and methods

A total of 30 samples of different commercial brands ($n = 9$) of Italian mineral water packaged in plastic containers (PET) purchased from local markets were analysed for estrogenic activity using the Yeast Estrogen Screen (YES). Ninety-three percent of the analysed samples had total dissolved solids (TDS) values between 39 and 276 mg/L and a pH range of 7.21–8.2. According to TDS values at 180 °C, the Italian legislation classifies mineral natural water as low mineral content water (TDS < 500 mg/L), as medium mineral content water (500 < TDS < 1500 mg/L), as high mineral content water (> 1500 mg/L). More than 50% of mineral water commercially sold in Italy is of low mineral content, about 25% is of medium mineral content.

Water samples were extracted by solid-phase extraction (SPE) on C18 cartridges (Supelco, Bellefonte, USA) previously conditioned with 15 mL methanol (Carlo Erba, Milan, Italy) followed by 15 mL *n*-hexane (Carlo Erba, Milan, Italy), at a flow rate of 15 mL/min. The volume was chosen assuming an average daily consumption of 1.5 L per capita. Depending on the climate and on physical activity the daily water requirement of an individual can vary, but the daily assumption of 1.5–2 L for an adult and 1 L for children is recommended by WHO (2000).

The cartridges were then dried under a gentle flow of nitrogen for 1 h and eluted sequentially with 5 mL of methanol and 5 mL of *n*-hexane. Recovery efficiency (average of three determinations ± SD) of estrogenic compounds has been assessed with standard diethylstilbestrol (DES) (Sigma, Milan, Italy). Adsorbates were dissolved in dimethyl sulfoxide (DMSO) for the in vitro assay.

The yeast strain used was *S. cerevisiae* RMY326 (His3 Leu2-3, 112 trp1-1 ura3-52/hER-TRP1-2μ[pG/ER(G)], ERE-CYC-Lac*Z*-URA3-2μ[pUCΔSS-ERE], HIS-3CEN/ARS[pRS423]). This strain contains the human estrogen receptor α (hERα) and an estrogen-responsive element (ERE) bound to the reporter gene lac*Z* encoding for the enzyme β-galactosidase (Liu and Picard, 1998).

The activation of the receptor due to the formation of a complex receptor–ligand causes expression of the reporter gene lac*Z*. The production of the enzyme β-galactosidase is measured with a spectrophotometer (OD_{420} nm) (Pinto et al., 2004; Garritano et al., 2006), normalised to the number of cells assayed and expressed as Miller units using the following formula (Miller, 1972):

$$\beta - \text{gal units (M.U.)} = (1000 \times OD_{420})/(t \times V \times OD_{600})$$

t is the length of incubation (min); V is the volume of culture used in the assay (mL). Water samples were analysed at final concentrations $100X$ and $200X$.

Ultra-pure "endotoxin-free" water (Sigma-Aldrich, Milan, Italy) was used as a control of critical points in extraction procedure (blank). Vehicle was used as a negative control. For comparison, tap water samples from local distribution networks (treated surface water, groundwater and spring water) were analysed.