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REVIEW ON GENOTOXICITY OF HOSPITAL WASTEWATERS

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ABSTRACT

With the augmentation of new technologies, changing demographics, economic forces, heightened patient expectations and legislative actions, the healthcare sector is evolving rapidly. Being the centre of cure, health-care facilities are important centers of infectious and cytotoxic waste generation. Major health care facilities such as hospitals use a variety of chemical substances such as pharmaceuticals, radionuclides, solvents, disinfectants for medical purposes such as diagnostics, disinfections and research. After application, some of these substances and excreted nonmetabolized drugs by the patients enter into the hospital effluents which generally reach the urban wastewater and thereby to surface waters. As genotoxic pharmaceutical compounds, including cytostatic agents, are discharged in wastewaters, assessment of genotoxic potential of wastewaters from hospital discharges is a domain of interest. Therefore through this review, the authors attempted to throw light on severity of environmental risk associated with mutagenic and cytotoxic hospital wastewater due to their careless and improper management.

Key words: Hospital wastewaters, Pharmaceuticals, Genotoxicity, Cytotoxicity

INTRODUCTION

Tons of pharmacologically active drugs in medicinal therapy are being consumed annually for diagnosis and treatment of diseases. In proportion to this consumption, large volumes of waste are produced by hospitals and other healthcare entities. Almost 80% of the waste generated by healthcare activities is general non-hazardous waste and only 20% remaining waste is expected to comprise hazardous fraction. High-income countries can generate up to 6 Kg of hazardous waste per person per year. In majority of low-income countries, health care waste is usually not separated into hazardous or non-hazardous waste. In these countries, the total healthcare waste per person per year is anywhere from 0.5 to 3

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Kg (WHO Fact Sheet, 2000). Apart from solid waste, a significant amount of liquid waste or waste water is also discharged from hospitals. The amount of waste water discharged from hospital varies from hospital to hospital but it has been estimated at 400 to 120 liters/bed/day[1]. Tsakona et al.[2] reported an estimate on per capita production of waste water in hospital to be 1000 liters/person/day.

The Indian scenario about health care waste generation reveals that as the health care sector is expanding rapidly in terms of revenue and employment, the waste generation by these facilities is likely to be on increase. In India, total waste generated by health care units is estimated to be 0.33 million tons annually or 0.5-2.0 Kg/bed/day[3]. The national government has promulgated Bio Medical Waste (handling and management) Rules, 1998, prepared national guidelines, and implemented a national training program. Consequent upon amendments made in the year 2000 and subsequently in 2003 in BMW rules, the State Pollution Control Boards (SPCBs) and Pollution Control Committees (PCCs) are in the process of re-inventorising hazardous waste generated[4]. Despite such regulations, health care establishments in India are not giving due attention to their waste management. The absence of waste management, lack of awareness about the health hazards, insufficient financial and human resources and poor control of waste disposal are the most common problems connected with health care wastes. An essential issue is the clear attribution of responsibility of appropriate handling and disposal of waste (WHO Fact Sheet, 2000).

Hospital wastewaters comprising variety of chemicals

Hospitals use a variety of chemical substances such as pharmaceuticals, radionuclides, solvents and disinfectants for medical purposes such as diagnostics, disinfections and research[5,6]. After application, these substances get washed from the body and enter the water systems, ultimately winding up in the effluent of municipal waste water treatment plants and aquatic ecosystems. Buhner[7] stated that high percentages of many pharmaceuticals can be excreted from the body unmetabolized and enter wastewater as biologically active substances. Since medical substances are developed with the intention of performing some sort of biological function, they have a tendency to bioaccumulate and induce effects in aquatic and terrestrial ecosystems[8]. Occurrence of pharmaceutical residues in the environment may also be caused by agriculture applying large amounts of Pharmaceutically Active Compounds (PhACs) as veterinary drugs and feed additives in livestock breeding[9].

Many of these chemical compounds resist normal wastewater treatment. Residues of pharmaceuticals can be found in all wastewater treatment plant (WWTP) effluents, due to their inefficient removal in the conventional systems[10-14]. Several investigations have shown

some evidence that substances of pharmaceutical origin are often not eliminated during municipal wastewater treatment and also not biodegraded in the environment[15,16]. They end up in surface waters where they can influence the aquatic ecosystem and interfere with the food chain[17]. Such wastes are universally accepted as potential danger to human health and environment if they are not handled and managed in an environmentally safe manner[18].

Most important chemicals in hospital wastewater are antibiotics, cytostatic agents, anaesthetics, disinfectants (due to their major use in hospital practice), platinum, mercury (in preservatives in diagnostic agents and as active ingredients of disinfectants), rare earth elements (gadolinium, indium, and osmium) and iodinated X-ray contrast media. Hospitals, in fact, are the fourth largest source of mercury discharged into the environment[19]. Of the antibiotics used for human purposes, 26% are used in hospitals (Kümmerer, 2001). Antibiotics and their metabolites end up in the Waste Water reatment Plant (WWTP), since they are excreted with urine and faeces in wastewater. Kümmerer[10] estimated the total antibiotic load of municipal wastewater (which contains the contribution of hospitals) ats 50 µg/L. He also reported that 90% of the drug, propofol found in anesthesia, is excreted unmetabolized. Unmetabolized pharmaceuticals are often the most non-biodegradable substances in the environment[20]. Jolibois et al.[21] contribute the genotoxic effect of 55% of the samples to anticancer drugs (e.g. ifosfamide, cisplatin) and antibiotics (e.g. ciprofloxacin). This genotoxic effect correlates with the findings of Kümmerer et al.[22] in the Closed Bottle Test (CBT). The authors could not detect biodegradation of ciprofloxacin, ofloxacin and metronidazole in the CBT.

Presence of antibiotics at levels could not only alter the ecology of the environment but also give rise to antibiotic resistance. Hospital effluent with its high content of multidrug resistant enterobacteria and the presence of enteric pathogens could pose a grave problem for the community. The occurrence of strongly selective environments for antimicrobials, such as hospitals, promotes not only the growth of resistant bacteria but also leads to an increase in the frequency of resistance bacterial genes and genetic elements such as plasmids. The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is of special concern. MRSA strains acquire multi resistance by means of additional resistant factors, such as conjugative gentamycin resistance plasmids[23]. Ruiz et al.[24] reported higher antibiotic susceptibility of environmental *Pseudomonas aeruginosa*, which was collected from the hospital tap water and in the garden, relatively to clinical isolates from the same hospital. These studies clearly demonstrate that hospital wastewaters are a source of bacteria with acquired resistance against antibiotics and this with at least a factor of 2-10 higher than domestic wastewater.

Earlier investigations of drug residues in WWTP effluents were focused on clofibric acid, the major metabolite of three lipid regulators (etofibrate, etofyllinclofibrate and clofibrate)[8,25]. Clofibric acid is a metabolite of a blood lipid regulator used to lower blood cholesterol levels. Numerous studies reported the relatively nonbiodegradable nature of this pharmaceutical[7,16]. Buhner[7] insinuates that the increasing levels of estrogen in the environment, via pharmaceuticals for purposes such as menopause symptom relief and birth control pills, could be causing adverse effects on humans, such as reduced male sperm counts and sperm motility and younger ages of puberty in girls. Many literature sources were encountered that supported his statements about the negative effects of increasing aquatic estrogen levels on fish[26,27]. However, estrogen levels in hospital waste water has not been analysed quantitatively yet to our knowledge.

Acetaminophen (paracetamol), acetylsalicylic acid (ASA) and diclofenac or ibuprofen are the most common analgesics (i.e. pain killers, anti-inflammatory and antipyretic drugs) found in sewage treatment plants (STPs). However, these compounds were found to be efficiently removed by the municipal STPs and detected at very low concentrations in sewage effluents and also in rivers[28-30].

The contamination of waters with genotoxic chemicals is a worldwide problem. Cytostatic agents, commonly found in hospital waste water due to anticancerous activities (such as treatment of cancer), represent a danger because of their proven carcinogenicity, mutagenicity and embryotoxic properties[17]. Residues of cytostatic drugs almost exclusively originate from hospital applications and may occur in hospital sewage at concentrations up to the low µg/L level[31]. Steger-Hartmann et al.[32] detected ifosfamide and cyclophosphamide in sewage samples from a university hospital at concentrations of 24 and 146 ng/L, respectively. Kümmerer et al.[6] found ifosfamide at mean concentrations of 109 ng/L in effluents from an oncologic hospital. In the influents and effluents of the receiving municipal STP, it was measured that there was not any significant reduction during sewage treatment. In four out of 16 effluent samples from German STPs, Ternes[28] detected cyclophosphamide at maximum concentrations of 20 mg/L. Ifosfamide was only detected in two samples but in one of these samples with a concentration of 2.9 µg/L.

0.1%–34% of the selected cytostatic agents (cancerostatic platinum compounds (CPC) cisplatin, carboplatin, oxaliplatin, 5-fluorouracil (5-FU) and the anthracyclines (doxorubicin, daunorubicin and epirubicin) administered in the oncologic in-patient treatment ward could be analysed in the oncologic wastewater. Wastewater treatment by means of a MBR-system was able to remove the selected cytostatic drugs due to different mechanisms[33].

Besides pharmaceuticals used in treatment of diseases, many chemical compounds are employed in disease diagnosis. Iodinated contrast media (ICMs) are used for X-ray imaging of soft tissues. The AOI (absorbable organic iodinated compounds) are biologically inert and stable towards metabolism during their passage through the body. They are excreted almost completely within a day after administration, ending up in the WWTP, where they are poorly removed (0–85% removal)[34]. Since not much is known about their fate and long term effects, there is a risk connected to their spread in the environment. They could end up in groundwater. More research is needed on this topic and precautionary measures should be taken.

Microorganisms from hospital wastewater can cause outbreaks of diarrhoea and diseases like cholera[35]. Chemical disinfectants are effective for killing harmful microorganisms in drinking water, but they are also powerful oxidants, oxidizing the organic matter, anthropogenic contaminants, and bromide/iodide naturally present in most source waters (rivers, lakes, and many groundwaters)[36]. Chlorine, ozone, chlorinedioxide, and chloramines are the most common disinfectants in use today; each produces its own suite of disinfection by-products (DBPs) in drinking water, with overlapping constituents[37]. Some epidemiologic studies have shown that a life time exposure to chlorinated water is associated with an increased risk for cancer, especially of the urinary bladder and colorectum[38,39].

Humans are particularly exposed to above described chemical pollutants by the drinking water, produced from contaminated surface water[17]. Consumption of drinking water that contains mutagens may lead to adverse health effects including cancer in humans[40]. Besides, environmental pollution may also affect the stability of aquatic ecosystems[41,42]. All individuals exposed to hazardous health care waste are potentially at risk, including those within health care establishments that generate hazardous waste, and those outside these sources who either handle such waste or are exposed to it as a consequence of careless management[43].

Analysing hospital wastewaters

The hospital liquid waste discharges have been analysed physico-chemically in many studies[44-51], El-Gawad and Aly, 2011. These studies reveal that organic matter can reach up to high concentrations in these effluents. Mahvi et al.[49] detected the presence of heavy metals such as Pb, Cd, Cr, Cu, Fe, Mn and Zn in a concentration exceeding the standard range prescribed by FEPA (Federal Environmental Protection Agency) and USEPA (United States Environmental Protection Agency). Contrary to most of these studies,

physiochemical parameters studied by El-Gawad and Aly[51] revealed that the hospital wastewaters showed most of parameters values within WHO acceptable limits. But the authors recommended that the hospitals have to select onsite separate wastewater treatment alternative as the outcome of their study was that it not applicable to all hospitals.

As genotoxic pharmaceutical compounds, including cytostatic agents, are discharged in wastewaters, the mutagenic potential of wastewaters from various origins (hospital and municipal) is a domain of interest. It is extremely difficult to quantify the risk associated with these chemical pollutants because they usually occur in the concentrations too low to allow analytical determination and putative mutagens, with few exceptions have never been identified. Thus, only physico-chemical analysis is not sufficient to estimate the potential harmful effects of hospital effluents. In order to efficiently assess the presence of mutagens in the water, in addition to the chemical analysis, mutagenicity/genotoxicity assays should be included as additional parameters in water quality monitoring programs[52].

A large number of studies on the genotoxic effects of drinking water, surface and ground waters have been carried out globally and numerous DNA-damaging compounds have been identified[41,52]. However, extensive attention is being paid lately towards the use of toxicological characterization and disposal of hospital liquid effluents. Even if no standard are followed protocols for sample collection, sample processing, or selection of tests exist, all the studies done so far show that the hospital wastewater could have a genotoxic potential[21,31,43,45,48,53-57].

Bioassays do not require prior information about chemical composition and can effectively, economically and rapidly assess the genotoxicity of complex waste materials. Such assays have proven to be of significance in envisaging the genotoxic and mutagenic potential of hospital wastewaters. The development of bioassays employing bacteria aims mainly at reducing both the costs and the duration of the experiments, besides improving the sensitivity of the test to the toxicants present in the samples. The Ames mutagenecity assay with *Salmonella typhimurium* is considered by many researchers as the most sensitive one for a wide array of substances, when compared to other bacterial assays. The Ames test being simple, quick and relatively easy to perform is suggested to be used as an initial screening test to assess the suitability of hospital waste waters to be released into the environment[43]. Testing of chemicals for mutagenecity in Ames assay is based on the knowledge that a substance that is mutagenic in the bacterium in the presence of animal liver enzymes metabolizing chemicals is likely to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans[58].

Gupta et al.[43] conducted the *Salmonella*/microsome reversion assay using the plate incorporation procedure described by Ames et al.[59] and revised by Maron and Ames[60]. This study revealed that the untreated wastewater samples from the hospitals under study showed positive mutagenecity ratio much higher than 2.0. Treated samples collected from Effluent treatment plant (ETP) of Escorts hospital, one of the Indian hospital studied by authors, showed weak mutagenicity activity with all the three strains studied. Earlier, Hartmann et al.[54] found two out of 25 of the composite samples mutagenic using the standard Ames strains (8%).

Earlier studies used to perform the umuC test for the toxicity estimation of hospital wastewaters[31,53,54]. The umuC test makes use of a genetically modified *S. typhimurium* strain. The umuC gene that is linked to the *lacZ* gene is part of the SOS-repair system which becomes active in case of DNA-damage. Hartmann et al.[61] studied the specific induction of the umuC test by fluoroquinolones (FQs) in hospital wastewater. 10 samples out of 25 (40%) were umuC-positive and ciprofloxacin concentrations ranged from 0.7 to 124.5 µg/L.

Jolibois and Guerbet[62] executed their study with the *Salmonella* fluctuation test, a version in liquid medium of the *Salmonella* mutagenecity test usually performed in agar plate[60] as well as with SOS chromotest. They conducted the assay without metabolic activation. Major disadvantages of fluctuation test are that it is marginally slower and slightly more labour intensive than the Ames protocol. For certain applications, however, these disadvantages may be offset by the advantages of somewhat greater sensitivity, ability to be automated, and facility for using hepatocytes for metabolic activation. The test is particularly suitable for the testing of aqueous samples containing low levels of mutagen and hence, well adapted for mutagenicity testing in waste water samples. For SOS chromotest, the authors used a genetically engineered bacterium *E. coli* PQ37, which allows the detection of primary DNA damaging agents. The principle is similar to that of umuC test. The two genotoxicity tests had different sensitivities. Indeed, the *Salmonella* fluctuation test allowed the detection of 68% of the samples as genotoxic while the SOS chromotest indicated 45% samples genotoxic.

To detect the toxic effects of any environmental contaminant on a eukaryotic cell, the assays based on yeast systems have proven to be helpful. *Saccharomyces cerevisiae* D7 assay is significant in detecting the gene conversion, point mutation and mitochondrial DNA mutability[63]. The potential of hospital effluent samples to induce genome rearrangements was investigated by the use of *S. cerevisiae* D7 strain by Paz et al.[54] The results showed only the induction of gene reversion with summer 2003 wastewater samples collected by them.

One study organized by Emmanuel et al.[48] has made use of *Daphnia magna* for the evaluation of genotoxicity of hospital originated wastewaters and reported high toxicity of samples. Among the higher plant bioassays employed for the evaluation of hospital wastewater genotoxicity, the one with *Allium cepa* was the most common. The chromosomal aberration method in *A. cepa* roots have been validated by the International Program on Chemical Safety (IPCS), as an efficient test for the analysis and *in situ* monitoring of the genotoxicity of environmental substances. In a study carried out by Bagatini et al.[56], *A. cepa* test was used to evaluate the genotoxicity of a hospital effluent from Brazil to monitor the risks of environmental contamination. The results demonstrated that there was a decrease in the mitotic index for each of the samples indicating, the presence of compounds with some degree of cytotoxicity present in the effluent. Paz et al.[54] also determined the toxicity of hospital wastewater with the observation of mitotic index in *Allium* root tips.

Cytogenetic effects can be studied either in whole organisms (*'in vivo'*) or cells grown in culture (*'in vitro'*). So far, there exist two studies, to our knowledge, which had make use of animal cell lines[54,57]. A strikingly high incidence of mutagenicity was found in the V79 chromosomal aberration assay by Hartmann et al.[57]. Ferk et al.54 performed the single-cell gel electrophoresis (SCGE) assay with primary rat hepatocytes and reported positive genotoxicity. They also found that membrane filtration resulted in a substantial (62–77%) reduction of these effects, while additional treatments (activated carbon filtration and UV-irradiation) did not lead to a further decrease of the genotoxic activity of the samples.

Recently Alabi and Shokunbi[45] accomplished their research to study the toxicological effects of hospital wastewater using male swiss albino mice. This study is indispensible because it is the one and only study, found in literature till now, utilizing animals. For Chromosomal aberration (CA), there was dose dependent and statistically significant ($p < 0.05$) inhibition of mitotic index (MI) in bone marrow cells of mice. The authors also observed various abnormalities in sperm morphology and the mean sperm count was also found to be declined significantly in dose dependent manner. The results of this study point out precisely that hospital waste poses a significant impact on health and environment, and therefore proper waste management strategy is needed to ensure health and environmental safety.

CONCLUSION

Concluded from above discussed literature, untreated wastewaters from health care centers have been proved to present a potential risk to aquatic ecosystems because of the content of toxic and genotoxic chemicals and properly designed waste treatment systems can

remove or destroy many of the harmful contaminants in wastewaters. And by using a battery of bioassays systems, each with different mechanisms of toxicity, the composite toxicological response to a wastewater sample can be characterised. Although there are species differences in metabolism, DNA repair, and other physiological processes affecting chemical mutagenesis, the universality of DNA and genetic code provides rationale for using various non-human test systems to predict the intrinsic mutagenicity of test chemicals.

The disadvantages associated with animal and plant bioassays such as problem with standardization of the organisms, requirements for special equipment and skilled operators, long duration of the assay and lack of reproducibility make the bacterial assays more attractive. The growing interest in these tests is due to the fact that despite the existence of different toxicity for various organisms of different species, a substance that is toxic for an organism often demonstrates similar toxic effects on the other organisms[64]. Therefore, evaluation of biological effects using a rapid, simple, sensitive and cost effective method could indicate specific information on genotoxicity and ecotoxicity and allow incorporation of toxicity parameters in the regulatory framework[65] and hence, short term assays employ microbes, for testing a toxicant or mutagen, having the aforesaid qualities, seem to be relevant tools for assessing the mutagenic potential and health hazard caused by hazardous effluents (as from hospitals) to human beings and other higher aquatic and terrestrial organisms, upto a considerable extent.

REFERENCES

1. O. M. Iyekhoetin, I. I. Nnanna, O. I. Blessing, B-O. O. Idowu, Eur. J. Sci. Res., **52(2),** 226-235 (2011).
2. M. Tsakona, E. Anagnostopoulou and E. Gidarakos, Waste Manage., **27(2)**, 912-920 (2006)
3. A. D. Patil and A. V. Shekdar, J. Environ. Manage., **63**, 211-220 (2001).
4. B. Onursal, Health Care Waste Management in India, Lessons From Experience, The World Bank (2003).
5. B. Erlandsson and S. Matsson, Water Air Soil Poll., **9(2),** 199-206 (1978).
6. K. Kümmerer, M. Meyer and T. Steger-Hartmann, Wat. Res., **31(11),** 2705-10 (1997).
7. S. H. Buhner, Chelsea Green Publishing: White River Junction, Vermont (2003).
8. B. Halling-Sørensen, Chemosphere, **36(2)**, 357-393 (1998).

9. T. Heberer, Toxicol. Lett., **131**, 5-17 (2002).

10. K. Kümmerer, Chemosphere, **45**, 957-969 (2001).

11. D. Kolpin, E. T. Furlong, M. T. Meyer, E. M. Thurmann, S. D. Zaugg, L. B. Barber and H. T. Buxton, Environ. Sci. Technol., **36**, 1202-1211 (2002).

12. M. Petrovic, S. Gonzalez and D. Barcelo, TrAC, **22(10)**, 685-696 (2003).

13. S. A. Snyder, P. Westerhoff, Y. Yoon and D. L. Sedlak, Environ. Engg. Sci., **20(5)**, 449-469 (2003).

14. M. Carballa, F. Omil, J. M. Lema, M. Llompart, C. Garcia-Jares, I. Rodriguez, M. Gomez and T. Ternes, Wat. Res., **38**, 2918-2926 (2004)

15. C. G. Daughton and T. A. Ternes, Environ. Health Persp., **107(6)**, 907-938 (1999)

16. C. Zwiener, T. Glauner and F. H. Frimmel, HRC-J. High Res. Chromatogr., **23**, 474-478 (2000).

17. B. Pauwels and W. Verstraete, J. Water Health, **4(4)**, 405-416 (2006).

18. B. K. Sharma, Draft Report – Maldives Mission (2007).

19. Emerging Environmental Risks in the Healthcare Industry (2012), http://www.acegroup.com/Attachments/Emerging-Environmental-Risks-in-Healthcare.pdf

20. F. Stuer-Lauridsen, M. Birkved, L. P. Hansen, H. C. Holten Lutzhoft and B. Halling Sorensen, Chemosphere, **40(7)**, 783-793 (2000).

21. B. Jolibois and M. Guerbet, Mutat. Res., **565,** 151-162 (2005).

22. K. Kümmerer, A. Al-Ahmad and V. Mersch-Sundermann, Chemosphere, **40**, 701-710 (2000).

23. K. Ohlsen, T. Ternes, G. Werner, U. Wallner, D. Löffler, W. Ziebuhr, W. Witte and J. Häcker, Environ. Microbiol. **5(8)**, 711-716 (2003).

24. L. Ruiz, M. A. Dominguez, N. Ruiz and M. Viñas, Arch. Med. Res., **35**, 251-257 (2004).

25. Stumpf, Ternes, Wilken, Rodrigues and Baumann, Sci. Total Env., **225**, 135-141 (1999).

26. S. Webb, T. A. Ternes, M. Gibert, and K. Olejniczak, Toxicol. Lett., **142**, 157-167 (2003)

27. F. M. Christensen, Regul. Toxicol. Pharm., **28(3)**, 212-221 (1998)

28. T. A. Ternes, Wat. Res., **32**, 3245-3260 (1998).

29. T. A. Ternes, M. Stumpf, B. Schuppert and K. Haberer, Vom Wasser, **90**, 295-309 (1998).

30. T. A. Ternes, TrAC, **20(8)**, 419-434 (2001)

31. T. Steger-Hartmann, K. Kümmerer and A. Hartmann, Ecotoxicol. Environ. Saf., **36**, 174-9 (1997).

32. T. Steger-Hartmann, K. Kümmerer and J. Schecker, J. Chromatogr. A., **726**, 179-184 (1996).

33. K. Lenz, S. N. Mahnik, N. Weissenbacher, R. M. Mader, P. Krenn, S. Hann, G. Koellensperger, M. Uhl, S. Knasmüller, F. Ferk, W. Bursch and M. Fuerhacker, Water Sci. Technol., **56**, 141-149 (2007).

34. T. Steger-Hartmann, R. Länge, H. Schweinfurth, M. Tschampel and I. Rehmann, Wat. Res., **36**, 266-274 (2002).

35. A. K. Gautam, K. Sunil and P. C. Sabumon, J. Environ. Manage., **83**, 298-306 (2006).

36. S. D. Richardson, M. J. Plewa, E. D. Wagner, R. Schoeny and D. M. DeMarini, Mutat. Res., **636,** 178-242 (2007).

37. S. D. Richardson, The Encyclopedia of Environmental Analysis and Remediation, **3**, 1398-1421 (1998).

38. IARC, IARC Monogr. Eval. Carcinog. Risks Hum., **84**, 1-477 (2004), PMID:15645577.

39. G. E. Bove, P. A. Rogerson and J. E. Vena, Int. J. Health Geogr., **6**, 18 (2007).

40. F. J. Lu, C.L. Hong, M. F. Lu and H. Shimizu, Bull. Environ. Contam. Toxicol., **51**, 545-550 (1993).

41. P. A. White and J. B. Rasmussen, Mutat. Res., **410(3),** 223-236 (1998)

42. K. L. Dearfield, M. C. Cimino, N. E. McCarrol, I. Mauer and L. R. Valcovic, Mutat. Res., **521,** 121-135 (2002).

43. P. Gupta, N. Mathur, P. Bhatnagar, P. Nagar and S. Srivastava, Ecotoxicol. Environ. Saf., **72**, 1925-1932 (2009).

44. I. N. Ibeh and M. I. Omoruyi, JASR, **1(1),** 7-17 (2011).

45. O. A. Alabi and O. S. Shokunbi, Ann. Biol. Res., **2(2)**, 265-275 (2011).

46. F. O. Ekhaise and B. P. Omavwoya, Am. Eurasian J. Agric. Environ. Sci., **4(4)**, 484-488 (2008).

47. E. Emmanuel, J. M. Blanchard, G. Keck and Y. Perrodin, Revue Francophone D'écologie Industrielle, N° 22 – 2ème Trimestre, 31-33 (2001).

48. E. Emmanuel, J. M. Blanchard, G. Keck, Y. Perrodin and P. Vermande, XXVIII Congreso Interamericano De Ingeniería Sanitaria y Ambiental Cancún, México (2002).

49. A. Mahvi, A. Rajabizadeh, A. Fatehizadeh, N. Yousefi, H. Hosseini and M. Ahmadian, WASJ, **7(12)**, 1521-1525 (2009).

50. M. Paz, H. Muzio, V. Gemini, A. Magdaleno, S. Rossi, S. Korol, and J. Moretton, Higiene y Sanidad Ambiental, **4,** 83-88 (2004).

51. H. A. Abd El-Gawad and A. M. Aly, Aust. J. Basic Appl. Sci., **5(7)**, 474-782 (2011).

52. T. Ohe, T. Watanabe and K. Wakabayashi, Mutat. Res., **567**, 109-149 (2004).

53. F. Giuliani, T. Koller, F. E. Würgler and R. M. Widmer, Mutat. Res., **368,** 49-57 (1996).

54. M. Paz, H. Muzio, A. Mendelson, A. Magdaleno, C. Tornello, N. Balbis and J. Moretton, J. Braz. Soc. Ecotoxicol., **1(1),** 1-6 (2006).

55. M. G. S. Ortolan and M. A. Z. Ayub, Brazil. Arc. Bio. Technol., **50**, 637-643 (2007).

56. M. D. Bagatini, T. G. Vasconcelos, H. D. Laughinghouse, A. F. Martins and S. B. Tedesco, Bull. Environ. Contam. Toxicol., **82**, 590-592 (2009).

57. F. Ferk, M. Mi¡sík, T. Grummt, B. Majer, M. Fuerhacker, C. Buchmann, M. Vital, M. Uhl, K. Lenz, B. Grillitsch, W. Parzefall, A. Nersesyan and S. Knasmüller, Mutat. Res., **672**, 69-75 (2009).

58. N. Mathur, P. Bhatnagar, and P. Bakre, Appl. Ecol. Env. Res., **4(1)**, 111-118 (2005).

59. B. N. Ames, J. McCann and E. Yamasaki, Mutat. Res., **31**, 347-364 (1975).

60. D. M. Maron, and B. N. Ames, Mutat. Res., **133**, 173-215 (1983).

61. A. Hartmann, A. C. Alder, T. Koller and R. M. Widmer, Environ. Toxicol. Chem., **17**, 377-382 (1998).

62. B. Jolibois and M. Guerbet, Ann. Occup. Hyg., **50(2)**, 89-196 (2005).

63. C. Pellacani, E. Branchi, A. Buschini, M. Furlini, P. Poli and C. Rossi, Epidemiol. Prev., **29(5-6 Suppl),** 34-6 (2005).

64. K. L. E. Kaiser, Environ. Health Prospect., **106,** 583-91 (1998).

65. S. Parvez, C. Venkataraman and S. Mukherji, Environ. Int., **32**, 265-268 (2006).

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