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Short Communication

The evaluation of reactive textile dyes regarding their potential to cause organ-specific cyto- and geno-toxicity

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Abstract

The textile industry extensively uses synthetic chemicals such as dyes. Several studies report the deleterious effects (e.g., cell death and DNA damage) of dispersive dyes on humans. Humans can be exposed to toxic dyes by ingesting contaminated waters or dermal contact with colored garments. Thus, toxicity evaluations of textile dyes using organ-specific cell lines are relevant to estimating their hazard. Cyto- and geno-toxicity of the dyes Reactive Green 19 (RG19), Reactive Blue 2 (RB2), Reactive Blue 19 (RB19), Reactive Red 120 (RR120) and Reactive Orange 16 (RO16) were evaluated by the *In Vitro* MicroFlow® kit with immortalized human keratinocyte cell line (HaCaT) and immortalized human hepatic cell line (HepaRG). Concentration-dependent cytotoxicity was observed for HaCaT cells exposed to three of the five tested dyes (RB2, RB19, RO16), while in HepaRG cells, cytotoxic effects were only verified after exposure to RB19 and RO16 at the highest tested concentration (1000 µg/mL). Genotoxicity was not detected in any tested textile dyes under both test conditions (HaCaT and HepaRG). In conclusion, our data provide evidence that, although the tested reactive dyes are not genotoxic, which is in agreement with published literature, they can cause cytotoxicity in both target tissue, and the effect can be more severe in epidermal cells (HaCaT) than in liver cells (HepaRG). This differential cytotoxicity data emphasizes the need to assess the toxicity of textile dyes to the target organ specificity.

Keywords: cytotoxicity; skin cells; liver cells; flow cytometry-based micronucleus assay.

INTRODUCTION

The dyeing of fabrics is an art that began thousands of years ago, and the commercial availability of dyes is enormous (≥ 10,000 colors) (Ferraz *et al.,* 2012). Reactive dyes are the main class of dyes used in dyeing cotton fibers. However, their application may cause undesirable levels of environmental contamination and harmful effects on living organisms, including humans (Leme *et al.*, 2014, 2015b; Fernandes *et al.,* 2019). The environmental problems of reactive dyes are related to their high solubility in water and nondegradable under the typical aerobic conditions of conventional and biological treatment systems (Hassan *et al.*, 2009; Oliveira *et al.,* 2010). Ingestion of dispersive dye-polluted waters can compromise human health by inducting DNA damages (Oliveira *et al*., 2006, Chequer *et al.,* 2011; Ferraz *et al.*, 2013; Vacchi *et al*., 2013, 2017; Domingues *et al.,* 2020). Apart from the oral route of exposure, humans

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can also be affected by textile dyes by dermal contact with colored garments (Leme *et al.*, 2014, 2015b; Brüschweiler *et al.*, 2014).

Based on the association between DNA damages and cancer development, the safety assessment of chemicals comprises genotoxicity studies (Bolognese *et al.*, 2017). *In vitro* genotoxicity tests are frequently performed with transformed or permanently growing cell lines (*e.g.,* V79 and CHO) (Corvi & Madia, 2017). However, the choice of cell type for genotoxicity testing does not consider hazard identification in different routes of exposure; and chemical toxicity can differ depending on the target organ. For instance, the textile dye Disperse Red 1 is genotoxic for human hepatoma cells (HepG2) but not for dermal equivalents (Leme *et al.,* 2015b).

The *in vitro* micronucleus (MN) assay tests clastogenicity/aneugenicity, and it is an endpoint required to predict chemical genotoxicity (Corvi & Madia, 2017). Studies using flow cytometry-based MN assay demonstrated several advantages in using this method: high throughput, high number of cells capable of being evaluated, good performance in detecting chemical genotoxicity, identification of genotoxic mode of action (clastogenic and/or aneugenic effects), and detection of cytotoxic effects (Avlasevich *et al.,* 2011; Bryce *et al*., 2011; Yao *et al.,* 2013; García-Rodríguez *et al*., 2019). Thus, flow cytometry-based MN assay allows assessing cytotoxicity and genotoxicity, which are predictive parameters essential for characterizing chemical toxicity (García-Rodríguez *et al*., 2019).

In this study, the organ-specific cyto- and geno-toxicity of the textile dyes Reactive Green 19 (RG19), Reactive Blue 2 (RB2), Reactive Blue 19 (RB19), Reactive Red 120 (RR120) and Reactive Orange 16 (RO16) was investigated using *In Vitro* MicroFlow® kit in miniaturized (96-well plate) format. The HaCaT (immortalized human keratinocyte cell line) and HepaRG (immortalized human hepatic cell line) cells were employed as representative models of dermal and oral routes of exposure, respectively.

MATERIAL AND METHODS

Tested chemicals

The textile dyes Reactive Green 19 (RG19 – CAS No. 61931-49-5, dye content 65%), Reactive Blue 2 (RB2 – CAS No. 12236-82-7, dye content 60%), Reactive Blue 19 (RB19 – CAS No. 2580-78-1, dye content ~50%), Reactive Red 120 (RR120 – CAS No. 61951-82-4, dye content 50-70%) and Reactive Orange 16 (RO16 – CAS No. 12225-83-1, dye content ≥ 70%) (Fig. 1) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS) was used as a vehicle to prepare the working dye solutions.

In vitro MicroFlow® kit (Litron) assay

Cell culture. HaCaT cells (Cell line services, Germany) and HepaRG (Biopredic International, Rennes, France, acquired from Fisher Scientific) were grown in culture medium at 37°C, flushed with 5% CO_2 in air. Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) (all from Gibco®, Life Technologies, Grand Island, USA) and addition of 100 IU/mL penicillin G, 100 mg/mL streptomycin and 1 µg/mL amphotericin, was used for HaCaT cells. Williams' E medium, supplemented with 10% fetal calf serum (FCS), 1% insulin, 1% hydrocortisone hemisuccinate and addition of 0.1% streptomycin/penicillin, was used for HepaRG.

Chemical treatments. Tested concentrations were determined according to Leme et al. (2015b). HaCaT and HepaRG cells, at $1x10^5$ and $2x10^5$ cells/mL, respectively, were exposed in a 96-well format to six concentrations, of the test dyes (at 31.25 to 1000 µg/mL); PBS 20%-v/v (negative control); Mitomycin C (MMC) at 2.5 to 10 µg/Ml (positive control) and Vinblastine (VB) at 12.5 -50 ng/mL (positive control) for 48 and 24 h, for both cell lines, respectively. The experiments were carried out in duplicate/treatment and repeated twice.

Flow cytometry-based MN measurements. A sequential staining method was applied to the treated samples according to the instructions described in the *In Vitro* MicroFlow® Kit (Litron Laboratories Ltd., Rochester, New York, United States). Briefly, the medium was removed, and the treated cells were stained with the photo-activated dye ethidium monoazide (EMA). The cells were washed, then lysed, and stained with lysis solutions composed of a nonionic detergent, pan-nucleic acid dye SYTOX Green and RNase.

The plates were immediately analyzed with a BD Biosciences (San Jose, CA) FACSCanto II flow cytometer. The percentages (%) of relative survival, EMA-positive, hypodiploid nuclei and MN were determined based on the acquisition of at least 10,000 gated nuclei per well.

Data evaluation. The % and fold-increase calculations were made according to Bryce et al. (Bryce et al., 2011), using Excel Office 2007 (Microsoft, Seattle, WA). A result was deemed positive if the following criteria were met: (i) cytotoxicity (EMA-positive events) showed greater than 3-fold increase compared with the concurrent solvent control values; (ii) genotoxicity – not overly cytotoxic concentration (without exceeding 60% reduction of relative survival) resulting in ≥ 3-fold increase in mean % MN relative to control value.

RESULTS AND DISCUSSION

Three of the five tested reactive dyes (RB2 (1000 µg/mL), RB19 (500 and 1000 µg/mL), RO16 (1000 µg/mL)) were cytotoxic (*i.e.*, significant increase in EMA-positive) for HaCaT cells (Figure 1). In contrast, in HepaRG cells, cytotoxic effects were observed only for RB19 and RO16 at the highest tested concentration (1000 µg/mL) (Figure 2).

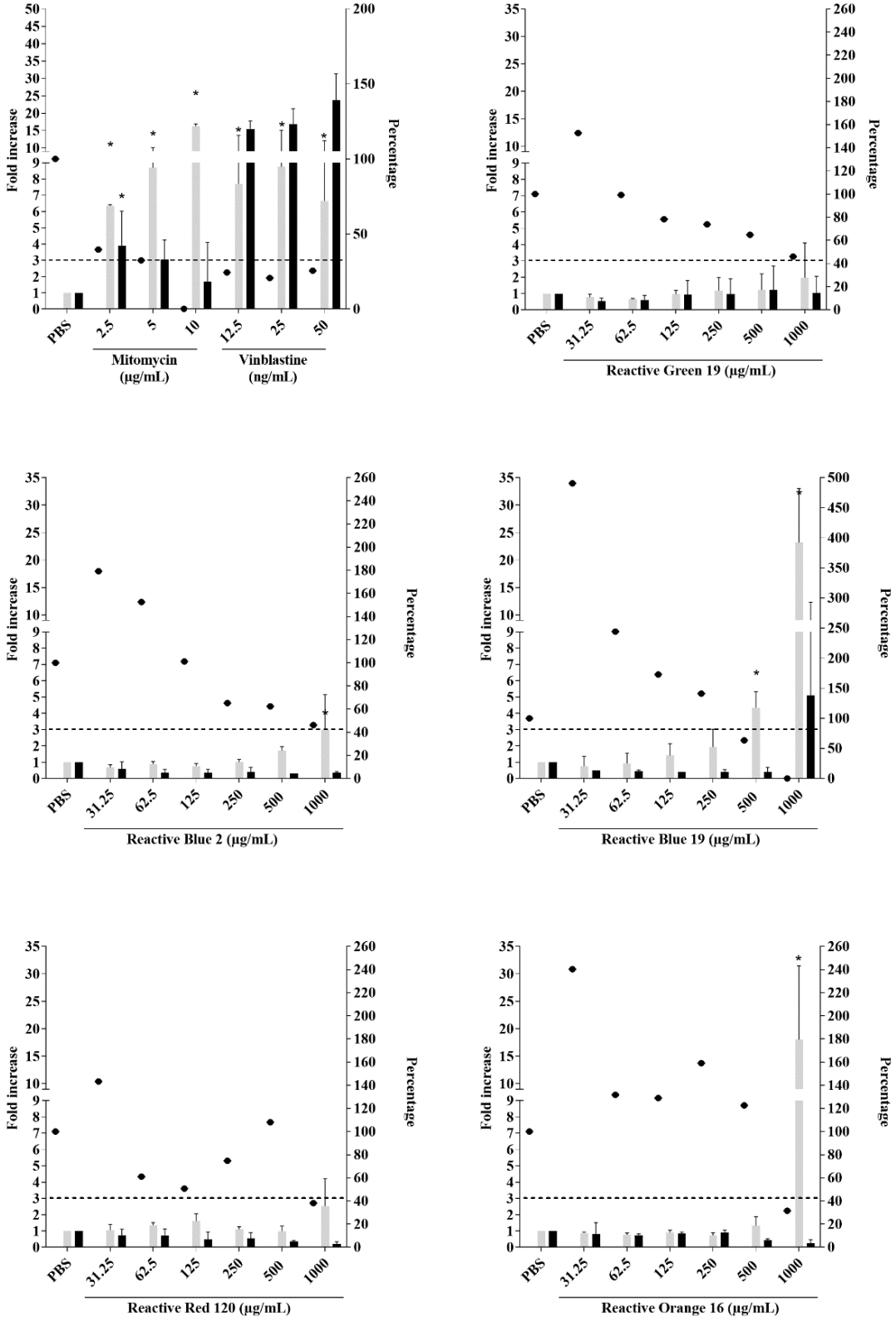

Figure 1. Cyto- and geno- data of flow cytometry-based in vitro MN assay with HaCaT cells are graphed for the tested reactive textile dyes. The Y-axis shows fold increase values of EMA+ and MN. The YY-axis showed the percentage of Relative Survival. *≥3-Fold over the concurrent solvent control (PBS).

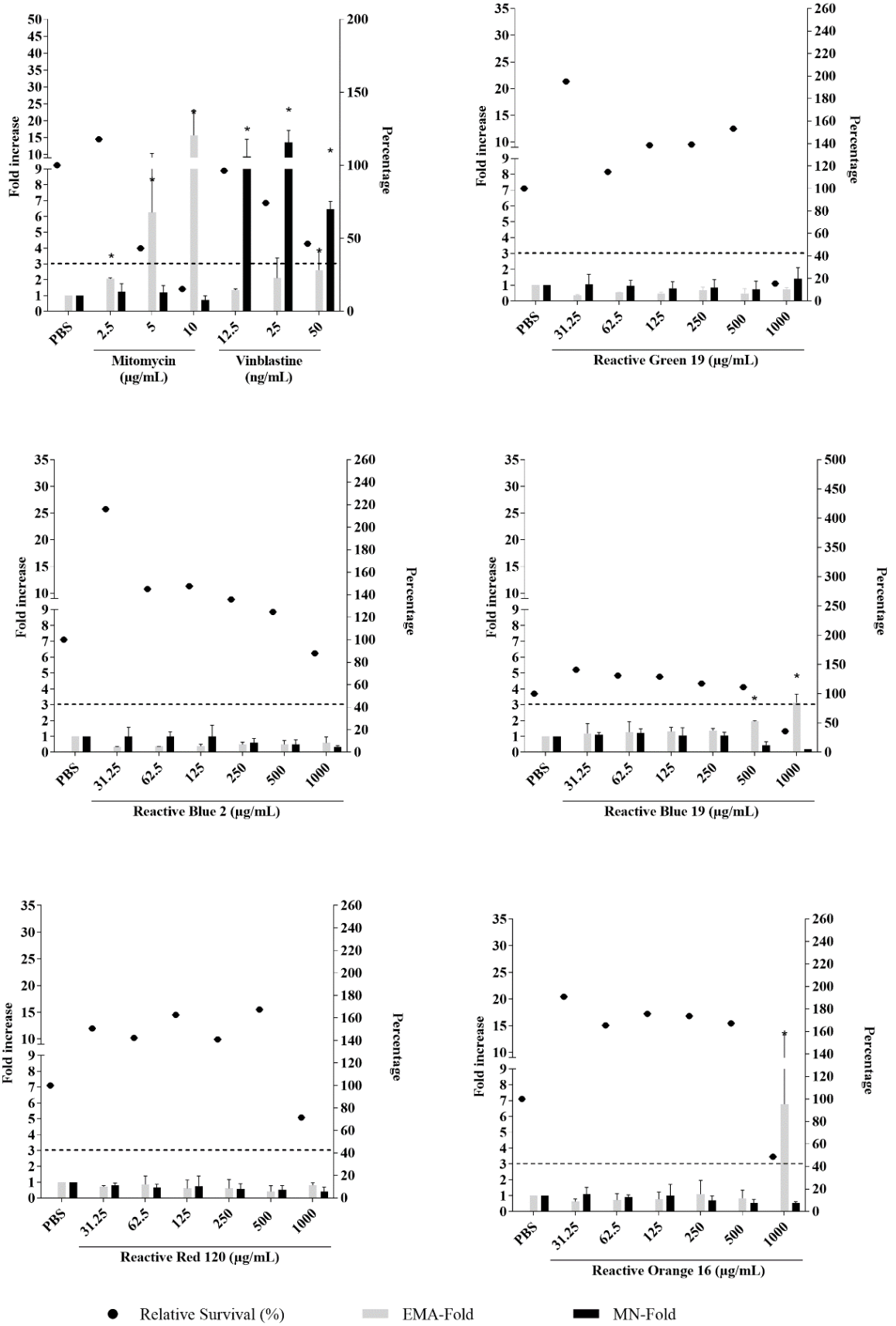

Figure 2. Cyto- and geno-toxicity data of flow cytometry-based in vitro MN assay with HepaRG™ cells are graphed for the tested reactive textile dyes. The Y-axis shows fold increase values of EMA+ and MN. The YY-axis showed the percentage of Relative Survival. *≥3-Fold over the concurrent solvent control (PBS).

Cytotoxic effects were correlated to organ-specific toxicity, as observed in hepatic, cardiac and nephrotoxicity. Thus, assessing cell cytotoxicity *in vitro* helps in the earlier prediction of toxic effects on specific organs (Lin *et al.*, 2012). In this context, the first step is to identify differences in sensitivity for each particular cell line. Specific cytotoxicity is related to the intrinsic cellular, physiological and metabolic differences between different cell lines, emphasizing the need to study the toxicity in an organ-specific context (Yeasmin *et al.*, 2017).

The results showed that HaCaT cells appear to be more sensitive to reactive dyes than HepaRG cells. Other studies have indicated that HaCaT cell line tends to be more sensitive than other human cell lines, such as HepG2, HPL-1D, A431 (NTP, 2019; Eremin *et al*., 2018). In addition, HepaRG is a metabolic competent cell line with a metabolizing rate greater than HaCaT. This may contribute to faster detoxification of these chemicals, causing a lower level of damage in the cell. Furthermore, it is important to note that RB19 and RO16 are the dyes with the lowest molecular weights among those tested, and these dyes presented the highest level of cytotoxic effects in both cell lines. Thus, our data suggest that molecular weight might be an important contributor factor to the cytotoxicity of reactive dyes, as with other types of compounds (Monnery *et al.*, 2017; Huang *et al.*, 2004).

Regarding the genotoxic effects, for both cell lines, genotoxicity was not verified in any of the tested dyes compared to the negative control (Figure 1-2). Studies with reactive dyes using alkaline Comet assay generally report no genotoxicity for these dyes with mammalian cell line cultures (Leme *et al*., 2014; Janović *et al*., 2017), except RG19 and RB5 that presented genotoxicity in a three-dimensional (3D) human dermal equivalent and human lung epithelial cell line (Leme *et al*., 2015b; Janović *et al.*, 2017). This may occur due to the metabolic activities specific to each cell type and the test setup systems used in each study; 3D tissue-like are more likely to suffer genotoxic damages when compared to monolayer cell cultures (Mandon *et al*., 2019; Behravesh *et al*., 2005). Moreover, differences between the genotoxicity test methods (Comet assay and MN assay) may explain the divergent results for RG19 and RB5. The Comet assay can detect primary DNA damages (*e.g.*, single and double DNA strand breaks), which are likely to suffer the rapid action of the DNA repair systems (Collins, 2014). Therefore, as the chemical can cause DNA damage by different mechanisms of action, genotoxicity testing needs to cover all types of DNA damages (DNA strand breaks, chromosomal aberrations, sister-chromatid exchanges and micronuclei) to precisely determine the genotoxic nature of a chemical (Al-Saleh *et al*., 2017; Tafurt-Cardona *et al*., 2015).

CONCLUSION

The manufacturing of textiles commonly utilizes reactive dyes for dyeing cotton and other cellulose-based fibers. Given its widespread use and different human exposure pathways, it is important to evaluate the toxicity of textile dyes considering their different context of human exposure. Although the tested reactive dyes do not cause genetic damage (genotoxicity), they can cause cytotoxic effects in keratinocytes and hepatic cells and may pose risks to human health. The cytotoxicity effects of the reactive dyes are organ-specific, and it seems that epidermal cells (HaCaT) are more prone to the cytotoxic effects of these dyes than the hepatic cells (HepaRG). In order to better understand the present results, additional mechanistic studies need to be performed to elucidate the cellular uptake of tested dyes and their mode of action, particularly those related to cytotoxicity and genotoxicity. Nevertheless, the findings of this study show the need to perform investigations on the toxicity of textile dyes, considering local toxicity in target organs relevant for human exposure.

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AUTHOR CONTRIBUTION STATEMENT

Enzo Zini Moreira Silva – Formal analysis, writing - review & editing.

Andrea Sehr - Investigation, methodology, validation, visualization, data curation, formal analysis, writing - review & editing.

Tamara Grummt † - Conceptualization, supervision, funding acquisition, resources, validation, writing- review.

Danielle Palma de Oliveira - Conceptualization, resources, supervision, writing - review & editing.

Daniela Morais Leme - Conceptualization, formal analysis, validation, supervision, writing - review & editing, project administration.

REFERENCES

Al-Saleh, I., Al-Rajudi, T., Al-Qudaihi, G., & Manogaran, P. (2017). Evaluating the potential genotoxicity of phthalates esters (PAEs) in perfumes using in vitro assays. Environmental science and pollution research international, 24(30), 23903–23914. https://doi.org/10.1007/s11356-017-9978-1.

Avlasevich, S., Bryce, S., De Boeck, M., Elhajouji, A., Van Goethem, F., Lynch, A., Nicolette, J., Shi, J., & Dertinger, S. (2011). Flow cytometric analysis of micronuclei in mammalian cell cultures: past, present and future. Mutagenesis, 26(1), 147–152. https://doi.org/10.1093/mutage/geq058.

Behravesh, E., Emami, K., Wu, H., & Gonda, S. (2005). Comparison of genotoxic damage in monolayer cell cultures and three-dimensional tissue-like cell assemblies. Advances in space research : the official journal of the Committee on Space Research (COSPAR), 35(2), 260–267. https://doi.org/10.1016/j.asr.2005.01.066.

Bolognesi, C., Castoldi, A. F., Crebelli, R., Barthélémy, E., Maurici, D., Wölfle, D., Volk, K., & Castle, L. (2017). Genotoxicity testing approaches for the safety assessment of substances used in food contact materials prior to their authorization in the European Union. Environmental and molecular mutagenesis, 58(5), 361–374. https://doi.org/10.1002/em.22094.

Brüschweiler, B. J., Küng, S., Bürgi, D., Muralt, L., & Nyfeler, E. (2014). Identification of non-regulated aromatic amines of toxicological concern which can be cleaved from azo dyes used in clothing textiles. Regulatory toxicology and pharmacology : RTP, 69(2), 263–272. https://doi.org/10.1016/j.yrtph.2014.04.011.

Bryce, S. M., Avlasevich, S. L., Bemis, J. C., & Dertinger, S. D. (2011). Miniaturized flow cytometry-based CHO-K1 micronucleus assay discriminates aneugenic and clastogenic modes of action. Environmental and molecular mutagenesis, 52(4), 280–286. https://doi.org/10.1002/em.20618.

Chequer, F. M., Lizier, T. M., de Felício, R., Zanoni, M. V., Debonsi, H. M., Lopes, N. P., Marcos, R., & de Oliveira, D. P. (2011). Analyses of the genotoxic and mutagenic potential of the products formed after the biotransformation of the azo dye Disperse Red 1. Toxicology in vitro : an international journal published in association with BIBRA, 25(8), 2054–2063. https://doi.org/10.1016/j.tiv.2011.05.033.

Collins A. R. (2014). Measuring oxidative damage to DNA and its repair with the comet assay. Biochimica et biophysica acta, 1840(2), 794–800. https://doi.org/10.1016/j.bbagen.2013.04.022.

Corvi, R., & Madia, F. (2017). In vitro genotoxicity testing-Can the performance be enhanced?. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 106(Pt B), 600–608. https://doi.org/10.1016/j.fct.2016.08.024.

Domingues, G., Düsman E., Vicentini, V.E.P. (2020). Cytotoxicity of Crude and Treated Liquid Effluents from Textile Industry Dyeing Using Bioindicator Allium cepa L. Water, Air & Soil Pollution, 231, 448. doi:https://doi.org/10.1007/s11270-020-04818-1.

Eremin A. V., Suezov E. V., Grishina P. S., Ponyaev A. I., Medvedskiy N. L. (2018). Relative cytotoxicity of complexes of platinum(II) and palladium(II) against pure cell culture Paramecium caudatum and human cell lines A431 and HaCaT. Mediterranean Journal of Chemistry, 7, 28-38. https://doi.org/10.13171/mjc71/01803131026-eremin.

Fernandes, F. H., Umbuzeiro, G. A., & Salvadori, D. (2019). Genotoxicity of textile dye C.I. Disperse Blue 291 in mouse bone marrow. Mutation research. Genetic toxicology and environmental mutagenesis, 837, 48–51. https://doi.org/10.1016/j.mrgentox.2018.10.003.

Ferraz, E. R., Li, Z., Boubriak, O., & de Oliveira, D. P. (2012). Hepatotoxicity assessment of the azo dyes disperse orange 1 (DO1), disperse red 1 (DR1) and disperse red 13 (DR13) in HEPG2 cells. Journal of toxicology and environmental health. Part A, 75(16-17), 991–999. https://doi.org/10.1080/15287394.2012.696513.

Ferraz, E. R., Oliveira, G. A., Grando, M. D., Lizier, T. M., Zanoni, M. V., & Oliveira, D. P. (2013). Photoelectrocatalysis based on Ti/TiO2 nanotubes removes toxic properties of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1 from aqueous chloride samples. Journal of environmental management, 124, 108–114. https://doi.org/10.1016/j.jenvman.2013.03.033.

García-Rodríguez, A., Kazantseva, L., Vila, L., Rubio, L., Velázquez, A., Ramírez, M. J., Marcos, R., & Hernández, A. (2019). Micronuclei Detection by Flow Cytometry as a High-Throughput Approach for the Genotoxicity Testing of Nanomaterials. Nanomaterials (Basel, Switzerland), 9(12), 1677. https://doi.org/10.3390/nano9121677.

Hassan, S. S., Awwad, N. S., & Aboterika, A. H. (2009). Removal of synthetic reactive dyes from textile wastewater by Sorel's cement. Journal of hazardous materials, 162(2-3), 994–999. https://doi.org/10.1016/j.jhazmat.2008.05.138.

Huang, M., Khor, E. & Lim, LY. Uptake and Cytotoxicity of Chitosan Molecules and Nanoparticles: Effects of Molecular Weight and Degree of Deacetylation. Pharm Res 21, 344–353 (2004). https://doi.org/10.1023/B:PHAM.0000016249.52831.a5

Janović, B. S., Collins, A. R., Vujčić, Z. M., & Vujčić, M. T. (2017). Acidic horseradish peroxidase activity abolishes genotoxicity of common dyes. Journal of hazardous materials, 321, 576–585. https://doi.org/10.1016/j.jhazmat.2016.09.037.

Leme, D. M., de Oliveira, G. A., Meireles, G., dos Santos, T. C., Zanoni, M. V., & de Oliveira, D. P. (2014). Genotoxicological assessment of two reactive dyes extracted from cotton fibres using artificial sweat. Toxicology in vitro : an international journal published in association with BIBRA, 28(1), 31–38. https://doi.org/10.1016/j.tiv.2013.06.005.

Leme, D. M., Oliveira, G. A., Meireles, G., Brito, L. B., Rodrigues, L., & Palma de Oliveira, D. (2015a). Eco- and genotoxicological assessments of two reactive textile dyes. Journal of toxicology and environmental health. Part A, 78(5), 287–300. https://doi.org/10.1080/15287394.2014.971208

Leme, D. M., Primo, F. L., Gobo, G. G., da Costa, C. R., Tedesco, A. C., & de Oliveira, D. P. (2015b). Genotoxicity assessment of

reactive and disperse textile dyes using human dermal equivalent (3D cell culture system). Journal of toxicology and environmental health. Part A, 78(7), 466–480. https://doi.org/10.1080/15287394.2014.999296.

Lin, Z., & Will, Y. (2012). Evaluation of drugs with specific organ toxicities in organ-specific cell lines. Toxicological sciences: an official journal of the Society of Toxicology, 126(1), 114–127. https://doi.org/10.1093/toxsci/kfr339.

Mandon, M., Huet, S., Dubreil, E., Fessard, V., & Le Hégarat, L. (2019). Three-dimensional HepaRG spheroids as a liver model to study human genotoxicity in vitro with the single cell gel electrophoresis assay. Scientific reports, 9(1), 10548. https://doi.org/10.1038/s41598-019-47114-7.

Monnery, B. D., Wright, M., Cavill, R., Hoogenboom, R., Shaunak, S., Steinke, J., & Thanou, M. (2017). Cytotoxicity of polycations: Relationship of molecular weight and the hydrolytic theory of the mechanism of toxicity. International journal of pharmaceutics, 521(1-2), 249–258. https://doi.org/10.1016/j.ijpharm.2017.02.048

NTP. National Toxicology Program. (2019). Research Report on Synthetic Turf/Recycled Tire Crumb Rubber: Characterization of the Biological Activity of Crumb Rubber In Vitro: Research Report 12 [Internet]. Research Triangle Park (NC): National Toxicology Program. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545085

Oliveira, D. P., Carneiro, P. A., Rech, C. M., Zanoni, M. V., Claxton, L. D., & Umbuzeiro, G. A. (2006). Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water. Environmental science & technology, 40(21), 6682–6689. https://doi.org/10.1021/es061020p.

Oliveira, G. A., Ferraz, E. R., Chequer, F. M., Grando, M. D., Angeli, J. P., Tsuboy, M. S., Marcarini, J. C., Mantovani, M. S., Osugi, M. E., Lizier, T. M., Zanoni, M. V., & Oliveira, D. P. (2010). Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mutation research, 703(2), 200–208. https://doi.org/10.1016/j.mrgentox.2010.09.001.

Tafurt-Cardona, Y., Suares-Rocha, P., Fernandes, T. C., & Marin-Morales, M. A. (2015). Cytotoxic and genotoxic effects of two hair dyes used in the formulation of black color. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 86, 9–15. https://doi.org/10.1016/j.fct.2015.09.010.

Vacchi, F. I., Albuquerque, A. F., Vendemiatti, J. A., Morales, D. A., Ormond, A. B., Freeman, H. S., Zocolo, G. J., Zanoni, M. V., & Umbuzeiro, G. (2013). Chlorine disinfection of dye wastewater: implications for a commercial azo dye mixture. The Science of the total environment, 442, 302–309. https://doi.org/10.1016/j.scitotenv.2012.10.019.

Vacchi, F. I., Vendemiatti, J., da Silva, B. F., Zanoni, M., & Umbuzeiro, G. A. (2017). Quantifying the contribution of dyes to the mutagenicity of waters under the influence of textile activities. The Science of the total environment, 601-602, 230–236. https://doi.org/10.1016/j.scitotenv.2017.05.103.

Yao, J., Gao, Q., Mi, Q., Li, X., Miao, M., Cheng, P., & Luo, Y. (2013). In vitro micronucleus assay for the analysis of total particulate matter in cigarette smoke: comparison of flow cytometry and laser scanning cytometry with microscopy. Mutation research, 755(2), 120–125. https://doi.org/10.1016/j.mrgentox.2013.05.017.

Yeasmin S., K. H., Chaudhuri S., Malik D., Bandyopadhyay A. (2017). In-vitro anti-cancer activity of shape controlled silver nanoparticles (AgNPs) in various organ specific cell lines. Journal of Molecular Liquids, 242, 757-766. https://doi.org/10.1016/j.molliq.2017.06.047.