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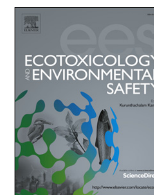

Evaluation of genotoxic effects of surface waters using a battery of bioassays indicating different mode of action

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ABSTRACT

With the burgeoning contamination of surface waters threatening human health, the genotoxic effects of surface waters have received much attention. Because mutagenic and carcinogenic compounds in water cause tumors by different mechanisms, a battery of bioassays that each indicate a different mode of action (MOA) is required to evaluate the genotoxic effects of contaminants in water samples. In this study, 15 water samples from two source water reservoirs and surrounding rivers in Shijiazhuang city of China were evaluated for genotoxic effects. Target chemical analyses of 14 genotoxic pollutants were performed according to the *Environmental quality standards for surface water* of China. Then, the *in vitro* cytokinesis-block micronucleus (CBMN) assay, based on a high-content screening technique, was used to detect the effect of chromosome damage. The SOS/*umu* test using strain TA1535/pSK1002 was used to detect effects on SOS repair of gene expression. Additionally, two other strains, NM2009 and NM3009, which are highly sensitive to aromatic amines and nitroarenes, respectively, were used in the SOS/*umu* test to avoid false negative results. In the water samples, only two of the genotoxic chemicals listed in the water standards were detected in a few samples, with concentrations that were below water quality standards. However, positive results for the CBMN assay were observed in two river samples, and positive results for the induction of *umu*C gene expression in TA1535/pSK1002 were observed in seven river samples. Moreover, positive results were observed for NM2009 with S9 and NM3009 without S9 in some samples that had negative results using the strain TA1535/pSK1002. Based on the results with NM2009 and NM3009, some unknown or undetected aromatic amines and nitroarenes were likely in the source water reservoirs and the surrounding rivers. Furthermore, these compounds were most likely the causative pollutants for the genotoxic effect of these water samples. Therefore, to identify causative pollutants with harmful biological effects, chemical analyses for the pollutants listed in water quality standards is not sufficient, and single-endpoint bioassays may underestimate adverse effects. Thus, a battery of bioassays based on different MOAs is required for the comprehensive detection of harmful biological effects. In conclusion, for genotoxicity screening of surface waters, the SOS/*umu* test system by using different strains combined with the CBMN assay was a useful approach.

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1. Introduction

The increasing contamination of surface water with thousands of industrial and natural compounds is a key problem for environmental safety and human health (Schwarzenbach et al., 2006). With some surface waters being a supply for drinking water, contamination can affect human health indirectly. Based on epidemiological evidence, links are found between contaminants in drinking water and colorectal and bladder cancers (Boffetta, 2006; Zhou et al., 2002). Although many guidelines for water quality include a long list of pollutants, it is difficult to estimate the entire suite of adverse effects. Bioassays that evaluate potential genotoxic effects of source and river waters are essential (Charalampous et al., 2015; Radic et al., 2010; Warren et al., 2015; Yu et al., 2011). *In vitro* bioassays provide high-throughput, short-term, and low-cost measurements of potential toxicity of chemicals and have been used frequently in water quality assessments in

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recent decades (Jiang et al., 2012; Mehinto et al., 2015; Wei et al., 2012). A single bioassay can detect specific effects of chemicals that have the identical endpoint or mode of action (MOA). However, because of the complex components in environmental samples, a single bioassay cannot examine all the pathways of bioactivity of samples that are mixtures of contaminants. Therefore, the guidelines published by responsible agencies typically suggest at least two endpoints (gene mutation and chromosome alterations) be evaluated in the genotoxicity assessment of chemicals (COM, 2000; ICH, 2011). Furthermore, applications of a battery of bioassays to rapidly screen for potential genotoxic effects and discriminate water samples of different quality continue to be a trend in water quality assessment (Escher et al., 2014; Yan et al., 2014b). Although the genotoxic responses induced by environmental water samples have been observed in previous studies (Wang et al., 2011; Warren et al., 2015), many of these targeted a single endpoint or ignored a particular MOA for a chemical, which may both lead to underestimating of genotoxic effects.

The cytokinesis-block micronucleus (CBMN) assay is widely used to detect effects of chromosome damage. Recently, a high-content screening (HCS) technique was developed in the rodent cell line CHO-K1 that complements the CBMN assay by automatically analyzing cells treated with fluorescent dyes in microplates (Diaz et al., 2007; Ogata et al., 2011; Westerink et al., 2011). The HCS provides several key advantages over the traditional method used with the bioassay. In particular, the user can customize multiplex endpoints and gates for specified cells for an integrated assessment of cellular toxicity.

The SOS/*umu* test established by Oda et al. (1985) is based on the ability of DNA-damaging agents to induce *umu*C gene expression in strain *Salmonella typhimurium* (*S. typhimurium*) TA1535/pSK1002. This assay has been frequently used to detect the genotoxic effects of chemicals and water samples (Escher et al., 2014; Yasunaga et al., 2004). Moreover, the *O*-acetyltransferase (*O*-AT)-overproducing strain *S. typhimurium* NM2009 with S9 and the *O*-AT- and nitroreductase (NR)-overproducing strain NM3009 have extremely sensitive responses to aromatic amines and nitroarenes, respectively, compared with the parental strain TA1535/pSK1002 (Oda et al., 1993, 1995).

The aim of this study was to provide new and comprehensive insight into the genotoxic effects of source and river waters. Fifteen water samples were collected from two source water reservoirs and surrounding rivers in Shijiazhuang city of China, and target chemical analyses and *in vitro* bioassays were used in succession to determine the genotoxicity of the water samples. The target chemical analyses for genotoxic pollutants were conducted following the *Environmental quality standards for surface water* of China (MEPC, 2002). The CBMN assay based on the HCS technique and the SOS/*umu* test were used to examine genotoxic effects that resulted from different MOAs. To avoid false negative results, not only the parental strain TA1535/pSK1002 but also the strains NM2009 and NM3009 were used in the SOS/*umu* test.

2. Materials and methods

2.1. Materials

S. typhimurium strains TA1535/pSK1002, NM2009 and NM3009 used in the tests were obtained from Professor Yoshimitsu Oda. CHO-K1 cells were purchased from the Cell Culture Center, Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences, Beijing, China.

The chemicals including mitomycin C (MMC, CAS 50-07-7), 4-nitroquinoline-*N*-oxide (4-NQO, CAS 56-57-5), 2-aminoanthracene (2-AA, CAS 613-13-8), benzo[α]pyrene (B[α]P, CAS 50-32-8), 1-nitropyrene (1-NP, CAS 5522-43-0), dimethylsulfoxide (DMSO, CAS 67-68-5), Hoechst 33342 (CAS 23491-52-3), cytochalasin B (CAS 14930-96-2), and *o*-nitrophenyl-D-galactopyranoside (ONPG, CAS 396–07-3) were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). HPLC-grade dichloromethane, *n*-hexane and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ham's F12 medium and fetal calf serum were obtained from HyClone (Thermo Scientific, Logan, UT, USA). Other reagents and chemicals were purchased from reliable sources and were the highest quality available.

2.2. Sample collection and processing

As shown in Fig. 1, water samples were collected from 15 sites in Shijiazhuang, China. The sites S5-S15 were on the rivers that flowed into two reservoirs. The site S1 was at the entry point of the Gangnan reservoir for the rivers with sites S8 and S10, S3 was at the entry of the Huangbizhuang reservoir for the river with site S9, and S2 and S4 were at the outlets of the two reservoirs. Sample collection and processing for chemical analyses followed the standard methods according to the *Environmental quality standards for surface water* of China (MEPC, 2002), each method was listed in Table S1. Sample collection and processing for bioassays were performed as described by Yan et al. (2014b) with slight modification. Briefly, a total of 30 L of water per site was collected in amber glass containers and transported to the laboratory at the sites immediately for processing. Water was filtered through 0.45 μm glass filters (APFF; Millipore, USA) to remove insoluble materials. Five Oasis HLB cartridges (Waters, Milford, USA) were conditioned and used to isolate the organic extracts from 30 L of water per sample. Then, 10 mL of dichloromethane was used to elute each cartridge. The elute extracts of each water sample were dried under gentle flow of nitrogen and dissolved in 300 μL of DMSO (extracts of the S3 sample were dissolved in 500 μL). Then, the extract solutions in DMSO were diluted by 1:1 (6 steps) for bioassays.

2.3. Target chemical analyses

Of the chemicals listed in the *Environmental quality standards for surface water* of China (MEPC, 2002), those known to cause a positive response in the SOS/*umu* test and MN assay were analyzed by standard chemical methods as shown in Table S1.

2.4. CBMN assay

The CBMN assay was conducted according to Yan et al. (2014a) and Westerink et al. (2011). Briefly, a complete culture medium was prepared with 90% F12 medium, 10% fetal calf serum, and 1% penicillin–streptomycin (10,000 U/mL, HyClone). Cells were maintained in complete culture medium with an atmosphere of 5% CO_2 at 37 °C. For the MN test, CHO-K1 cells were seeded at a density of 4000 cells/well in a 96-well microplate and cultured for 24 h at 37 °C under 5% CO_2. Then, the culture medium was gently removed, and 100 μL of fresh culture medium was added that contained 0.5% of the extract solutions. DMSO was used as the solvent control, and MMC was used as the positive control. The final DMSO concentration was 0.05%, and all samples were analyzed in triplicate. After 24 h, the exposure medium was replaced with 100 μL of fresh culture medium containing 3 μg/mL of cytochalasin-B, and cells were cultured for an additional 24 h. After exposure, the medium was removed, and 100 μL of 4% paraformaldehyde in PBS was added to fix the cells for 10 min at room temperature. Following fixation, the cells were gently washed with PBS, and 100 μL of staining solution containing Hoechst 33342 (1 μM) was added to stain the nuclei at room temperature for

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