Check for updates

Monitoring hospital wastewaters for their probable genotoxicity

Asma Beltifa, Sana Alibi and Hedi Ben Mansour

ABSTRACT

Hospitals' effluents contain a considerable amount of chemicals. Considering the significant volume of wastewater discharged by hospitals, the presence of these chemicals represents a real threat to the environment and human health. Thus, the aim of this study was to evaluate the *in vivo* and *in vitro* genotoxicities of three wastewater effluents collected from Tunisian hospitals. The liver of *Swiss albino* male mice, previously treated with different doses of the hospital wastewaters, was used as a model to detect DNA fragmentation. Our results showed all the hospital effluents caused significant qualitative and quantitative hazards in hepatic DNA. The wastewater collected from Sfax hospital exhibited the highest genotoxic effect, which may be explained by the presence in this effluent of some toxic micropolluants. There was a significant increase in genotoxicity, proportionally to the concentration of effluent. However, the vitotox assay did not show any significant genotoxicity on *Salmonella typhimurium* TA104 in the presence or absence of microsomal fraction S9. The ratio gentox/cytox was lower than the threshold 1.5. This study assessed the toxicological risk issued from Tunisian hospital wastewaters, which is potentially very harmful, and it has been pointed out that wastewater treatment requires special attention.

Key words | biomonitoring, genotoxicity, hospital wastewater, water pollution

Asma Beltifa
Sana Alibi
Hedi Ben Mansour (corresponding author)
Research Unit of Analysis and Process Applied to the Environment – APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia,
University of Monastir, Mahdia,
Tunisia
E-mail: *hdbenmansour@gmail.com*

Asma Beltifa
Faculty of Mathematical, Physical and Natural Sciences,
University Tunis,
El Manar,
Tunisia

INTRODUCTION

In contemporary lifestyle, pharmaceutical products are increasingly and inevitably consumed. However, considerable amounts of these products are released in the environment and are responsible for aquatic pollution, including pollution of river environments, drinking water sources, and lakes. The rapid and the considerable spread of pharmaceutical-based pollution has become a real threat and is receiving significant interest worldwide.

There is growing concern regarding the toxic effects of pharmaceutical products on human health, because they are designed to elicit specific physiological functions in targeted regions of the human body at relatively low concentrations. Hospitals constitute the principal place where pharmaceuticals are consistently employed to treat diseases (Azuma 2018). Previous studies (Sdiri-Loulizi *et al.* 2010; Nasri *et al.* 2017a, 2017b; Souza *et al.* 2018) have shown that hospital wastewaters contain large amounts of pathogenic organisms, dangerous and persistent substances such as pharmaceuticals, radionuclides, solvents, disinfectants, and metals. More recently, the discharge of pharmaceutical residues, which may eventually be discharged into the aquatic environment, raises the question of their impact on human and environmental health. In fact, hospital effluent may release a significant amount of pollutants with teratogenic, carcinogenic, and mutagenic hazards (Jobling *et al.* 2002; Phillips & Foster 2008; Pérez-Alvarez *et al.* 2018). On the other hand, many studies have detected the presence of endocrine disrupting compounds and antibiotics causing genotoxic and cytotoxic effects and inducing much damage in the tissue of marine species (Zouiten *et al.* 2016; Nasri *et al.* 2017b; Tahrani *et al.* 2017, 2018). Data according to health hazards associated with long-term exposure to

doi: 10.2166/wh.2019.103

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

hospital wastewaters are scant. In fact, hospital effluents are generally discharged directly into the public sanitation network. Currently, there are no rules or limits on hospital wastewater treatment before discharging (Yilmaz *et al.* 2017).

Therefore, our study focused on the characterization of three different hospital effluents in Tunisia and the evaluation of their genotoxic effects. Genotoxicity and cytotoxicity assays were performed using *Salmonella typhimurium* TA104 and the hepatic tissue of an animal model periodically treated with wastewater.

MATERIAL AND METHODS

Effluents obtention

Wastewater samples were collected from three Tunisian hospitals: Hedi Chaker University Hospital in Sfax, Tahar Sfar University Hospital in Mahdia, and the Regional Hospital of Gafsa, during the same period (March 2017).

The sampling points chosen received the highest amount of wastewater and collected discharges from different services including: general medicine, general surgery, intensive care units, maternity, gynecology, oncology, psychiatry, rheumatology, hematology, hepatic-gastroenterology, several radiology units, and laboratories. Wastewater samples collected were transported to the laboratory prior to determining the different physicochemical parameters and/or immediately stored at −20 °C until further analysis.

Vitotoxicity assay

The vitotox test was carried out on genetically modified *Salmonella typhimurium* TA104 as previously described by Verschaeve *et al.* (1999). Briefly, an overnight pre-culture containing 100 μL of each strain, 625 μL of tetracycline (0.8 mg mL^{-1}), and 312.5 mL of ampicillin (8 mg mL^{-1}) were placed in two Erlenmeyer's flasks in a water bath (300 rpm, 36 °C) for 16 h. Next, the two Erlenmeyer's flasks were placed on ice for 15 min and 20 mL of mineral medium, 160 μL of previously cultured bacteria were added and stirred during 1 h in an appropriate water bath (300 rpm, 36 °C). These genox and cytox work-cultures were then ready for testing. A mixture of 2.125 mL of mineral medium, 350 μL of bacterial suspension, and 1 mL of the post-mitochondrial supernatant fraction S9 was prepared and transferred to a black 96-well microplate with 100 μL from each sample (diluted at ½ and ¼ in distillate water).

In the absence of S9, 1 mL of mineral medium was added. Positive controls 4-NQO (0.4 mg mL^{-1}) and B(a)P (0.8 mg mL^{-1}) were used in the presence and absence of S9, respectively. A microplate illuminometer was used to measure genotoxicity and cytotoxicity; the light was analyzed every 5 min over a 4-h time span. All the measurements were automatically performed between 60 and 240 min of incubation. The samples were added to the bacteria in the presence and the absence of the S9 mix (fraction obtained from the livers of aroclor-treated rats). The signal to noise ratio (S/N) was measured using the formula of Verschaeve *et al.* (1999): $S/N =$ the light production of exposed cells divided by the light production of nonexposed cells for each measurement and for each strain separately. It was previously demonstrated that genotoxicity always takes place when the maximum S/N (genox)/maximum S/N (cytox) is higher than 1.5. Cytotoxicity is assumed when S/N in genox/cytox is lower than 0.8 (Verschaeve *et al.* 1999, 2012; Verschaeve 2002). This test shows DNA damage by emitting a readily detectable signal of bioluminescence after exposure to mutagenic factors.

In vivo genotoxicity test

Animals model

Sixty adult male mice (weighing 180–200 g) were purchased from SIPHAT, Tunisia. The standard pellet diet was purchased from the Industrial Society of Rodents' Diet (SNA, Sfax, Tunisia). The animals were maintained under constant temperature (22 ± 1 °C) and humidity (50%), with diurnal lighting (12 h light/12 h dark). Mice were fed a standard laboratory diet and given free access to tap water. All animals were treated according to the local Institute Ethical Committee Guidelines for the Care and Use.

In vivo assay

Animals were left for 2 weeks for adaptation, and then were randomly separated into ten groups of six mice in each

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

group (Table 1) and effluents were administrated orally to all the groups by gavage during 28 consecutive days.

Biological samples collection

After 28 days of the administration of effluents, the mice were sacrificed by cervical decapitation. Liver tissues were excised immediately from the animals and stored in a liquid nitrogen container to avoid protein degradation and were used for molecular studies.

DNA fragmentation assay

Qualitative damage to genomic DNA was estimated by agarose gel electrophoresis, as previously described by Kanno *et al.* (2004). DNA was extracted in duplicate from the liver with equal volumes of phenol-chloroform-isoamyl alcohol (25:24:1), and precipitated with twice the volume of ethanol. DNA samples (3 μg of DNA/lane) were electrophoresed on 1.4% agarose gel containing ethidium bromide (final concentration, 0.16 μg/ml), and the fragmentation was visualized under UV light.

RESULTS

The monitoring of water contamination for potentially genotoxic compounds represents a major concern for human health. This study provides important data to consider such a concern using samples from three hospital wastewaters and different toxicity bioassays.

Vitotox test

All the wastewater samples showed a direct genotoxic effect (genox/cytox ratio >1.6) in a dose-dependent manner when tested in the absence of S9 mix (Figure 1). However, no genotoxic effect was observed when hospital wastewaters were tested in the presence of S9 mix. In fact, the genox/cytox ratio does not exceed 1.5 (Figure 2).

Effects of pharmaceutical effluent on the DNA ladder fragmentation in liver tissue of mice

Liver DNA electrophoresis showed the hallmark of apoptosis, characterized by a mixed smearing and laddering of the DNA extracted from mice treated with 100% concentrations of the pharmaceutical effluent (lanes 4 and 8). Treated animals with 25% and 50% concentrations of the pharmaceutical effluent collected from Gafsa and Mahdia (lanes 2, 3, 6, and 7) and control group (lanes 1, 5, and 9) revealed an intact band (Figure 3).

Effluents collected from the hospital in Sfax caused DNA fragmentation in the livers of treated mice (lanes 10, 11, and 12) independently from the sample concentration.

DISCUSSION

Many studies have reported the important occurrence of micropollutants and toxic substances in hospital wastewaters (Emmanuel *et al.* 2009; Verlicchi *et al.* 2010). Nowadays, the detection and the quantification of these

Table 1 | Characteristics of the mice groups used in this study

Groups	Characteristics
Group 1	Control group receiving distilled water
Group 2	Group receiving 25% concentration of a pharmaceutical effluent collected from the hospital of Gafsa
Group 3	Group receiving 50% concentration of a pharmaceutical effluent collected from the hospital of Gafsa
Group 4	Group receiving 100% concentration of a pharmaceutical effluent collected from the hospital of Gafsa
Group 5	Group receiving 25% concentration of a pharmaceutical effluent collected from the hospital of Sfax
Group 6	Group receiving 50% concentration of a pharmaceutical effluent collected from the hospital of Sfax
Group 7	Group receiving 100% concentration of a pharmaceutical effluent collected from the hospital of Sfax
Group 8	Group receiving 25% concentration of a pharmaceutical effluent collected from the hospital of Mahdia
Group 9	Group receiving 50% concentration of a pharmaceutical effluent collected from the hospital of Mahdia
Group 10	Group receiving 100% concentration of a pharmaceutical effluent collected from the hospital of Mahdia

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

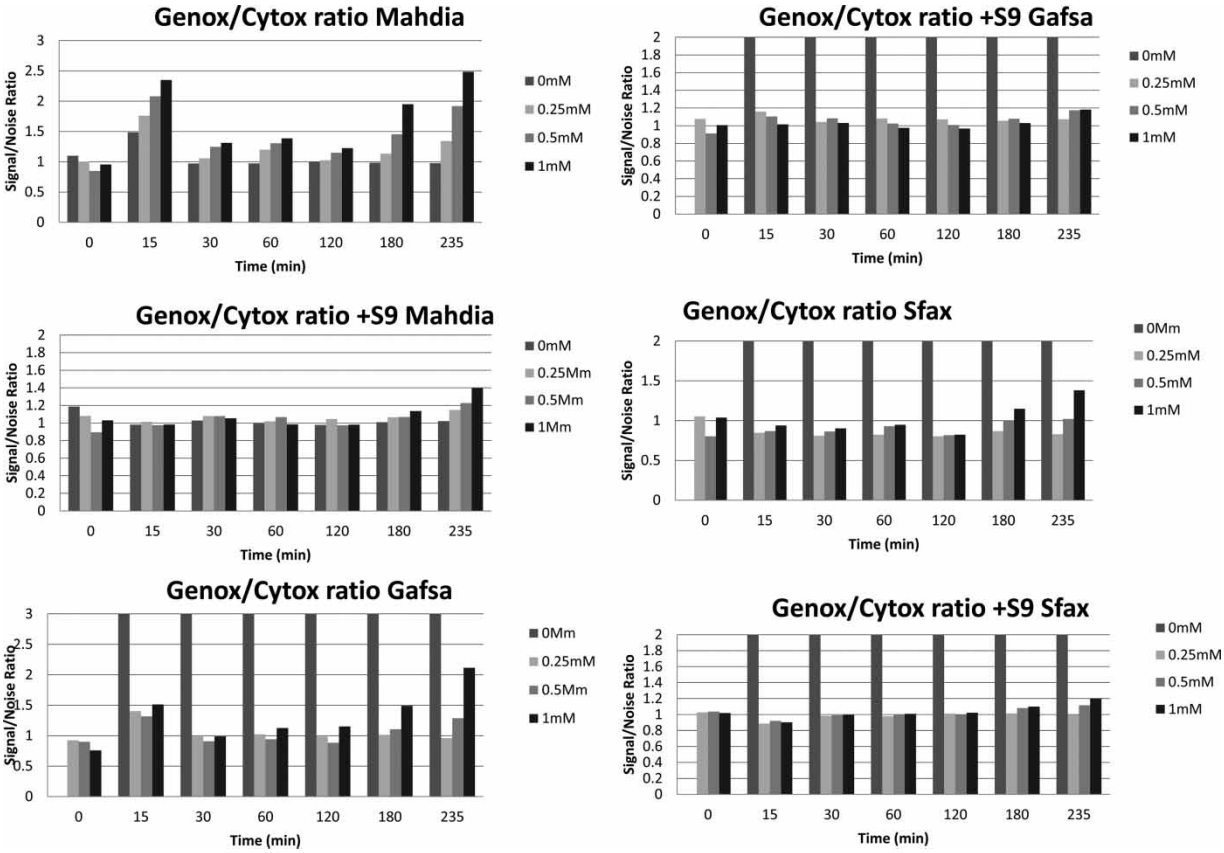

Figure 1 | The genotoxic effects of hospital wastewater samples on *Salmonella typhimurium* TA104 the vitotox test. Genotoxicity always take place when the maximum S/N (genox)/maximum S/N(cytox) is greater than 1.5 and cytotoxicity is assumed when S/N in genox/cytox decreases far below 0.8. HWM: hospital wastewater Mahdia; HWG: hospital wastewater Gafsa; HWS: hospital wastewater Sfax. (1) 100 μL of hospital wastewater; (0.5): 100 μL of HW diluted ½ in distillate water; (0.25) 100 μL of HW dilated ¼ in distillate water; (0) 100 μL of distillate water.

residues in hospital effluents require particular attention. These substances are potentially genotoxic and may cause harmful environmental effects even at very low concentrations (Kummerer *et al.* 2009; Toolaram *et al.* 2014). In a previous study, our team detected heavy metals and antibiotics in hospital wastewaters collected from three different regions in Tunisia (Nasri *et al.* 2017a).

It is evident now that prescription trends and patients' consumption are closely reflected by the pharmaceutical residues found in hospital effluents (Dorival-Garcia *et al.* 2013). The presence of high concentrated pharmaceutical products such as enrofloxacin, marbofloxacin, oxytetracycline, pipemidic, acid sulfamethoxazole, sulfonamides, acetaminophen, mefenamic acid, atenolol, carbamazepine, and caffeine was revealed.

Personal care products such as benaophenone-3 and benzotrizole were the most frequently detected in the three hospitals' effluents (results not shown). Effluents collected from the hospital in Sfax revealed the presence of ciprofloxacin, which may explain its highest genotoxicity (results not shown).

These observations confirm the fact that hospital effluents are the main source of heavy metals (e.g., Cr, Co, Ni, Cu, Zn, Cd, Pb, and Hg) release into the environment (Nasri *et al.* 2017a).

It is well known that hospital effluents, industrial effluents, and domestic wastewaters can contain pharmaceutical compounds, drugs, chemicals, and pesticides; detergents and disinfectants could also be detected in the wastewaters (Kummerer 2001).

The genotoxic activity of such mixtures has already been demonstrated in many previous studies (Hamer *et al.* 2000; Dizer *et al.* 2002; Durusoy & Kambur 2003; Zegura *et al.* 2009).

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

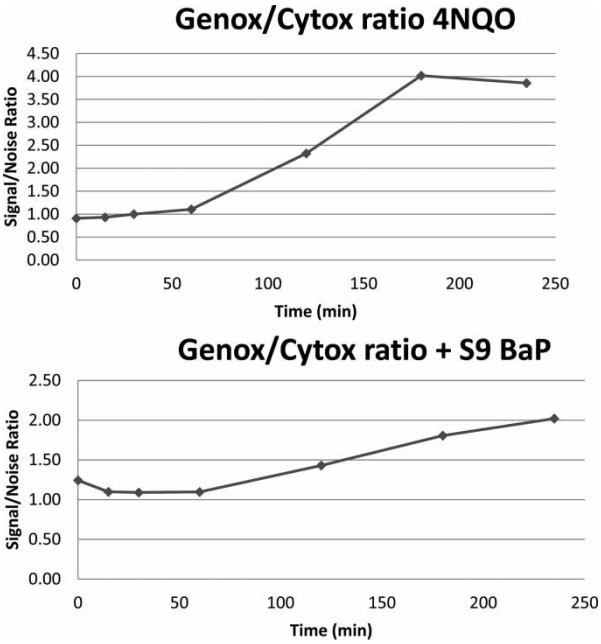

Figure 2 | Vitotox results for the positive controls 4-NQO (without S9 mix) and B(α)P (with S9 mix). The genox strain clearly shows that all are genotoxic (S/N > 1.5) whereas the cytox strain shows no significant deviation from S/N¼ 1.

The genotoxic activity was generated by all the effluents tested, suggesting the presence of different genotoxic compounds' formation.

The hospital wastewaters; genotoxic response was not affected by the addition of S9 mix addition which is in agreement with other studies which showed that the putative genotoxins in surface waters and municipal wastewaters are primarily direct-acting, i.e., S9 addition does not advance the response and the municipal wastewaters having a low genotoxic response. However, we were in disagreement with Kittinger *et al.* (2015), where a toxic signal could only be found after addition of S9 mix.

Indeed, data provided by the conventional water quality indices and chemical analysis are not sufficient to confirm possible aftermaths and the numerous formed substances that can threaten human health (Dizer *et al.* 2002; Wang *et al.* 2011). Therefore, to detect changes in cellular organics, further histological assays should be performed, such as for the presence of micronuclei after exposure to different concentrations of wastewaters. Thus, chemical agents can induce micronuclei through spindle disturbance or chromosome breaks (Ventura-Camargo *et al.* 2011).

The process of programmed cell death, or apoptosis, is generally characterized by distinct morphological characteristics and energy-dependent biochemical process, which plays an important role in the development and maintenance of homeostasis in most of the multicellular organisms (Smaili *et al.* 2003). To detect the genotoxic and mutagenic assessment of xenobiotics, DNA fragmentation test is the most widely used because of its technical simplicity and rapidity (Krishna *et al.* 2000). Our study revealed that sub-acute exposure to different concentrations of the pharmaceutical effluent collected from Gafsa, Sfax, and Mahdia increased the DNA fragmentation in mice livers, a hallmark of apoptosis which joins previous observations in suggesting a link between genotoxicity and pharmaceutical effluent poisoning (Houk 1992; Snyder &

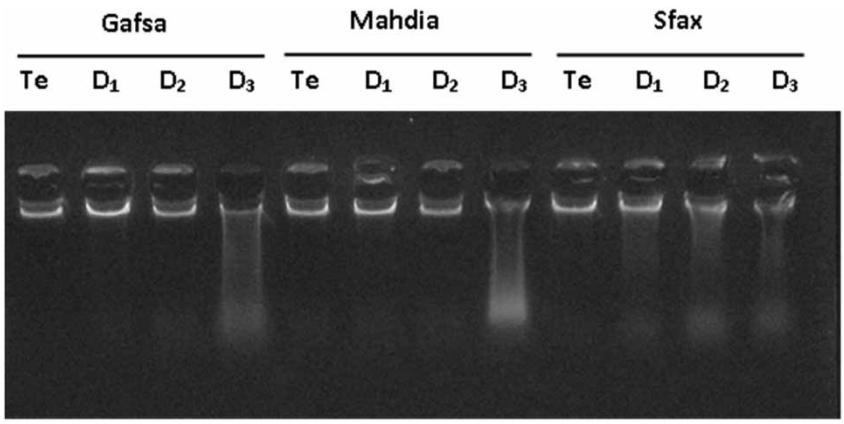

Figure 3 | The agarose gel electrophoresis of the DNA isolated from experimental liver tissues was loaded into 1% (w/v) agarose gels. Lane Te: DNA isolated from control liver tissue; lane D_1: DNA isolated from 25% concentrations of the pharmaceutical effluent-treated liver samples; lane D_2: DNA isolated from 50% concentrations of the pharmaceutical effluent-treated liver samples; lane D_3: DNA isolated from 100% concentrations of the pharmaceutical effluent-treated liver samples.

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

Green 2001; Babatunde & Bakare 2006; Siddique *et al.* 2008; Adeoye *et al.* 2015).

This cellular process can be triggered by the activation of pro-inflammatory cytokines that ultimately culminates in the activation of the caspase family of proteases (Yano *et al.* 2007), or by activation of free radicals, either through pharmacodynamics, auto-oxidation, or enzyme-catalyzed oxidation of electrophilic molecules of the pharmaceutical effluent (Bakare *et al.* 2009). Indeed, reactive oxygen species, endogenously generated on exposure to the effluent, could induce lipid peroxidation in the tissues by reacting with the lipid content of the cell membrane, thereby causing breakage of the DNA chain by oxidating the base component of the membrane. In addition, the free radicals generated by effluent could also have reacted with those protein-enzymes involved in the DNA repair mechanism, the alteration of repair enzyme activity resulting in increased frequency in DNA damage (Marnett & Burcham 1993).

CONCLUSION

In conclusion, our study focused on the detection of heavy metals, antibiotics, and plasticizers in hospital wastewaters from three hospitals in Tunisia. The results obtained assessed the toxicological risk and pointed to cytotoxic and genotoxic hazards caused by hospital effluents. Although there was a low concentration, harmful damage was observed; therefore, wastewater treatment requires special attention. It is mandatory to acquire additional information about the quality of hospital effluents and environmental control programs must be designed for the pre-treatment of wastewater before its release into municipal sewage systems. Controlling wastewaters should start by reducing wastewater generation and by using natural biological processes to treat them. Bioremediation by microorganisms, algae, and aquatic organisms should be promoted for wastewater treatment.

REFERENCES

Adeoye, G. O., Alimba, C. G. & Oyeleke, O. B. 2015 The genotoxicity and systemic toxicity of a pharmaceutical effluent in Wistar rats may involve oxidative stress induction. *Toxicology Reports* **2**, 1265–1272.

Azuma, T. 2018 Newly designed water treatment systems for hospital effluent. *Yakugaku Zasshi* **138**, 289–296.

Babatunde, B. B. & Bakare, A. A. 2006 Genotoxicity screening of wastewaters from Agbara industrial estate, Nigeria evaluated with the *Allium* test. *Pollution Research* **25**, 227–234.

Bakare, A. A., Okunola, A. A., Adetunji, O. A. & Jenmi, H. B. 2009 Genotoxicity assessment of a pharmaceutical effluent using four bioassays. *Genetics and Molecular Biology* **32** (2), 373–381. doi:10.1590/S1415-47572009000200026.

Dizer, H., Wittekindt, E., Fischer, B. & Hansen, P. D. 2002 The cytotoxic and genotoxic potential of surface water and wastewater effluents as determined by bioluminescence, umu-assays and selected biomarkers. *Chemosphere* **46**, 225–233.

Dorival-Garcia, N., Zafra-Gomez, A., Cantarero, S., Navalon, N. & Vilchez, J. L. 2013 Simultaneous determination of 13 quinolones antibiotic derivatives in wastewater samples using solid-phase extraction and ultra performance liquid chromatography–tandem mass spectrometry. *Microchemical Journal* **106**, 323–333.

Durusoy, M. & Kambur, S. 2003 The application of the umu test system for screening mutagenicity of surface water. *Turkish Journal of Biochemistry* **28**, 3–7.

Emmanuel, E., Pierre, M. G. & Perrodin, Y. 2009 Groundwater contamination by microbiological and chemical substances released from hospital wastewater: health risk assessment for drinking water consumers. *Environment International* **35**, 718–726.

Hamer, B., Bihari, N., Reifferscheid, G., Zahn, R. K., Muller, W. E. & Batel, R. 2000 Evaluation of the SOS/umu-test posttreatment assay for the detection of genotoxic activities of pure compounds and complex environmental mixtures. *Mutation Research* **23**, 161–171.

Houk, V. S. 1992 The genotoxicity of industrial wastes and effluents: a review. *Mutation Research* **227**, 91–138.

Jobling, S., Coey, S., Whitmore, J. G., Kime, D. E., Van Look, K. J. W. & McAllister, B. G. 2002 Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biology of Reproduction* **67**, 515–524.

Kanno, S., Shouji, A., Hirata, R., Asou, K. & Ishikawa, M. 2004 Effects of naringin on cytosine arabinoside (Ara-C)-induced cytotoxicity and apoptosis in p388 cells. *Life Science* **75**, 353–365.

Kittinger, C., Baumert, R., Folli, B., Lipp, M., Liebmann, A., Kirschner, A., Farnleitner, A. H., Grisold, A. J. & Zarfel, G. E. 2015 Preliminary toxicological evaluation of the River Danube using in vitro bioassays. *Water* **7**, 1959–1968.

Krishna, G. & Hayashi, M. 2000 In vivo rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research* **455**, 155–166.

Kummerer, K. 2001 Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – the review. *Chemosphere* **45**, 957–969.

Kummerer, K. 2009 Antibiotics in the aquatic environment-a review: part I. *Chemosphere* **75**, 417–434.

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest

Marnett, L. J. & Burcham, P. 1993 Endogenous DNA adducts: potential and paradox. *Chemical Research in Toxicology* **6**, 771–785.

Nasri, E., Machreki, M., Beltifa, A., Aroui, S., Ghorbel, A., Saad, A., Feriani, A., Borgi, M. A., Ghazouani, L., Sire, O., Balcázar, J. L. & Ben Mansour, H. 2017a Cytotoxic effects of seven Tunisian hospital wastewaters on the proliferation of human breast cancer cell line MDA-231: correlation with their chemical characterization. *Environmental Science and Pollution Research International* **24**, 20422–20428.

Nasri, E., Subirats, J., Sànchez-Melsió, A., Mansour, H. B., Borrego, C. M. & Balcázar, J. L. 2017b Abundance of carbapenemase genes (bla_{KPC}, bla_{NDM} and bla_{OXA-48}) in wastewater effluents from Tunisian hospitals. *Environmental Pollution* **229**, 371–374. doi: 10.1016/j.envpol.2017.05.095. Epub 12 June 2017.

Pérez-Alvarez, I., Islas-Flores, H., Gómez-Oliván, L. M., Barceló, D., López De Alda, M., Pérez Solsona, S., Sánchez-Aceves, L., SanJuan-Reyes, N. & Galar-Martínez, M. 2018 Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. *Environmental Pollution* **240**, 330–341.

Phillips, K. P. & Foster, W. G. 2008 Key developments in endocrine disrupter research and human health. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* **11**, 322–344.

Sdiri-Loulizi, K., Hassine, M., Aouni, Z., Gharbi-Khelifi, H., Chouchane, S., Sakly, N., Guédiche, M. N., Pothier, P., Aouni, M. & Ambert-Balay, K. 2010 Detection and molecular characterization of enteric viruses in environmental samples in Monastir, Tunisia between January 2003 and April 2007. *Journal of Applied Microbiology* **109**, 1093–1104.

Siddique, H. R., Sharma, A., Gupta, S. C., Murthy, R. C., Dhawan, A., Saxena, D. K. & Chowdhuri, D. K. 2008 DNA damage induced by industrial solid-waste leachates in *Drosophila melanogaster*: a mechanistic approach. *Environmental and Molecular Mutagenesis* **49**, 206–216.

Smaili, S. S., Hsu, Y. T., Carvalho, A. C. P., Rosenstock, T. R., Sharpe, J. & Youle, R. J. 2003 Mitochondria, calcium and proapoptotic proteins as mediators in cell death signaling. *Brazilian Journal of Medical and Biological Research* **36**, 183–190.

Snyder, R. D. & Green, J. W. 2001 A review of the genotoxicity of marketed pharmaceuticals. *Mutation Research* **488**, 151–169.

Souza, D. M., Reichert, J. F. & Martins, A. F. 2018 A simultaneous determination of anti-cancer drugs in hospital effluent by DLLME HPLC-FLD, together with a risk assessment. *Chemosphere* **201**, 178–188.

Tahrani, L., Van Loco, J., Anthonissen, R., Verschaeve, L., Ben Mansour, H. & Reyns, T. 2017 Identification and risk assessment of human and veterinary antibiotics in the wastewater treatment plants and the adjacent sea in Tunisia. *Water Science and Technology* **76** (11–12), 3000–3021.

Tahrani, L., Mehri, I., Reyns, T., Anthonissen, R., Verschaeve, L., Khalifa, A. B. H., Van Loco, J., Abdenaceur, H. & Ben Mansour, H. 2018 UPLC-MS/MS analysis of antibiotics in pharmaceutical effluent in Tunisia: ecotoxicological impact and multi-resistant bacteria dissemination. *Archives of Microbiology* **4**, 553–565.

Toolaram, A. P., Kummerer, K. & Schneider, M. 2014 Environmental risk assessment of anticancer drugs and their transformation products: a focus on their genotoxicity characterization-state of knowledge and short comings. *Mutation Research/Reviews in Mutation Research* **760**, 18–35.

Ventura-Camargo, B. C., Maltempi, P. P. & Marin-Morales, M. A. 2011 The use of the cytogenetic to identify mechanisms of action of an azo dye in *Allium cepa* meristematic cells. *Journal of Environmental and Analytical Toxicology* **1**, 1–12.

Verlicchi, P., Galletti, A., Petrovic, M. & Barceló, D. 2010 Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. *Journal of Hydrology* **389**, 416–428.

Verschaeve, L. 2002 Genotoxicity studies in groundwater, surface waters, and contaminated soil. *Scientific World Journal* **2**, 1247–1253.

Verschaeve, L., Van Gompel, J., Thilemans, L., Regniers, L., Vanparys, P. & Van der Lelie, D. 1999 VITOTOX® bacterial genotoxicity and toxicity test for the rapid screening of chemicals. *Environmental and Molecular Mutagenesis* **33**, 240–248.

Verschaeve, L., Mertens, B., Ndhlala, A. R., Anthonissen, R., Gorissen, B. & Van Staden, B. J. 2012 Extracts from Hypoxis species and a commercially available Hypoxis preparation. *Phytotherapy Research* **27**, 350–356.

Wang, D., Xu, Z., Zhao, Y., Yan, X. & Shi, J. 2011 Change of genotoxicity for raw and finished water: role of purification processes. *Chemosphere* **83**, 14–20.

Yano, T., Itoh, Y., Matsuo, M., Kawashiri, T., Egashira, N. & Oishi, R. 2007 Involvement of both tumor necrosis factor-alpha-induced necrosis and p53-mediated caspase-dependent apoptosis in nephrotoxicity of cisplatine. *Apoptosis* **12**, 1901–1909.

Yilmaz, G., Kaya, Y., Vergili, I., Beril Gönder, Z., Özhan, G., Ozbek Celik, B., Altinkum, S. M., Bagdatli, Y., Boergers, A. & Tuerk, J. 2017 Characterization and toxicity of hospital wastewaters in Turkey. *Environmental Monitoring and Assessment* **189**, 55.

Zegura, B., Heath, E., Cernosa, A. & Filipic, M. 2009 Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere* **75**, 1453–1460.

Zouiten, A., Beltifa, A., Van Loco, J., Ben Mansour, H. & Reyns, T. 2016 Ecotoxicological potential of antibiotic pollution-industrial wastewater: bioavailability, biomarkers, and occurrence in *Mytilus galloprovincialis*. *Environmental Science and Pollution Research International* **23**, 15343–15350.

First received 11 May 2019; accepted in revised form 15 November 2019. Available online 31 December 2019

Downloaded from http://iwaponline.com/jwh/article-pdf/18/1/1/720470/jwh0180001.pdf
by guest