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## ► To cite this version:

G Caorsi, F Candotto Carniel, Emmanuel Flahaut, E Vázquez, M Prato, et al.. Applicability of OECD test guideline 201 to graphene-related materials (GRMs): new insights into GRMs dispersions stability and possible modifications of the standard protocol. International Congress of Toxicology (ICT2022), Sep 2022, Maastricht, Netherlands. hal-03850073

**HAL Id: hal-03850073**

**<https://hal.science/hal-03850073>**

Submitted on 12 Nov 2022

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## **Applicability of OECD test guideline 201 to graphene-related materials (GRMs): new insights into GRMs dispersions stability and possible modifications of the standard protocol**

G. Caorsi<sup>1</sup>, F. Candotto Carniel<sup>1</sup>, E. Flahaut<sup>2</sup>, E. Vázquez<sup>3</sup>, M. Prato<sup>4</sup>, M. Tretiach<sup>1</sup>

*Proceedings of the 16<sup>th</sup> International Congress of Toxicology (ICT2022), Sept. 18-21, 2022, Maastricht, The Netherlands*

*1 University of Trieste, Department of Life Sciences, Trieste, Italy;*

*2 University Paul-Sabatier, CIRIMAT, Toulouse, France;*

*3 University of Castilla-La Mancha, Department of Organic Chemistry, Ciudad Real, Spain;*

*4 University of Trieste, Department of Chemical and Pharmaceutical Sciences, Trieste, Italy*

Graphene-related materials (GRMs) are used in many innovative applications thanks to their unique physicochemical properties. The increasing development of GRMs-containing products and the resulting future widespread availability could lead to the unintended release of GRMs with unknown consequences for the environment.

According to REACH, all new substances manufactured or imported in the EU must undergo a battery of ecotoxicological bioassays [OECD Test Guidelines (TG)] to assess their potential risk to aquatic organisms. However, these tests were developed to assess the toxicity of water-soluble substances, whereas GRMs are not. GRMs dispersed in aqueous media tend to aggregate and settle, changing their bioavailability over time. Therefore, this work aims to assess the applicability of TG201 to GRMs by studying the GRMs Dispersions Stability (GDS) in the TG201 medium. GDS was evaluated by examining the sedimentation and aggregation of GRMs by Turbiscan and flowcytometry (FCM) analyses, respectively, over the 72 hours test period. The following factors affecting GDS were tested: (i) particle composition and (ii) concentration, (iii) turbulence, and (iv) medium composition. The effects of (i)–(iii) were tested using dispersions of few-layer graphene (FLG), graphene oxide (GO), and reduced GO (rGO) at 4–20 mg L<sup>-1</sup>, that were or were not subjected to various methods to maintain turbulence (bubbling, shaking, and stirring). The effect of (iv) was tested by comparing GDS in distilled water, 5 commercial mineral waters with different ionic contents, and TG201 medium eventually added with dispersants [Suwannee River Natural Organic Matter (SRNOM), carboxymethylcellulose (CMC), and sodium deoxycholate (SDC)]. In addition, GRMs dispersions were used to evaluate the algal growth inhibition, i.e. the TG201 endpoint. Turbiscan analyses revealed that FLG had the fastest sedimentation rate in the first 3 hours, while GO was the most stable, independent of (ii). Visual inspections and FCM revealed that magnetic stirring increased GDS the most. However, turbulence always increased the formation of loose, stable, and more complex aggregates. Similarly, GRMs dispersions in high ionic content media had a greater increase in GRMs particles with high complexity over the 72 hours than in those with low content, e.g. the TG201 medium. In contrast, the addition of SRNOM to the TG201 medium increased the GDS of all dispersions. Our results show that GRMs dispersions are not stable under the standard TG201 conditions and therefore do not meet the TG requirement of a stable and homogeneous dispersion throughout the test. Therefore, modifications of the test protocols to improve the applicability of TG201 to GRMs, such as the use of SRNOM, will be presented. Finally, these data on GDS will help predict the GRMs behaviour during ecotoxicological testing, an important factor for the correct interpretation of TG201 results, and the fate of GRMs in freshwater environments.