

***In vitro* studies on the genotoxicity of reduced graphene oxide**

Oskar Cebadero-Dominguez*, C Medrano-Padial, M Puerto, A M Cameán and Angeles Jos
University of Seville, Spain

The interest in graphene derivatives, as reduced graphene oxide (rGO), has increased as they possess unique physical and chemical properties for applications in different fields. However, exposure to graphene materials could be a risk for human health. Thus, the European Food Safety Authority in its guidance on risk assessment of nanomaterials to be applied in the food and feed chain, recommends to perform genotoxicity and mutagenicity studies to assure the safety of materials. The aim of this work is to define the potential in vitro genotoxic and mutagenic effects of rGO on a human colorectal adenocarcinoma cell line (Caco-2) and L5178Y Tk+/- mouse lymphoma cells. For this purpose, a battery of different in vitro assays was used: micronucleus test (MN), mouse lymphoma assay (MLA), and comet assay. L5178Y Tk+/- cells were used for MN and MLA. Cells were exposed at different rGO concentrations (0-250 µg/mL) for 24h in the MN test and for 4-24h in MLA. In the comet assay, Caco-2 cells were exposed to EC50 (176.3 ± 7.56 µg/mL), EC50/2 and EC50/4 for 24h and 48h. The test concentrations were based on previous cytotoxicity studies. No genotoxic effects were observed in the MN and comet assays at any concentration tested. Nevertheless, rGO caused statistical differences in the mutant frequency on L5178Y Tk+/- cells from 125 µg/mL after 4h of exposure. In conclusion, our results evidence mutagenic effects of rGO. Therefore, further studies are necessary before its potential commercial application. Acknowledgement: Project US-1259106 cofunded by Programa Operativo FEDER 2014-2020 and Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía. And project P18-RT-1993 (PAIDI-2020, Junta de Andalucía). Biology Services of CITIUS are acknowledged for technical assistance.

Recent Publications

1. O. Cebadero et al., (2022). In vitro toxicity evaluation of graphene oxide and reduced graphene oxide on Caco-2 cells. Toxicology reports.
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3. OECD (2016), Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.
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Biography

Óscar Cebadero is graduated in Biology at University of Salamanca with postgraduate studies in Physiology and [Neuroscience](#) at the University of Sevilla. At present, he is PhD student in the Department of Nutrition and Bromatology, Toxicology, and Legal Medicine at the University of Sevilla. Their studies are based on the development and toxicological evaluation of graphene derivatives for their potential use as food contact materials.

Received: January 16, 2022; **Accepted:** January 20, 2022; **Published:** March 09, 2022
