A preliminary Ames fluctuation assay assessment of the genotoxicity of drinking water that has been solar disinfected in polyethylene terephthalate (PET) bottles

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ABSTRACT

Though microbially safe, concerns have been raised about the genotoxic/mutagenic quality of solar-disinfected drinking water, which might be compromised as a result of photodegradation of polyethylene terephthalate (PET) bottles used as SODIS reactors. This study assessed genotoxic risk associated with the possible release of genotoxic compounds into water from PET bottles during SODIS, using the Ames fluctuation test. Negative genotoxicity results were obtained for water samples that had been in PET bottles and exposed to normal SODIS conditions (strong natural sunlight) over 6 months. Under SODIS conditions, bottles were exposed to 6 h of sunlight, followed by overnight room temperature storage. They were then emptied and refilled the following day and exposed to sunlight again. Genotoxicity was detected after 2 months in water stored in PET bottles and exposed continuously (without refilling) to sunlight for a period ranging from 1 to 6 months. However, similar genotoxicity results were also observed for the dark control (without refill) samples at the same time-point and in no other samples after that time; therefore it is unlikely that this genotoxicity event is related to solar exposure.

Key words | Ames fluctuation assay, mutagenicity/genotoxicity, PET bottles, SODIS, solar water disinfection

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INTRODUCTION

The importance of genotoxic testing of drinking water is justified by epidemiological studies that have shown a link between increased cancer risk and genotoxicity in drinking water, particularly during repeated and extended use (Koivusalo *et al.* 1995; Haider *et al.* 2002; Lah *et al.* 2005*a*). The potential presence of genotoxins in water results not only from anthropogenic activities such as pharmaceutical, biocidal and industrial chemical contamination, but also from water treatment methods (Lah *et al.* 2005*b*). Disinfection of drinking water to remove and inactivate pathogens by chlorination, ozone and UV-irradiation have been shown to release disinfection by-products that were found to be potentially genotoxic on testing with short-term mutagenicity tests (Zoeteman *et al.* 1982; Haider *et al.* 2002).

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Solar disinfection (SODIS) is a water treatment method that has been demonstrated as an appropriate point-of-use water disinfection method. The SODIS technique involves filling transparent containers with biologically contaminated water and exposing the containers to direct sunlight. The water is considered microbiologically safe to drink after a minimum of 6 h exposure (Acra *et al.* 1980; McGuigan *et al.* 1998; Dejung *et al.* 2007). It is recommended that solar disinfected water is consumed within 24 h of exposure. Most SODIS bottles are made from PET because of its robustness, efficient transmittance of UV-A, light weight and ease of availability in most communities (Dejung *et al.* 2007). During the SODIS process, prolonged solar exposure may lead to photoproducts leaching from the PET container material resulting in the generation of

genotoxic substances. Until recently this risk has remained unexamined.

Numerous studies have been conducted on PET because of its increased use as a packaging and bottling material for water, beverages and food. Previous studies explored the effect of consumer use, long-term storage and storage conditions on the migration of compounds from PET into water and food (Nawrocki *et al.* 2002; Monturi *et al.* 2007; Morrison *et al.* 2008). The thermal degradation products of PET identified include: formaldehyde, acetaldehyde, acetone, CO_2 and water (Villian *et al.* 1994; Nawrocki *et al.* 2002; Mutsuga *et al.* 2006) and plasticisers such as di(2-ethylhexyl)phthalate, di(2-ethylhexyl)adipate, phthalic acid, dimethyl phthalate, diisobutyl phthalate and dibutyl phthalate (Monturi *et al.* 2007). Analytical methods such as high performance liquid chromatography (HPLC), gas chromatography (GC) and mass spectrometry (MS) proved invaluable in identifying and quantifying these compounds. However, the biological effects and toxicological relevance of these compounds at varied concentrations remains uncertain (Evandri *et al.* 2000); some compounds might be released at high concentrations but not necessarily have a genotoxic effect, while others might be found at low concentrations and have a highly mutagenic effect. Therefore, short-term genotoxicity tests were conducted to determine the genotoxic risk presented by leaching of compounds from PET. Results obtained varied according to the genotoxic test used, physicochemical conditions to which the PET bottles were exposed, the type of water stored and the PET bottle used (Ames *et al.* 1975).

Using the prokaryotic Ames test (in solid agar medium) with *Salmonella typhimurium* (TA98 and TA100 strains), De Fusco *et al.* (1990) found that slight mutagenic activity occurred only in mineral water stored for 1 month. Higher mutagenic activity was observed for mineral water that was stored in bottles exposed to sunlight compared with those in the dark (De Fusco *et al.* 1990). A similar study conducted by Monarca *et al.* (1994) revealed no mutagenic activity in the mineral water after 1 month of storage. Evandri *et al.* (2000) and Biscardi *et al.* (2003) used two plant-based genotoxic assays, *Allium cepa* and *Tradescantia*/micronuclei, respectively, to evaluate migration of mutagens from PET bottles. These plant assays have been used to test genotoxicity in drinking water and can be used for unconcentrated samples as well. Evandri *et al.* (2000) showed that genotoxic activity was present in water samples after 8 weeks regardless of light exposure. Biscardi *et al.* (2003) observed mutagenic behaviour not only in water samples without light exposure during mineral bottle storage but also from pipes supplying water for the bottling process. None of these exposure conditions (temperature and sunlight) resembled those experienced by PET bottles during SODIS use. The Comet assay is a DNA-based genotoxic assay. It is a sensitive method used to detect low-level damage in DNA due to genotoxins and has been used to test surface water and by-products of drinking water disinfection (Biscardi *et al.* 2003; Lah *et al.* 2005*a*,*b*).

The short-term mutagenicity test used in this study was the *Salmonella* Ames fluctuation test developed by Green *et al.* (1976) and is a more sensitive, liquid-based version of the *Salmonella* Ames test developed by Ames *et al.* (1975). The Ames test is well validated, widely used and allows for comparison with the results of researchers who commonly use the Ames test as the sole assay for testing genotoxicity in water (Lah *et al.* 2005*a*,*b*). In the fluctuation assay a greater amount of sample volume can be tested without the need for concentration, thereby avoiding concentration methods that might change the original genotoxicity of the water sample (Monarca *et al.* 1985; Stahl 1991; Le Curieux *et al.* 1996). In addition, testing large volumes of water will result in a more accurate estimate of the genotoxic risk to which a SODIS user will be exposed since SODIS users do not concentrate their water before drinking. It is recommended that SODIS users consume the disinfected water from the PET bottles within 24 h of the end of exposure.

The *S. typhimurium* strain used for the mutagenicity testing was TA100. The TA100 strain detects a point mutation which involves the substitution of base pairs and is specific to G → A transition in the *hisG46* gene. It is also capable of detecting G → T and G → C transitions. Acetaldehyde and formaldehyde, which have previously been shown to migrate from PET bottles, give a mutagenic response in TA100 without the need for metabolic inactivation with S9 (Dillion *et al.* 1998). The Ames fluctuation assay has been used to evaluate genotoxicity in different types of water (Le Curieux *et al.* 1996; Jolibois & Guerbet 2005); however, prior to this study it has not been used to determine the genotoxicity of water subjected to SODIS conditions in PET bottles.

The primary aim of this research was to determine if there is an observable genotoxic activity associated with prolonged use of PET bottles as SODIS containers while adhering to standard SODIS protocols (daily refill of PET containers, minimum of 6 h exposure to natural sunlight and water consumed within 24 h). Genotoxicity testing ensures that SODIS users are not drinking water containing genotoxins, thereby exposing them to health hazards and the possibility of developing cancer. An additional aim was to determine whether genotoxicity could be observed in samples subjected to prolonged exposure for extended periods of time up to 6 months, without refilling.

MATERIALS AND METHODS

Samples

Two litre volumes of mineral water in PET bottles were purchased in Almería, Spain, in May 2007, and were used for the duration of the experiment. The main physicochemical parameters of the water were listed on the labels of the bottles. We verified the given concentrations using ion chromatograph methods, running water samples from two separate bottles in duplicate. Cation concentrations were determined with a Dionex (Dionex Corporation, Sunnyvale, California) DX-120 ion chromatograph equipped with a Dionex Ionpac CS12A 4 × 250 mm column at a flow rate of 1.2 ml min^{-1}. Anion concentrations were determined with a Dionex DX-600 ion chromatograph using a Dionex Ionpac AS11-HC 4 × 250 mm column. The gradient programme for anion determination was pre-run for 5 min with 20 mM NaOH, an 8 min injection of 20 mM of NaOH, and 7-min with 35 mM of NaOH, at a flow rate of 1.5 ml min^{-1}. Table 1 provides a comparison between concentrations listed on the labels and those obtained by our analysis.

Table 1 | Chemical parameters of water as given on bottle labels compared with concentrations obtained by ion chromatograph methods conducted at the Plataforma Solar de Almería (PSA)

	Concentration (mg l^{-1})	
Ion	**Manufacturer**	**Laboratory**
Bicarbonate	314	309 ± 1
Sulphate	26.6	28.6 ± 0.7
Chloride	10.8	15.6 ± 0.8
Calcium	82.8	91.6 ± 0.4
Magnesium	24.2	26.3 ± 0.4
Sodium	4.5	6.8 ± 0.6

Sunlight exposure and storage conditions of water

During the months of June to December 2007, bottles containing mineral water were exposed in triplicate to sunlight for 1, 2, 3, 4, 5 and 6 months. The bottles were placed in a horizontal position on the roof of a laboratory at the Plataforma Solar de Almería (latitude 37° 05′ N, longitude 2° 21′ W, altitude 500 m). Solar UV irradiance was measured with a global UV radiometer (295–385 nm) (Model CUV3, Kipp & Zonen, Netherlands) inclined at 37°. The solar UV dose ($Dose_{UV}$, J m^{-2}) delivered onto the bottles was obtained by integration of solar UV irradiance (I_{UV}, W m^{-2}) over a given period of time (dt, s) in 1 min intervals (Equation (1)).

$$Dose_{UV} = \int_{t_1}^{t_2} I_{UV}\, dt \quad (1)$$

The typical variation in UV-A irradiance during the exposure period is illustrated in Figure 1. The end of daylight saving time in October 2007 accounts for the left shift in irradiance curve observed in December 2007.

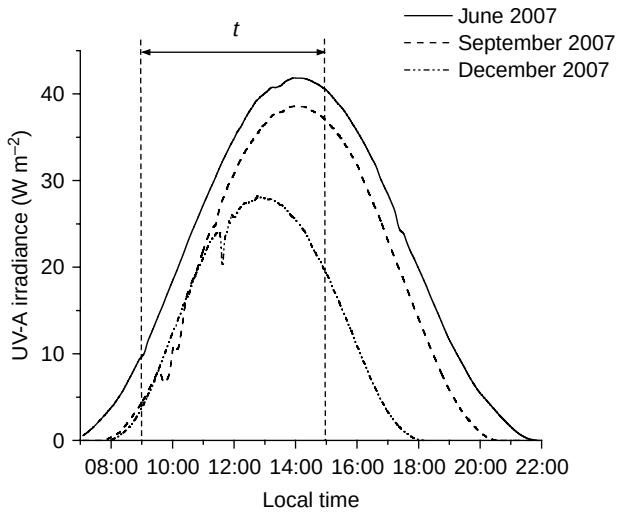

Figure 1 | UV-A irradiance curves for the experimental period of June 2007–December 2007; t represents the exposure period for SODIS daily refill samples.

Two sets of samples were prepared:

(i) *SODIS protocol (daily-refill) samples*: In order to simulate the way in which PET bottles are used during SODIS, three 2-l bottles filled with distilled water were exposed to sunlight for 6 h and then stored in the dark. The following day (approximately 24 h after initial exposure to sunlight), the bottles were emptied, refilled and then exposed to the sun again. Exposure occurred on 5 consecutive days of each week. Over weekends, bottles were emptied and kept in the dark until the following Monday. The water was collected after each month and tested. Controls were prepared and maintained in a similar manner but were kept in the dark rather than exposed.

(ii) *Continuous exposure (no-refill) samples:* Twenty-one sealed 2-l mineral water bottles were placed outside for exposure in the manner described previously. At each time point (months 0, 1, 2, 3, 4, 5 and 6) three bottles were retrieved and tested. Twenty-one control bottles also containing mineral water were stored in the dark at room temperature (23–25°C) for the duration of the experiments.

Ames fluctuation test

The Ames fluctuation test was performed using reagents from the commercially available Muta-ChromoPlate™ Ames test kit (EBPI Inc., Mississauga, Ontario, Canada) (EBPI 2008). The tester strain *S. typhimurium* TA100 without S9 mix was used. Lyophilised bacteria were transferred into nutrient broth and grown overnight for 16 to 18 h. The liquid reaction medium consisted of Davis-Mingioli salts, D-glucose, D-biotin, L-histidine and bromocresol purple, sterile distilled water and *S. typhimurium* TA100. Un-concentrated water samples were added to the reaction medium and the suspension was then distributed into each well of a 96-well microplate (200 μl per well). Plates were incubated at 37°C for 5 days in sterile Ziploc bags to avoid evaporation. All yellow, partially yellow or turbid wells were considered positive, and all purple wells were recorded as negative. For each experiment a blank and two controls were run. The blank (did not contain bacteria) was performed to ensure sterility of the experiment; all wells in the blank were expected to be purple. The positive control was conducted using the standard mutagen sodium azide (0.5 μg/100 μl); all wells were expected to be yellow. DMSO (dimethyl sulfoxide) was used as a negative control to estimate the number of spontaneous reversions that would occur in the bacterial population.

Data analysis

The number of positive (yellow) wells out of 96 wells per replicate was compared with the number of spontaneous revertant wells obtained with the negative control. The results were an average of three experiments and were expressed as a mutagenicity ratio (MR = number of positive wells in samples/number of positive wells in the negative control). A sample was considered genotoxic when a statistically significant increase occurred in the number of positive wells compared with spontaneous revertant wells. Statistical significance was determined using the chi-square (χ^2) analysis illustrated by Gilbert (1980).

UV measurements of PET bottles

After analysis of the contents of the plastic bottles, 2 × 3 cm sections were cut from the parts of the bottles that received the most sunlight. There were three samples for every time period, as bottles had been exposed to the sun in triplicate. Plastic samples were then covered with tissue paper to avoid scratching or further scratching in the case of already scratched samples and stored in the dark until ready for analysis. The transmittance of the PET samples was measured using a Unicam spectrometer (Unicam Limited, Cambridge, UK).

RESULTS

The effect of storage time and exposure to sunlight on the genotoxic content of water in PET bottles was examined. Genotoxicity was not observed in any of the daily-refill samples that were exposed to SODIS conditions or their corresponding control samples regardless of storage time and UV-A dose received (Figures 2a and 3a).

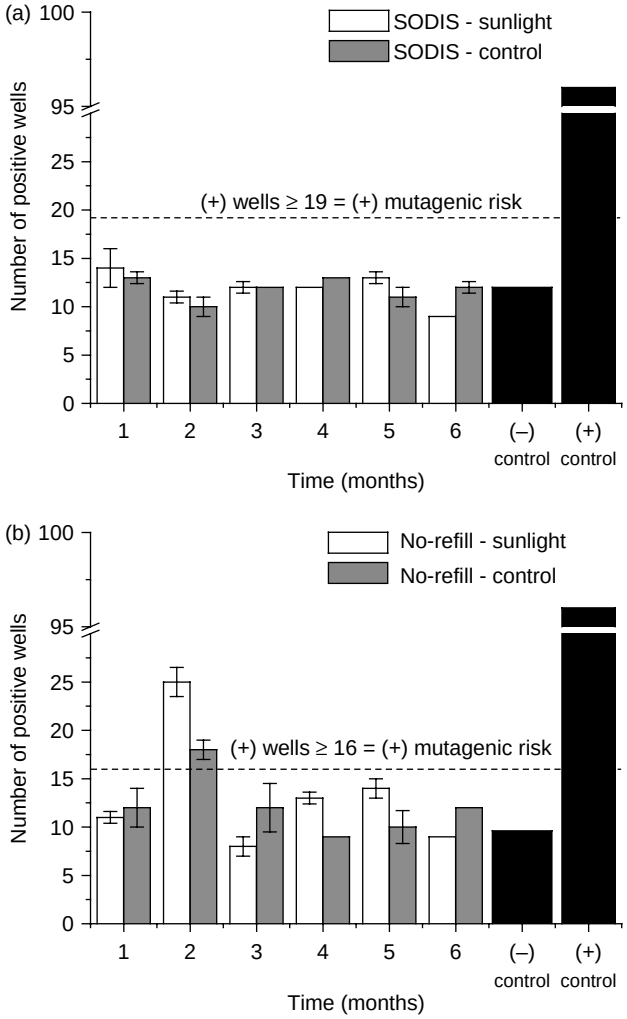

Figure 2 | Number of positive wells obtained for SODIS daily refill samples (a) and no-refill samples (b) exposed to sunlight and under dark conditions. Each column represents the average of triplicates and error bars show the standard error limits.

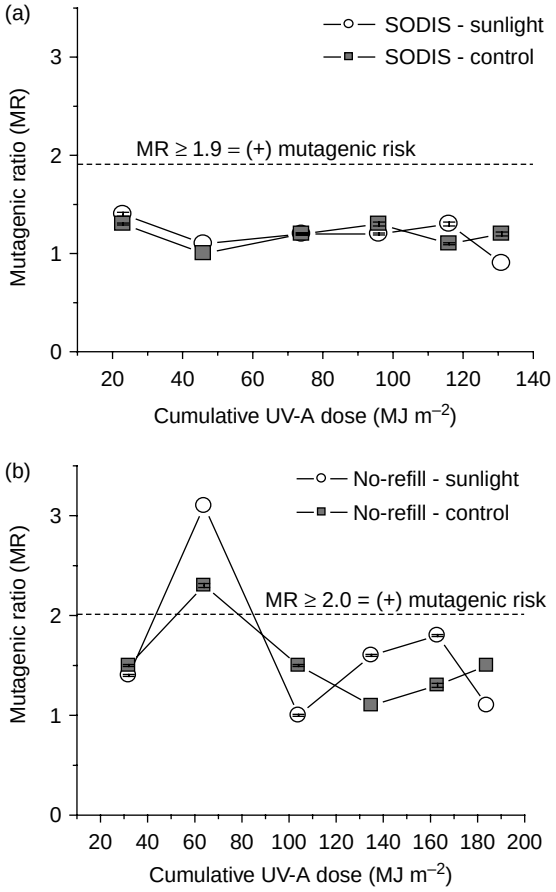

Figure 3 | Mutagenic ratios obtained for SODIS daily refill samples (a) and no-refill samples (b) exposed to sunlight and under dark conditions. Each point represents the average of triplicates and error bars show the standard error limits.

In the prolonged exposure (no-refill) samples (Figure 2b) significant ($p < 0.05$) genotoxic activity was observed after 2 months for both control (in the dark) and test (exposed to sunlight) samples. Sunlight increased genotoxic activity. Genotoxic samples received a cumulative UV-A dose of 64 MJ m^{-2} (Figure 3b). No other significant genotoxicity activity was observed at any other time.

DISCUSSION

During solar disinfection, PET bottles are subjected to two physical stresses: exposure to sunlight and an increase in water temperature. As the PET bottles age and are re-used, these factors could lead to a change in structure and composition not only of the PET bottle itself but also any photodegradation products that might migrate from the plastic bottle into the water. We observed the expected decrease in UV transmittance with sunlight exposure time as was also reported by Wegelin *et al.* (2001).

Nawrocki *et al.* (2002) report that, at room temperature, carbonyls (formaldehyde, acetaldehyde and acetone) migrated within a 2.5 h period from PET into water. Acetaldehyde concentrations were higher in newer bottles compared with bottles that were 1 month old. We did not detect genotoxicity in any SODIS protocol (daily-refill) water samples at any stage during this study (Figure 2a).

This may be due to the escape of volatile compounds into the atmosphere when PET bottles are opened (De Fusco *et al.* 1990; Monarca *et al.* 1994; Evandri *et al.* 2000). The water in SODIS bottles reached a maximum temperature of 43°C and was subsequently stored at room temperature (23–25°C). Potentially genotoxic compounds such as acetaldehyde may be highly volatile and could have been released into the air. However if the supposed volatility of photoproducts can be invoked to explain their absence from observed results then we would also expect this to occur during normal SODIS use and thus not present a risk to the SODIS user.

It should also be noted that usually a SODIS user consumes water that has been treated on the previous day while tomorrow's water is treated today. Thus, under normal conditions of use, SODIS containers are only exposed to sunlight on every second day. Our daily-refill samples were exposed daily from Monday to Friday but not over weekends. For a typical 28-day period one would then expect daily-refill SODIS containers to be exposed on 20 days, which is ~17% more frequently than the 14 days exposure experienced by containers under normal SODIS usage conditions. Since our experiments did not detect any genotoxicity in the daily-refill samples over a 6-month time frame it is reasonable to suggest that under realistic conditions no toxicity would have been detected after 7 months (7.01 months = 6 months × 1.17).

Our results support those of De Fusco *et al.* (1990) who, despite concentrating their samples using the solid agar Ames test, only observed significant mutagenicity in water samples in PET bottles stored for 1 month in the dark and increased mutagenicity on exposure to sunlight. Subsequent experiments also performed by the same research group did not detect any further mutagenicity. The difference in mutagenicity was attributed to the use of different PET bottles and different types of mineral water (Monarca *et al.* 1994). Evandri *et al.* (2000) showed genotoxic activity in water samples after 8 weeks of storage both in the dark and in the light, using a plant-based genotoxic assay. In our studies we also detected significant genotoxicity in both the dark and sunlight no-refill samples after 2 months (Figure 3b). However the mutagenic ratio was higher for the solar-exposed samples (3.1 ± 0.2) compared with the dark controls (2.3 ± 0.2). As no-refill bottles were not opened, the observed genotoxicity might be due not only to non-volatile compounds but also to volatile compounds as well (Evandri *et al.* 2000). Furthermore, genotoxicity was not observed after 2 months; it is therefore likely that genotoxic compounds detected after 2 months have undergone further degradation into non-genotoxic forms. High temperature and sunlight might increase the leaching of these products from PET and increase the rate of degradation of photoproducts. This results in the increased genotoxicity which is observed when bottles are exposed to sunlight. More importantly, under standard SODIS conditions, which included daily re-use of plastic bottles over a 6-month period, water contamination by genotoxic compounds was not observed. Genotoxic results obtained could be combined with previous studies carried out on migration of chemical compounds under SODIS conditions (Wegelin *et al.* 2001; Schmid *et al.* 2008) to give a better understanding of the overall health risk of drinking SODIS-treated water.

No indicator organisms were added to the sample bottles in this study to help monitor levels of disinfection. There is a possibility that organic compounds originating from the destruction of microbial cell membranes and organelles may contribute in some way to the proposed genotoxicity. All of the experiments reported here used sterile distilled water (for the daily refill samples) or the original commercially available mineral water (for the no-refill samples) rather than natural waters, in order to eliminate the possibility that the presence of extraneous compounds might interfere with our studies.

CONCLUSION

Our preliminary investigation did not identify any genotoxic risk associated with un-concentrated SODIS water (daily refill). Based on this study, if users apply the SODIS technique correctly, they are unlikely to experience any health hazards from genotoxins generated by SODIS if they replace their bottles every 6 months. Genotoxicity was detected after 2 months in water stored in PET bottles and exposed continuously (without refilling) to sunlight but also in PET bottles stored in the dark after 2 months. These results indicate the need for further study. In particular, an

evaluation of the genotoxicity of SODIS water over a range of sample concentrations, for a variety of different PET containers and with a more realistic microbiological profile, would be beneficial. Other intensive genotoxicity assay methods with different genetic end-points such as the Comet assay (DNA damage on human leukocytes) offer interesting alternative investigative routes. There are current plans for studies of this nature and funds will be sourced accordingly.

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REFERENCES

Acra, A., Karahagopian, Y., Raffoul, Z. & Dajani, R. 1980 Disinfection of oral rehydration solutions by sunlight. *Lancet* **2**, 1257–1258.

Ames, B. N., McCann, J. & Yamasaki, E. 1975 Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* **31**, 347–364.

Biscardi, D., Monarca, S., De Fusco, R., Senatore, F., Poli, P., Buschini, A., Rossi, C. & Zani, C. 2003 Evaluation of the migration of mutagens/carcinogens from PET bottles into mineral water by *Tradescantia*/micronuclei test, Comet assay on leukocytes and GC/MS. *Sci. Total Environ.* **302**, 101–108.

De Fusco, R., Monarca, S., Biscardi, D., Pasquini, R. & Fatigoni, C. 1990 Leaching of mutagens into mineral water from polyethyleneterephthalate bottles. *Sci. Total Environ.* **90**, 241–248.

Dejung, S., Fuentes, I., Almanza, G., Jarro, R., Navarro, L., Arias, G., Urquieta, E., Torrico, A., Fernandez, W., Iriarte, M., Birrer, C., Stahel, W. A. & Wegelin, M. 2007 Effect of solar water disinfection (SODIS) on model microorganisms under improved and field SODIS conditions. *J. Water Suppl. Res. Technol.-AQUA* **56**, 245–256.

Dillion, D., Combes, R. & Zeiger, E. 1998 The effectiveness of *Salmonella* strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. *Mutagenesis* **13**, 19–26.

EBPI 2008 *Muta-ChromoPlate™ Basic Test Kit Version 3.3. User's Manual*. EBPI, Mississauga, Ontario.

Evandri, M. G., Tucci, P. & Bolle, P. 2000 Toxological evaluation of commercial mineral water bottled in polyethylene terephthalate: a cytogenetic approach with *Allium cepa*. *Food Addit. Contam.* **17**, 1037–1045.

Gilbert, R. I. 1980 The analysis of fluctuation tests. *Mutat. Res.* **74**, 283–289.

Green, M. H., Muriel, W. H. & Bridges, B. A. 1976 Use of a simplified fluctuation test to detect low levels of mutagens. *Mutat. Res.* **38**, 33–42.

Haider, T., Sommer, R., Knasmuller, S., Eckl, P., Pribil, W., Cabaj, A. & Kundi, M. 2002 Genotoxic response of Austrian groundwater samples treated under standardized UV (254 nm): disinfection conditions in a combination of three different bioassays. *Water Res.* **36**, 25–32.

Jolibois, B. & Guerbet, M. 2005 Evaluation of industrial, hospital and domestic wastewater genotoxicity with the *Salmonella* fluctuation test and the SOS chromotest. *Mutat. Res.* **565**, 151–162.

Koivusalo, M., Vartianien, T., Hakulinen, T., Pukkala, E. & Jaakkola, J. J. K. 1995 Drinking water mutagenicity and leukemia, lymphomas and cancers of the liver, pancreas and soft tissue. *Arch. Environ. Health* **50**, 269–276.

Lah, B., Zinko, B., Narat, M. & Marinsek-Logar, R. 2005*a* Monitoring of genotoxicity in drinking water using *in vitro* Comet assay and Ames test. *Food Technol. Biotechnol.* **43**, 139–146.

Lah, B., Zinko, B., Tisler, T. & Marinsek-Logar, R. 2005*b* Genotoxicity detection in drinking water by Ames test, Zimmermann test and Comet assay. *Acta Chim. Slov.* **52**, 341–348.

Le Curieux, F., Giller, S., Marzin, D., Brice, A. & Erb, F. 1996 Use of three genotoxicity tests to evaluate the genotoxic activity of organohalides chlorinated fulvic acids and unconcentrated water samples collected from a drinking water treatment plant. *Rev. Sci. Eau* **1**, 75–95.

McGuigan, K. G., Joyce, T. M., Conroy, R. M., Gillespie, J. B. & Elmore-Meegan, M. 1998 Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *J. Appl. Microbiol.* **84**, 1138–1148.

Monarca, S., Pasquini, R. & Sforzolini, G. S. 1985 Mutagenicity assessment of drinking water supplies before and after treatments. *Bull. Environ. Contam. Toxicol.* **34**, 815–823.

Monarca, S., De Fusco, R., Biscardi, D., De Feo, V., Pasquini, R., Fatigoni, C., Moretti, M. & Zanardini, A. 1994 Studies of migration of potentially genotoxic compounds into water stored in PET bottles. *Food Chem. Toxicol.* **32**, 783–788.

Monturi, P., Jover, E., Morgantini, M., Bayona, J. M. & Triassi, M. 2007 Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. *Food Addit. Contam.* **25**, 511–518.

Morrison, E. D., Malvey, M. W., Johnson, R. D., Anacker, J. L. & Brown, K. A. 2008 Effect of chemical environments on stress cracking of poly(ethylene terephthalate) beverage bottles. *Polym. Test.* **27**, 660–666.

Mutsuga, M., Kawamura, Y., Sugita-Konishi, Y., Hara-Kudo, Y., Takatori, K. & Tanamoto, K. 2006 Migration of formaldehyde and acetaldehyde into mineral water in polyethylene terephthalate (PET) bottles. *Food Addit. Contam.* **23**, 212–218.

Nawrocki, J., Dabrowska, A. & Borcz, A. 2002 Investigation of carbonyl compounds in bottled waters from Poland. *Water Res.* **36**, 4893–4901.

Schmid, P., Kohler, M., Meierhofer, R., Luzi, S. & Wegelin, M. 2008 Does the reuse of PET-bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? *Water Res.* **42**, 5054–5060.

Stahl, R. G. J. 1991 The genetic toxicology of organic compounds in natural waters and waste waters. *Ecotoxicol. Environ. Saf.* **22**, 94–125.

Villian, F., Coudane, J. & Vert, M. 1994 Thermal degradation of poly(ethylene terephthalate) and the estimation of volatile degradation products. *Polym. Degrad. Stab.* **43**, 431–440.

Wegelin, M., Canonica, A., Alder, A., Suter, M., Bucheli, T. D., Haefliger, O. P., Zenobi, R., McGuigan, K. G., Kelly, M. T., Ibrahim, P. & Larroque, M. 2001 Does sunlight change the material and content of PET bottles? *J. Water Suppl. Res. Technol.–AQUA* **50**, 125–135.

Zoeteman, B. C. J., Hrubec, J., de Greef, E. & Kool, H. J. 1982 Mutagenic activity associated with by-products of drinking water disinfection by chlorine, chlorine dioxide, ozone and UV-irradiation. *Environ. Health Perspect.* **46**, 197–205.

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