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Assessment of the potential impact of graphene, graphene oxide and other 2D materials on health, and the environment

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Disclaimer

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Executive Summary

The European Union Observatory for Nanomaterials (EUON) provides information about existing nanomaterials on the EU market and aims to improve data transparency on nanomaterials' safety. One of the main ways this is achieved is by running studies to address important knowledge gaps on nanomaterials. The studies themselves aim at collecting and analysing information obtained mainly from systematic literature reviews or from surveys.

This document describes the methodology adopted by the contractor to carry out a systematic review and to critically assess the health and environmental effects of graphene, graphene oxide, and other two-dimensional (2D) materials, based on existing public information.

The key research questions addressed in this report can be divided into two groups:

- Structured literature review: potential adverse effects of graphene, graphene oxide and other 2D materials on **human health** and available methods for their assessment.
- Structured literature review: potential adverse effects of graphene, graphene oxide and other 2D materials on the **environment** and available methods for their assessment.

The systematic review covered publications, books, research reports, research and review papers. The table below provides an overview of whether specific information for each 2D materials were found in the context of the two groups described above and the section of the report where it is discussed.

2D Materials	Toxicity	Ecotoxicity
Graphene, few layer graphene, graphene nanosheets and graphene nanoflakes	Extensive information found. See section 7.1.1	Extensive information found. See section 7.1.2
Graphene oxide	Extensive information found. See sections 7.2.1 - 7.2.7	Extensive information found. See section 7.2.8
Reduced graphene oxide	Extensive information found. See section 7.3.1	Extensive information found. See section 7.3.2
Graphene nanoribbons	Limited information found. See section 7.5	No information found
MXenes	Limited information found. See section 7.6.1	Limited information found. See section 7.6.2
2D boron nitride	Limited information found. See section 7.7	No information found
Transition metal dichalcogenides	Limited. See section 7.8.1	Limited. See section 7.8.2
Black phosphorus	Limited information found. See section 7.9.1	Limited information found. See section 7.9.2
Graphitic carbon nitride	Limited information found. See section 7.10	No information found

Based on the findings, the following conclusions and recommendations have been formulated:

CONCLUSION 1.
It is mandatory to provide in any study a thorough characterization of each type of graphene and 2D material in terms of chemical composition, structure, lateral size, number of layers, in order to link any identified concern during use and disposal with the particular characteristic of the material. Not all 2D materials are graphene alike.

RECOMMENDATION 1. The application of the definitions and available documentary standards should allow to clearly identify the type of graphene and 2D materials used for the different applications and to evidence potential toxicity issues and risks.

CONCLUSION 2.
There is a strong need for specific and multiple analytical and spectroscopic methods for the detection and quantification of graphene and related carbon nanomaterials in biological and environmental matrices.

RECOMMENDATION 2. Multiple characterization techniques should be applied to clearly identify and quantify graphene materials in cells, tissues, organs and the environment.

CONCLUSION 3. Evidenced human and environmental toxic effects of graphene and 2D materials depend on their physicochemical characteristics.

RECOMMENDATION 3. Conclusions on toxicity and ecotoxicity should not be generalized and need to be associated to a precise description of the material used in the tests.

CONCLUSION 4. Cytotoxic effects have been identified for specific graphene and 2D materials both on health and environment, mainly dose dependent.

RECOMMENDATION 4. When health and environmental risks are reported or identified for a specific graphene or 2D material, doses and exposure scenarios should be considered for their manipulation and use.

CONCLUSION 5. Long-term/chronic studies are still limited, particularly for in vivo and repeated-dose administrations.

RECOMMENDATION 5. To assess chronic toxicity of graphene and 2D materials protocols for repeated-dose studies should be considered.

CONCLUSION 6. Studies using immune depressed or diseased models are still lacking, particularly for in vivo and repeated-dose administrations.

RECOMMENDATION 6. To assess potential toxicity of graphene and 2D materials relevant immune suppressed or diseased animal models should be considered.

CONCLUSION 7. Studies on genotoxicity of graphene and 2D materials are still very limited.

RECOMMENDATION 7. To assess the potential genotoxic risks, reliable testing methods should be developed; response mechanisms associated with genotoxicity should be evaluated in depth;

appropriate description of the type of graphene and 2D material tested should be reported; and different dosages and exposure times should be applied.

CONCLUSION 8. Toxicity studies of chemically exfoliated graphene and 2D materials lack of appropriate controls.

RECOMMENDATION 8. The solvents and the molecules used to exfoliate bulk materials into single- or few-layer graphene or 2D materials might remain as residues in the end-product, likely affecting the (eco)toxicity results. It is recommended to consider and include these potential impurities in the tests to exclude their implication and responsibility on (eco)toxicity.

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1. Glossary of terms

The following terms and definitions are based on the ISO terminology (ISO/TS 80004-13:2017(en) Nanotechnologies — Vocabulary — Part 13: Graphene and related two-dimensional (2D) materials) https://www.iso.org/obp/ui/#iso:std:iso:ts:80004:-13:ed-1:v1:en complemented by additional terms even where the ISO terminology is not used.

Table 1: Terms and definitions of the materials (or Test materials)

Material or Test material	Definition
2D heterostructure	Two-dimensional material consisting of two or more well-defined layers of different 2D materials
2D in-plane heterostructure	Two-dimensional material consisting of two or more well-defined layers of different 2D materials that are bonded to each other in the in-plane direction
2D material	Material, consisting of one or several layers with the atoms in each layer strongly bonded to neighbouring atoms in the same layer, which has one dimension, its thickness, in the nanoscale or smaller and the other two dimensions generally at larger scales.
2D vertical heterostructure	Two-dimensional material consisting of two or more well-defined layers of different 2D materials that are stacked out-of-plane
Aggregate	Particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components
Bilayer graphene/2LG	2D material consisting of two well-defined stacked graphene layers
Epitaxial graphene	Graphene layer grown on a silicon carbide substrate
Few-layer graphene/FLG	Two-dimensional material consisting of three to ten well-defined stacked graphene layers
Graphane	Single layer material consisting of a two-dimensional sheet of carbon and hydrogen with the repeating unit of (CH)n
Graphene nanoplate/graphene nanoplatelet/GNP	Nanoplate consisting of graphene layers
Graphene/graphene layer/single-layer graphene/monolayer graphene	Single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure
Graphite oxide	Chemically modified graphite prepared by extensive oxidative modification of the basal planes
Graphene oxide/GO	Chemically modified graphene prepared by oxidation and exfoliation of graphite, causing extensive oxidative modification of the basal plane
Graphite	Allotropic form of the element carbon, consisting of graphene layers stacked parallel to each other in a three-dimensional, crystalline, long-range order
Layer	Discrete material restricted in one dimension, within or at the surface of a condensed phase

MXenes	2D materials with the general formula of $M_{n+1}X_n$ where M is an early transition metal and X is carbon or nitrogen
Nanoplate	Nano-object with one external dimension in the nanoscale and the other two external dimensions significantly larger
Nanofoil/nanosheet	Nanosized sheet with extended lateral dimensions
Nanoribbon/nanotape	Nanoplate with the two larger dimensions significantly different from each other
Nanoscale	The size range from 1 nm to 100 nm (cfr. Commission Recommendation 2022/C 229/01)
Nanomaterial	Nanomaterials are chemical substances, materials or products with internal features or consisting of particles having external dimensions at the 'nanoscale' (size range of 1 nm to 100 nm, where 1 nm = 10-9 m)
Perfluorographane	Single layer material consisting of a two-dimensional sheet of carbon and fluorine with each carbon atom bonded to one fluorine atom with the repeating unit of (CF)n
Reduced graphene oxide/ rGO	Reduced oxygen content form of graphene oxide
Twisted bilayer graphene/ turbostratic bilayer graphene/tBLG/t2LG	2D material consisting of two well-defined graphene layers that are turbostratically stacked, with a relative stacking angle rather than Bernal (hexagonal) or rhombohedral stacking
Twisted few-layer graphene/ t(n+m)LG	2D material consisting of a few-layers of graphene of n Bernal stacked layers which are situated with a relative stacking angle upon m Bernal stacked layers
Trilayer graphene/3LG	2D material consisting of three well-defined stacked graphene layers

2. Abbreviations

Table 2: List of abbreviations

Extended term	Abbreviation
Adverse outcome pathways	AOPs
Bilayer graphene	2LG
Carbon black	CB
Carbon nanomaterials	CNs
Carbon nanotubes	CNTs
Carbonaceous nanomaterials	CNMs
Engineered nanomaterials	ENMs
European Chemicals Agency	ECHA
European Food Safety Authority	EFSA
European Union Observatory for Nanomaterials	EUON
Exogenous carbonaceous materials	ECM
Few-layer graphene	FLG
Graphene-based materials	GBMs
Graphene-family nanomaterials	GFNs
Graphene nanomaterials	GNMs
Graphene nanoplatelets	GNP
Graphene nanoribbon	GNR
Graphene nanosheets	GNS
Graphene oxide	GO
Graphene quantum dots	GQDs
Hexagonal boron nitride	hBN
International Organization for Standardization	ISO
Multi-layer graphene	MLG
Multi-walled carbon nanotubes	MWCNTs
Natural organic matter	NOM
Organisation for Economic Co-operation and Development	OECD
Population, exposure, comparator and outcome	PECO
Population, intervention, comparator and outcome	PICO

Pristine graphene	PG
Reduced graphene oxide	rGO
Reactive oxygen species	ROS
Test material	TM
Trilayer graphene	3LG
Twisted bilayer graphene	tBLG
Single wall carbon nanotubes	SWCNTs
Systematic review	SR
Web of Science	WoS

3. Abstract

The European Union Observatory for Nanomaterials (EUON) provides information about existing nanomaterials on the EU market and aims to improve data transparency on nanomaterials' safety, trying to cover important knowledge gaps. This report provides a comprehensive overview of the literature and existing public information of studies addressing the potential impact of graphene related materials and other 2D materials on health and the environment. Relevant reviews and significant articles, published in the last ten years, have been selected and presented as case studies. The results reported in these studies are meant to highlight the potential risks associated with the preparation and use of graphene and 2D materials, underlining possible data gaps and giving recommendations. The key research questions addressed in this report can be divided into two groups: 1) structured literature review describing potential adverse effects of graphene, graphene oxide and other 2D materials on human health and available methods for their assessment; and 2) structured literature review covering potential adverse effects of graphene, graphene oxide and other 2D materials on the environment and available methods for their assessment. The findings evidenced in this report should be made available to companies and other stakeholders with an interest in the use and placing on the EU market of graphene and 2D materials.

4. Introduction

Graphene, isolated and characterised in 2004 by Novoselov and Geim, is a single layer of monocrystalline graphite made of sp^2-hybridized carbon atoms. Graphene is part of a bigger family, which has been identified as the graphene family nanomaterials comprising few-layer graphene, graphene nanoplatelets, graphene quantum dots, graphene nanoribbons, graphene oxide (the oxidized form of graphene), and reduced graphene oxide (obtained by partial reduction of graphene oxide). The isolation and discovery of graphene have strongly motivated the search of new two-dimensional (2D) materials. The family of 2D materials comprises different chemical classes, the most popular of which include transition metal dichalcogenides [consisting of three-layer atomic structures, where the outside layers is made of chalcogens (*e.g.*, sulphur or selenium) covalently bound to a metallic atom (*e.g.* molybdenum or tungsten) inner layer], hexagonal boron nitride, graphitic carbon nitride, black phosphorous, and MXenes [constituted of transition metal carbides, nitrides, and carbon nitrides with a typical formula of $M_{n+1}X_n$ (with M an early d-transition metal; and X corresponding to carbon and/or nitrogen)].

Among the different 2D materials mentioned above, graphene is the one that has received the most extensive research and commercial attention in the last 2 decades due to its unique properties. Within a very short period, graphene and its derivatives have shown outstanding commercial applications in the field of composites, nanoelectronics, bioimaging, and nanomedicines. For example, functionalized graphene nanosheets have revealed enhanced interfacial interaction and binding properties with proteins, mammalian cells and bacteria, which make graphene a valuable nanosystem for the next-generation multifunctional bioengineering applications. However, time-dependent compatibility and interactions of graphene and its derivatives *in vitro* and *in vivo* is one of the most challenging tasks for the researchers working on different aspects of graphene. Therefore, we can say that graphene has shown great proficiency in every branch of science and technology, but a great support for further research is required from the governments and industries to harness the full potential of graphene and its derivatives. Since graphene is an inspiration for other 2D materials, materials like hexagonal boron nitride, germanene, silicene, different transition metal dichalcogenides, phosphorene, stanene, borophene, and boron nitride nanosheets are emerging as new nanostructures for the next generation nanoscience and nanotechnology research.

The European Union Observatory for Nanomaterials (EUON), hosted and maintained by the European Chemicals Agency (ECHA), provides information about nanomaterials on the EU market and aims to improve data transparency on their safety. One of the main ways this is achieved is by running studies to address important knowledge gaps on nanomaterials. The studies themselves aim at collecting and analysing information using systematic literature reviews. ECHA sought to conduct a service contract to assess the potential impact of graphene, graphene oxide and other 2D materials on health and environment. Based on existing public information, this study was aimed to conduct a systematic literature review (SR) of the health and environmental effects of graphene, graphene oxide, and other 2D materials. The study assessed also what general informed conclusions could be made regarding the potential toxicological and ecotoxicological impact of 2D materials. Finally, the review examined to what extent existing approaches to toxicological and ecotoxicological testing of chemicals apply to graphene, graphene oxide and other 2D materials, and what challenges and pitfalls exist surrounding the testing of these materials.

5. Methodology

This chapter describes the methodology adopted by the contractor to carry out the SR and critically identify the health and environmental potential impact of graphene, graphene oxide, and other 2D materials, based on existing public information.

Systematic review (SR) is one of the best approaches to ensure the coverage of the existent literature towards the selection, appraisal, and synthesis of relevant evidence found in relation to a specific research question or sub-questions. The methodology described below applies to two different objectives, namely:

- Objective 1. Structured literature review: potential adverse effects of graphene, graphene oxide and other 2D materials on human health and available methods for their assessment.

- Objective 2. Structured literature review: potential adverse effects of graphene, graphene oxide and other 2D materials on the environment and available methods for their assessment.

5.1 Developed search strategy

Preparatory steps were carried out to allow developing the full SR protocol to accomplish queries. These preparatory steps were essential to define and develop the appropriate questions, the eligibility criteria, as well as the inclusion and exclusion criteria. These steps were performed at beginning of the assignment jointly and in agreement with ECHA. The following points applied for the initial setup of this assignment.

5.1.1 Review of PICO/PECO

The identification of the appropriate safety questions is of the utmost importance to perform consistently the SR. The following key elements define PICO/PECO for both objectives.

• Populations (P). For both objectives, P identifies any cell-based living system, including cell-lines, animals, and humans. This large definition is appropriate to keep into account all possible studies related to the potential adverse effects of test materials (TMs) also in other domains than those strictly related to adverse effects in humans and/or environment.

• Interventions and exposure (I and E). For both objectives, I and E identify any intervention and/or exposure to which the population is exposed by any of the TM as defined in Table 1.

• Comparators (C). For both objectives, C identify control or reference group in experimental studies or documents not exposed to I or E and information on regulatory documents. For the scope of this assignment, the carbon nanotubes (CNTs) were used as benchmark material, being CNTs made of the same elements and structural organization. In addition, several data are currently available on CNTs, that are increasingly considered by the scientific community as reliable comparative materials.

• Outcomes (O). For Objective 1, O identifies: i) adverse effects of TMs on humans and/or animals relating to acute toxicity, repeated-dose toxicity, mutagenicity, carcinogenicity, reproductive or developmental toxicity; ii) adverse effects of TMs on humans and/or animals resulting from different routes of exposure (*e.g.* inhalation, dermal, ingestion, intravenous and other parenteral administration routes) with association of specific material parameters like dimensions, shape, functionalization, number of layers, average lateral size, thickness, surface chemistry, physicochemical properties, preparation methodology; iii) adverse effects of TMs in cells that would impact the normal and physiological cell proliferation and development (*e.g.* cytotoxicity, apoptosis, necrosis, migration, anoikis, differentiation); iv) in silico, *in vitro* or *in vivo* methods applied to TMs for assessing any of the adverse effects described in the previous points. For Objective 2, O identifies: any toxicological effects of TMs in animals or microorganism

or plants including the environmental fate of TMs in air, water and soil.

5.1.2 Database and structure to perform SR and literature searches

The table below reports the source of information included in the methodology.

Table 3: List of data source used for SR and type of search

Source	Type of search performed
Web of Science – core collection	Search with queries with the advanced online tool
PubMed	Search with queries with the advanced online tool
Graphene flagship[1] documents, in particular Health and Environment Work Package 4	Web site search
Website of national and international authorities, for instance: ECHA, EFFA, FDA, US EPA, Health Canada, FSANZ, China Food and Drug Administration	Grey literature search with selected keywords

5.1.3 Inclusion and exclusion criteria

The following definition applies to inclusion and exclusion criteria.

Inclusion criteria: all documents describing graphene, graphene oxide and other 2D materials that could be referred within the ISO terminology as listed in Table 1 or with synonyms or with ontologically related terms.

Exclusion criteria: defined as reported in the Table below.

Table 4: List of exclusion criteria and specific examples

Exclusion codes	Description of exclusion criteria	Examples of exclusions
EC1	Documents describing TMs preparations, manufacturing, and/or applications with no association with any kind of toxicological data	➢ Water treatment ➢ Optical properties ➢ Mechanistic studies ➢ Preparation processes
EC2	Articles describing toxicological and ecotoxicological data in cells, animals, human or relevant eco-systems that are not test-item related (*i.e.*, no clear link with TMs)	➢ Antitumoral applications ➢ Antibacterial applications, unless if linked to safer design ➢ Antimicrobial applications, unless if linked to safer design ➢ Biomedical applications ➢ *In vitro* tests on disease cell lines ➢ Ecotoxicity of other pollutants in presence of a 2D test material

[1] The Graphene Flagship is a European Union scientific research initiative that focuses on taking graphene and related two-dimensional materials and applying them in European technology (https://graphene-flagship.eu)

EC3	Documents describing toxicological data of materials that are not in the list of 2D materials and test materials of Table 1	➢ MOF ➢ Cd, Pb, Ag, Au, silicon carbide ➢ Nanoparticles ➢ Nano-onions ➢ Nanodiamonds ➢ Graphene quantum dots ➢ Nanopores ➢ 2D materials grafted, functionalized, coated or embedded with other chemicals, unless toxicity data of the unmodified 2D material is reported ➢ Graphite furnace ➢ Nanofibers
EC4	Documents with general speculation, general description, or historical description of TMs	Reviews not relevant for toxicity and linkage with 2D materials
EC5	Any other documents that cannot be categorized in inclusion criteria and cannot be excluded with the previous exclusion areas	No abstract available online

5.1.4 Performance of the SR - Queries

A combined query search related to TMs with additional specific search terms was performed to keep into account the two objectives of the assignment. The table below depicts query searches implemented in PubMed and Web of Science. Databases were interrogated on 01/02/2022.

Table 5: Syntax of queries applied to PubMed and WoS

N° query	Type of query	Query syntax
1	Query for Test materials	((graphene OR graphane OR silicene OR mxenes OR 2d mof OR 2d cof) OR ((transition metal OR boron nitride OR black phosporus) AND (2D material OR nanoplate OR nanofoil OR nanosheet OR nanoribbon OR nanotape OR quantum dot OR single layer OR monolayer OR bilayer OR trilayer OR nanoplatelet OR heterostructure OR 2D aggregate OR hexagonal)))
2	Query for toxicity and ecotoxicity	toxicity OR ecotoxicity

3	Toxicity related keywords	toxicokinetic OR pharmacokinetic OR ADME OR absorption OR distribution OR metabolism OR excretion OR genotoxicity OR mutagenicity OR reproductive toxicity OR developmental toxicity OR carcinogenicity OR teratogenicity OR epidemiologic OR exposure OR risk factor OR biomarkers OR cancer OR dietary OR tumors OR control study OR reports OR diet OR health OR gene activation OR protein production OR protein expression OR signalling OR alteration OR cell-cell interactions OR protein-protein interactions OR disease OR toxicity OR warning OR precaution OR adverse event OR adverse effect OR occurrence OR clinical OR causal OR reaction OR immunogenicity OR adjuvanticity OR allergen OR case control OR case study OR causality OR cohort OR cross-sectional OR safety OR harm OR damage OR hazard OR tolerability OR constituent OR additive OR impurity OR form OR purity OR composition OR functional OR size OR shape OR surface chemistry OR acute toxicity OR repeated dose toxicity OR short-term study OR sub-chronic study OR chronic study OR species OR animal OR strain OR model OR rat OR mouse OR dog OR monkey OR rabbit OR oral OR inhalation OR dermal OR infusion OR intramuscular OR subcutaneous OR intraperitoneal OR intravenous OR gavage OR vehicle OR excipient OR mortality OR clinical signs OR body weight OR food consumption OR hematology OR clinical biochemistry OR behavior OR gross pathology OR histopathology OR data OR array OR sequencing OR imaging OR MOA OR mode of action OR genomics OR DNA OR gene expression OR transcriptomics OR proteomics OR metabolomics OR proteins OR proteomics OR metabolites OR microbiome OR mRNA OR microRNA single nucleotide polymorphisms OR SNPs OR epigenomics OR microbiome OR ((toxicity OR modelling OR quantitative) AND ("in silico" OR "*in vitro*" OR "*in vivo*" OR QIVIVE)) OR HTS OR high throughput OR screening OR assay OR cell OR cell-free OR bioassay OR concentration OR dose OR response OR Hill-equation
4	Final query	#1 AND #2 AND #3

Combined searches, *i.e.* query #4, resulted in 4614 documents in PubMed and 4944 in WoS.

5.1.5 Repository and post-processing

Raw results were collected from the previous steps by exporting data from single databases in RIS files that were subsequently imported in Zotero software (V.6.0.4). After import, within the Zotero software a specific library was created to allow a straightforward post processing to remove duplicate, merge identical references and perform general data integrity check. A final list of 8019 documents was obtained.

5.1.6 Selection for relevance – title and abstract screening

The list of documents in the repository was used to assess the relevance of studies against inclusion and exclusion criteria defined previously. The work was organized in a way that at least two experts with different expertise (*i.e.*, nanotechnology and toxicological hazard assessment) could review independently each document. A decision on the relevance was made for each document if at least one expert judged it relevant with respect to the specific questions of the assignment. In particular, experts assessed:

- The relevance of each document against inclusion criteria.

- The relevance of each document against exclusion criteria.

Articles with a proposed exclusion code as defined in Table were marked irrelevant and were excluded for further steps of screening. When a missing consensus among experts was evidenced, a third expert performed the assessment to reach a conclusion. When documents could not provide sufficient information from the analysis of title and abstract, a conservative approach was adopted, and those documents were marked relevant in order to let expert analyses of the full-text in the subsequent steps.

The result of the selection of relevance by title and abstract is reported in Table and Table .

Table 6: Results of the selection of relevance by titles and abstract

SR result – title and abstract	N° of documents found
Total documents	8019
Excluded (with at least one exclusion code)	6756
Relevant	1263

Table 7: Occurrence of exclusion criteria in excluded documents.

Exclusion codes	N° of documents excluded[2]
EC1	2016
EC2	1167
EC3	4045
EC4	624
EC5	60

5.1.7 Selection for relevance – full-text examination and critical assessment

For documents that passed the relevance screening based on titles and abstracts or in cases when a final decision could not be made based on the title and/or abstract alone, the full-text was obtained and major conclusions were extracted. The table in Appendix 1 depicts the full list of relevant documents with the extracted major conclusion from the full-text analysis. It is worth to note that several documents resulted irrelevant after the analysis of the full text and were excluded from the critical assessment. The result of the selection of relevance by full text is reported in Table .

Table 8: Results of the selection of relevance by full text

SR result – full text examination	N° of documents found
Relevant from titles and abstract	1263
Excluded after full text examination	610
Final list of relevant documents (Appendix 1)	653

The final list of relevant documents in Appendix 1 was used to perform the critical assessment and the interpretation of the result in conformity with the following points:

i) Quantity of the evidence: total number of documents screened in relation to the two

[2] Documents could have more than one exclusion code associated. Documents were excluded if at least one exclusion code was identified.

objectives of the assignments (*i.e.* toxicity and ecotoxicity).

ii) Quality of the evidence: assessment of the quality of the body of evidence in terms of toxicity and ecotoxicity related to TMs in considerations of the methodological quality of the study. The extent by which the quality of a body of evidence regarding TMs could decrease was evaluated based on the scientific judgements about study limitations for each main outcome. When studies provided different evidence (*e.g.* contradicting conclusions from different studies), explanations were reported when available.

iii) Interpretation of the results. Chemical, biological and statistical significance of findings and assumptions made. Where few relevant data were found, the characterization and reporting of the knowledge gaps was remarked to support recommendations.

iv) Agreements/disagreements. Agreements or disagreements with other studies or reviews were discussed with possible reasoning and explanations for disagreements.

A general section describing review articles relating to 2D materials is provided in section 0 while significant information, mostly for graphene and graphene oxide (GO), from primary research articles was summarized in the subsequent sections where 2D materials are grouped as commonly referred in the recent scientific literature.

Within each section, documents are described in chronological order to provide a realistic and historical advancement of 2D materials in the scientific community. Discussion on the effects of 2D materials on toxicity (Objective 1) and ecotoxicity (Objective 2) are provided for each group.

6. Reviews on 2D materials

We describe here the results of the systematic literature search for review articles. It is worth to emphasize that no relevant reviews have been identified before 2012. The following Table is organized to report the class of 2D material as identified in **Error! Reference source not found.**, the specific endpoints linked to the general objectives of this study and the descriptive considerations with reference to toxicity and ecotoxicity.

Table 9: Relevant reviews describing 2D-materials

2D material	Endpoint	Considerations	Reference
graphene, few layer graphene, graphene oxide, reduced graphene oxide	toxicity, biomolecular interaction	Sanchez *et al.* discussed several unique modes of interaction between GFNs and nucleic acids, lipid bilayers, and conjugated small drugs and dyes along with relevant *in vitro* and *in vivo* studies. ROS are a potential mechanism for toxicity, although the extremely high hydrophobic surface area of some GFNs may also lead to significant interactions with membrane lipids leading to direct physical toxicity or adsorption of biological molecules leading to indirect toxicity. Limited *in vivo* studies demonstrate systemic biodistribution and biopersistence of GFNs following intravenous administration. Like other smooth, continuous, biopersistent implants or foreign bodies, it was hypothesized that GFNs can have the carcinogenicity potential, but no proofs have been reported since then.	(Sanchez *et al.*, 2012)
graphene, graphene oxide, reduced graphene oxide	toxicity	Jastrzebska *et al.* reported the effects of GFNs on bacteria, mammalian cells, animals, and plants. This article also reviewed *in vitro* and *in vivo* test results as well as potential anticancer activity and toxicity mechanisms of GFNs. The data are not sufficient to reach conclusions to connect potential hazards with risk assessment. The most likely the source of the apparent lack of uniformity is related to different physicochemical properties of GFNs. However, these parameters are not always well-controlled and, in some cases, even analysed. Moreover, some of these parameters may also be measured by different techniques, which makes the complied results almost impossible to compare.	(Jastrzebska *et al.*, 2012)
graphene	toxicity	Yang *et al.* reviewed the toxicity of graphene by describing the behaviour of graphene and its derivatives in microorganisms, cells, and animals. The results agree that the physicochemical properties such as surface functional groups, charges, coatings, sizes, and structural defects of graphene may affect its *in vitro*/*in vivo* behaviour as well as its toxicity in biological systems.	(Yang *et al.*, 2013)

graphene, graphene oxide, reduced graphene oxide	toxicity	Xu *et al.* reported a review investigating the potential effect of graphene has demonstrated its possible adverse effects on animals and bacteria. With the knowledge obtained in this study, it was envisioned that the physicochemical properties of graphene-based materials, such as concentration, size, shape, types of dispersants, etc., can influence the cytotoxicity of graphene. In particular, surface functionalization is an important factor that plays a critical role in biocompatibility.	(Xu *et al.*, 2013)
graphene, graphene oxide	toxicity, biomolecular interaction	Dinedayalane *et al.* reviewed the toxicity of graphene and graphene oxide focusing on the dependence on the exposure environment and mode of interaction with cells. In addition, biomedical applications of graphene and the biocompatible systems were reviewed. It was pointed out that, while graphene can be successfully used as a non-toxic nano-vehicle for efficient gene transfection and a novel gene delivery nano-vector with low cytotoxicity and high transfection efficiency, some form of graphene like carbon nanotubes might raise concerns about toxicity. In particular, it is recollected that studies have demonstrated that the shape of carbonaceous nanomaterials plays an extremely important role in how they interact with cells and potentially other biological systems, such as tissues and organisms and the toxicity of graphene and graphene oxide depends on the exposure environment and mode of interaction with cells.	(Dinadayalane, Leszczynska and Leszczynski, 2013)
graphene, graphene oxide	toxicity	Bianco clarified the existence of multiple graphene forms that should allow a better understanding of the differences between the components and eventually correlating their biological effects to the physicochemical characteristics of each material. It was discussed that new nanomaterial might become a health hazard, but chemical manipulation can alleviate the potential risks associated with the future development of GFNs for different applications. Nevertheless, it was pointed out that it is not possible to give a clear answer whether graphene is toxic or not, but there was strong evidence that the toxic effects are modular. In addition, a generalization on the toxicity of GFNs should be avoided as the risks associated with these new nanomaterials are dependent on the specific applications and development.	(Bianco, 2013)
graphene	toxicity, ecotoxicity	Hu and Zhou analysed the works regarding the health and ecosystem risks of graphene, scrutinizing its potential risks, reducing the scientific "blind spots" and knowledge gaps, and attempting to identify future directions in which this field is likely to develop. Generally, oxidative stress with generation of excess of ROS is viewed as a dominant mechanism of pathological changes induced by graphene.	(Hu and Zhou, 2013)

graphene	toxicity, ecotoxicity	Arvidsson *et al.* reviewed risk-related information on graphene with the purpose of outlining potential environmental and health risks and guide future risk-related research. The results from the studies covered by the review indicated that graphene could trigger potential toxicity in view of considerable emission of graphene from electronic devices and composites in the future. It was also suggested that graphene is both persistent and hydrophobic. Although these results indicated that graphene may cause adverse environmental and health effects, the subsequent and recent results foremost show that there are many risk-related knowledge gaps to be filled and that the emissions of graphene, the fate of graphene in the environment, and the toxicity of graphene should be further studied.	(Arvidsson, Molander and Sanden, 2013)
graphene, graphene oxide, reduced graphene oxide	toxicity, toxicity mechanism	Ma *et al.* reviewed the toxicity of different GNMs and related mechanisms at the molecular and cellular level. Various approaches to evaluate the *in vivo* toxicity of GNMs and major factors defining their toxicity is discussed and summarized. Variation in sample quality and diversity complicates drawing final conclusions on the toxicity of GNMs, especially for the *in vivo* toxicity. In addition, interpreting experimental data from the *in vivo* studies should be careful, because of certain limitations of the evaluating methods might apply, for instance the necessity to observe microstructural changes in certain tissues, *e.g.* major organs, in order to obtain more reliable conclusions on pathology.	(Ma *et al.*, 2014)
graphene, graphene oxide, reduced graphene oxide	toxicity	Guo and Mei tried to give a comprehensive understanding of the interaction of GFNs with living systems and their adverse effects *in vitro* and *in vivo*, as essential step for further development and safe use of graphene-based nanomaterials. Although surface modified GFNs with ultra-small sizes, excellent dispersibility, and stability in physiological environments are often less toxic, there are inconsistencies between studies. Dose is one of the most important factors and low doses of GFNs make them safer. It is difficult to compare the toxicological effects of GFNs between different studies due to the diversity in the sizes, shapes, surfaces, and fabrication of GFNs. For example, different production methods cause different amounts of oxygen to be bound to the surface of GFNs, which has proven to be correlated with their toxicity towards cells and other living systems.	(Guo and Mei, 2014)

graphene	toxicity	Orecchioni *et al.* reviewed the impact of functionalized carbon nanotubes (f-CNTs), graphene and carbon nanohorns on immune cells. Most of the works reviewed in this paper showed that (56%) of papers dealt with macrophages, followed by lymphocytes (30% of the studies). In the case of lymphocytes, T cells were the most investigated (22%) followed by monocytes and dendritic cells (7%), mixed cell populations (peripheral blood mononuclear cells, 6%), and B and natural killer (NK) cells (1%). These investigations have demonstrated that some f-CNTs can directly elicit specific inflammatory pathways. The interaction of graphene with the immune system was still at a very early stage of investigation.	(Orecchioni *et al.*, 2014)
graphene, graphene oxide	toxicity, ecotoxicity	Seabra *et al.* reviewed the results on *in vitro* and *in vivo* cytotoxicity and genotoxicity of graphene-related materials, and critically examined the methodologies employed to evaluate their toxicities. The environmental impact from the manipulation and application of graphene materials is also reported and discussed. Finally, this review presents mechanistic aspects of graphene toxicity in biological systems. The toxicity of graphene is dependent on its surface, size, number of layers, cell type, administration route (for *in vivo* experiments), dose, time of exposure, and synthesis methods. The toxicity profile of different types of graphene depends on several parameters, and generalizations should be avoided. The literature available until 2014 proposed that the generation of reactive oxygen species in target cells was the most important mechanism leading to cyctotoxicity of graphene. It was postulated that small and hydrophilic graphene nanomaterials (in particular, those capped with biocompatible molecules) tend to form a stable colloid dispersion, avoiding aggregation and, therefore, are more apt to be internalized and removed/excreted once administered intentionally or unintentionally.	(Seabra *et al.*, 2014)
graphene, graphene oxide	toxicity	Nezakati *et al.* reviewed chemically modified graphene and GO, and their impact when present in biological systems. It was showed that cells are very sensitive to size, shape, solubility, and concentration of graphene nanomaterials. In a comparative study, GO was considered more biocompatible than graphene due to its greater solubility/dispersibility, which resulted in less damage and toxicity in human cell types such as skin fibroblasts and red blood cells, and bacteria.	(Nezakati, Cousins and Seifalian, 2014)

graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Zhao *et al.* reviewed four critical processes determining GFN fate and disposition in aquatic environments. It is pointed out that GO preferentially adsorbs metal ions and positively charged organic molecules, whereas graphene and rGO adsorb hydrophobic and aromatic molecules. Because of their dissimilar surface properties, the materials exhibit distinct dispersion/aggregation behaviours under environmentally relevant conditions.	(Zhao *et al.*, 2014)
graphene	toxicity	Zhang *et al.* reviewed the toxicokinetics of carbon-based nanoparticles including graphene and FLG from both *in vitro* and *in vivo* studies. Some of the results are inconsistent because various groups have evaluated toxicity using different cellular or animal models, experimental conditions, and types of nanomaterials. The surface properties, shape, size, surface charge, stability and purity all contribute to the differential toxic effects observed.	(Zhang *et al.*, 2014)
graphene	toxicity	Vasyukova *et al.* reviewed the association, following exposure to carbon nanomaterials including carbon black, graphite nanoplatelets, graphene, single- and multi-walled carbon nanotubes, and fullerene to the adverse reproductive and developmental effects, *in vitro* and *in vivo* studies. It was shown that carbon nanomaterials revealed toxic effect on reproductive system and offspring development of the animals of various system groups to a certain degree depending on carbon crystal structure.	(Vasyukova, Gusev and Tkachev, 2015)
graphene, graphene oxide, reduced graphene oxide, transition metal dichalcogenides	toxicity	Chng and Pumera reviewed the *in vitro* toxicity studies on different 2D nanomaterials including transition metal dichalcogenides. In case of the transition metal dichalcogenides, the toxicity depended on the preparation method and resulted much lower than of graphene oxide.	(Chng and Pumera, 2015)
graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Jastrzebska and Olszyna summarized the information on the influence of GFNs on soil and water environment as well as identified the knowledge gaps and indicated the directions for the next generation of the original scientific investigations. When GFNs are released into the soil, they may interact with its components. However, in 2015 there was a huge knowledge gap on GFN fate and transport in soil environment which was related to the lack of investigations such as their interactions with soil organic matter, soil minerals, and soil solution which is composed of water and dissolved organic and inorganic matter.	(Jastrzebska and Olszyna, 2015)

graphene oxide	toxicity	Kiew *et al.* reviewed how the physicochemical characteristics (*e.g.*, size, surface area, surface properties, number of layers and particulate states) and surface coatings of GO affect *in vitro* and *in vivo* toxicity. This review provided an overview on the effects of GO properties on interactions with red blood cells, macrophages and cell lines, and rodents, rabbits and zebrafish. This review evidenced that GO could induce pulmonary toxicity because it has a tendency for high accumulation in the lungs upon administration. Moreover, the bio-persistence of plain GO in the human body could potentially trigger immunology and pathology effects.	(Kiew *et al.*, 2016)
graphene, graphene oxide	toxicity	Zhang *et al.* reviewed diverse types and various properties of graphene-based materials, and the methods for the surface modifications of the graphene-based materials were briefly described. In addition, the *in vivo* and *in vitro* cytotoxicity of graphene-based materials were comprehensively discussed. Based on the available experimental data, the cytotoxic effects were caused by oxidative stress and the damage of cell membrane. In 2016, few studies were available on the interactions between graphene-based materials and cell using *in vivo* models.	(Zhang, Wang and Zhai, 2016)
graphene, few layer graphene, graphene oxide, reduced graphene oxide	toxicity, ecotoxicity	Lalwani *et al.* summarized *in vitro* and *in vivo* studies and critically examines the methodologies used to perform graphene evaluations by highlighting the complex interplay of biological responses of graphene as a function of their physicochemical properties. In particular, the studies till date indicated that toxicity of graphene could be dependent on the shape, size, purity, post-production processing steps, oxidative state, functional groups, dispersion state, synthesis methods, route and dose of administration, and exposure times.	(Lalwani *et al.*, 2016)
graphene, graphene oxide	toxicity	Ema *et al.* summarized the findings of toxicity studies on GNMs in laboratory mammals. The inhalation of graphene induced only minimal pulmonary toxicity. Bolus airway exposure to graphene and GO caused acute and subacute pulmonary inflammation. Intratracheally administered graphene passed through the air-blood barrier into the blood oxidative stress and inflammation was indicated to be involved in the toxicity of GNMs. The surface reactivity, size, and dispersion status of GNMs play an important role in the induction of toxicity and biodistribution of GNMs.	(Ema, Gamo and Honda, 2017)

graphene, graphene oxide, reduced graphene oxide	toxicity	Volkov *et al.* published a critical analysis of 2015–2016 publications on graphene-related material biocompatibility and toxicity. Experimental findings from the diverse *in vitro* and *in vivo* model systems were analysed in the context of the most likely graphene exposure scenarios, such as respiratory inhalation, ingestion route, parenteral administration and topical exposure through the skin. Irrespective of the graphene used, it was largely accepted that the generation of ROS lies at the basis of graphene toxicity, further attenuated by structural and chemical properties of the material. There is a clear difference between the oxidised graphene forms and the pristine graphene, which appears to possess far less toxic potential.	(Volkov, McIntyre and Prina-Mello, 2017)
graphene, graphene oxide	ecotoxicity	Montagner *et al.* reviewed available ecotoxicology studies still evidencing a large knowledge gap, similar to a previous review published in 2015 by Jastrzebska and Olszyna	(Montagner *et al.*, 2017)
metal dichalcogenides, hexagonal boron nitride, black phosphorus	toxicity	Guiney *et al.* reviewed then the toxicity of TMDs as a function of their preparation methods and surface functionalization. This review underlined the lack of thorough material characterization, consistent design of *in vitro* assays, and mechanistic understanding of the cytotoxic response, often leading to contradictory results on the biocompatibility of many 2D materials. Additionally, the cytotoxicity of the 2D materials in most cases was reported as a simple quantification of cell viability, without details of the characterization of cell growth or cell morphology that would enable better comparison across studies.	(Guiney *et al.*, 2018)
graphene, graphene oxide, reduced graphene oxide	toxicity	Kenry reviewed the elucidation of the haemotoxicity of graphene nanomaterials through their interactions with blood proteins and cells. Conflicting findings were evidenced thought the literature and these apparent contradictions might stem from variations in the synthesis and processing of graphene nanomaterials, yielding graphene nanomaterials with a wide spectrum of morphological and physicochemical characteristics. Almost all haemocompatibility and haemotoxicity assessments were carried out over a short-term period while long-term haematological effects of graphene nanomaterials are poorly understood.	(Kenry, 2018)

graphene, graphene oxide, reduced graphene oxide	toxicity, ecotoxicity	Fadeel *et al.* comprehensively analysed the state-of-the-art of human and environmental hazard assessment of graphene-base materials by assessing toxicity and ecotoxicity data in the view of highlighting the importance of the structure−activity relationships. It is underlined that robust and validated assays for testing with respect to human health and environmental safety are of the utmost importance. In addition, it is suggested that systems biology approaches can provide useful insights to dissect the mechanisms of action in biological systems underlying adverse effects. Nonetheless, it is remarked that the chemical space of graphene-based materials is yet to be fully explored and a common framework (*e.g.* by means of GBM libraries) would be appropriate.	(Fadeel *et al.*, 2018)
graphene, few layer graphene, graphene oxide, reduced graphene oxide, graphene nanoplatelets	toxicity	Pelin *et al.* reviewed the most significant occupational exposure routes including inhalation, oral, cutaneous and ocular, inhalation being the most studied one. This review presented a critical analysis of the available *in vivo* toxicity data of the most significant GBMs, after using these exposure routes. The few *in vivo* inhalation toxicity studies indicated inflammatory/fibrotic effects at the pulmonary level, not always reversible after 14 to 90 days. More limited *in vivo* data were available for the oral and ocular exposure routes, whereas the studies on cutaneous toxicity were still at the initial stage in 2018. A long persistence of GBMs in rodents was observed, while contradictory genotoxic data were reported. Data gap identification is also provided. Based on the available data, the occupational exposure limits cannot be determined yet.	(Pelin *et al.*, 2018)
graphene oxide	ecotoxicity	De Marchi *et al.* compiled a review with up-to-date information on properties, applications and characterization methods of graphene family materials in aquatic environments and identified biological toxic impacts of these NMs, with special focus on graphene oxide based on the most recent literature.	(De Marchi *et al.*, 2018)
graphene, graphene oxide	ecotoxicity	Ren *et al.* presented an overview on how the exogenous carbonaceous materials (ECMs) affect bioavailability of organic pollutants to different organisms, such as microorganisms, plants and earthworms. This is affected by different biological response and properties of ECMs. Moreover, the possible risks of ECMs on soil biota are also discussed at different level.	(Ren *et al.*, 2018)

graphene	ecotoxicity	Freixa *et al.* reviewed the literature on the toxic effects of CNM in aquatic organisms as well as the toxic effects of CNM through influencing the toxicity of other micro-pollutants and outlined a series of research needs to reduce the uncertainty associated with CNM toxic effects. The results showed that environmental concentrations of CNMs do not pose a threat on aquatic organisms on their own. The observed concentrations of CNM in aquatic environments are in the order of nanogram per litre or even lower, much below than the lowest observed effect concentrations on different aquatic organisms (in the order of milligrams per litre). Toxic effects have been mainly observed in short-term experiments at high concentrations, and toxicity principally depends on the type of organisms, exposition time and CNM preparation methods. Moreover, we observed that CNM interact (establishing synergistic and/or antagonistic effects) with other micro-pollutants. Apparently, the resulting interaction is highly dependent on the chemical properties of each micro-pollutant, CNM acting either as carriers or as sorbents, thereby modifying the original toxicity of the contaminants.	(Freixa *et al.*, 2018)
graphene	ecotoxicity	Chen *et al.* reviewed the progress between 2015 and 2018 in the toxicity of various carbon nanomaterials to plants, animals and microbes. The toxicity mechanism of CNMs on microbes are similar to animals. The main toxicity mechanism is oxidative stress. The toxicity of CNMs to microbes is relatively simple compared with that of animals and plants. It mainly affects community structure, growth and diversity.	(Chen *et al.*, 2018)
graphene oxide	toxicicy	Palmieri *et al.* reviewed the travel of GO after intravenous injection, from the initial interactions with plasma proteins to the formation of the biomolecular corona, and biodistribution. Combining all the experimental results presented in this review, it was concluded that the biomolecular corona significantly affects the interactions of GO. This corona is able to inhibit the haemolytic effects of GO, regulate complement activation, and mediate immune response activity and biodistribution.	(Palmieri *et al.*, 2019)
graphene, graphene oxide, reduced graphene oxide	toxicity	Madannejad *et al.* reviewed various types of carbon-based nanomaterials and methods used for determining their toxic effects. Then, extensively discussed the toxic effects of these materials on the human and other living organisms and their toxicity routes including neurotoxicity, hepatotoxicity, nephrotoxicity, immunotoxicity, cardiotoxicity, genotoxicity, epigenetic toxicity, dermatotoxicity, and carcinogenicity. The results of CNMs indicated to the degree of toxicity is directly related to type, composition, shape, length, diameter, size and surface area. It can be concluded that the CNMs have advantages in human life, but they also can have destructive effects on human health.	(Madannejad *et al.*, 2019)

transition metal dichalcogenides, black phosphorus	toxicity	Tan *et al.* reviewed the biological and toxicity effects of different type of 2DM including BP, their associated mechanisms linking chemistry to biological end points. This review identified a need of proper standardization of the synthesis and testing of these materials to enable proper comparison, as well as protocols to be applied for hazard assessment under probable exposure scenarios.	(Tan *et al.*, 2019)
graphene, transition-metal dichalcogenides, boron nitride nanosheets, black phosphorus, g-C_3N_4 nanosheets	toxicity	Wang *et al.* reviewed the state-of-the-art progress of the fabrication, *in vitro* and *in vivo* toxicity research results of 2DMs including graphene and its derivatives, TMDs, BN, BP, metals nanosheets, g-C_3N_4 nanosheets, layered double hydroxide (LDH), clay nanosheets and other 2D nanomaterials.	(Wang *et al.*, 2019)
graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Wang *et al.* reviewed the accumulation and toxic effects of graphene-based nanomaterials in different developmental stages (embryos, larvae, and adults) of zebrafish, and on Japanese medaka and Cyprinus carpio. These materials were found to be toxic to the cellular environment even at very low concentrations. Moreover, their size has a significant impact on its toxicity. Smaller nanoparticles were more toxic than the larger particles. The surface properties of the graphene-based nanomaterials had a significant impact on toxicity. Presence of humic acid, L-cysteine, or biological secretion in the environment was able to modulate their toxic effects. The use of embryos, larvae, and adult zebrafish and embryos and larvae of Japanese medaka indicates that the toxic effects are mainly induced by oxidative stress due to the generation of ROS. Other mechanisms, such as apoptosis, metabolic disorders, neurodegeneration, and immunomodulation by graphene-based nanomaterials, were primarily mediated by ROS.	(Dasmahapatra, Dasari and Tchounwou, 2019)
graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Wang *et al.* reviewed the studies of toxicity caused by GFNs to plants, as well as its influencing factors. The phytotoxicity of GFNs was mainly manifested as a delay in seed germination and a severe loss of morphology of the plant seedling. Key mechanisms included physical effects (shading effect, mechanical injury, and physical blockage) and physiological and biochemical effects (enhancement of ROS, generation and inhibition of antioxidant enzyme activities, metabolic disturbances, and inhibition of photosynthesis by reducing the biosynthesis of chlorophyll).	(Wang *et al.*, 2019)

graphene	ecotoxicity	Chen *et al.* reviewed the progress about the effects of fullerenes, multi-walled carbon nanotubes, single- walled carbon nanotubes and graphene on microorganisms and their toxicity mechanisms. The main mechanism of toxicity of graphene to microorganisms involved oxidative stress. Graphene can attack cell membranes, lipids, proteins, etc. In microbial cells, graphene makes an imbalance between the anti-oxidation and the oxidation of microbial cells, leading to abnormal secretion of enzymes and other substances in the cells, resulting in cell damage. It was reported that the graphene toxicity was dependent on the production method.	(Chen *et al.*, 2019)
graphene, graphene oxide, reduced graphene oxide	toxicity	Gurcan *et al.* reviewed different genotoxicity studies performed with GBMs with specific focus on the different cell types and conditions. It is pointed out that the main mechanism of graphene toxicity is thought to be caused by reactive oxygen species produced in cells, which in turn interact with various biomolecules including DNA. However, it is concluded that there are not enough reports on the GBMs' genotoxicity effects on immune cells, which are the primary cell types that interact with foreign particles introduced to human body. It was also underlined that future studies focusing on the toxicity and genotoxicity of GBMs should also consider using physiologically relevant experimental conditions that will mimic an *in vivo* particle exposure scenario.	(Gurcan *et al.*, 2020)
graphene, graphene oxide, reduced graphene oxide	toxicity, ecotoxicity	Patil *et al.* described the interaction of graphene with cellular and sub-cellular components, and subsequent physiological signalling. The unresolved challenges include a) classifying the hazards associated with human and environmental exposure; b) correlating the toxicity with some defined intrinsic property of graphene; and c) developing a consensus on biosafety in compliance with the regulatory guidelines.	(Patil, Bahadur and Tiwari, 2020)
graphene, graphene oxide, reduced graphene oxide	toxicity	Fedel discussed again the haemocompatibility of different classes of carbon nanomaterials with the purpose of providing biomaterial scientists with a comprehensive vision of the interactions between CNs and blood components. Several studies often show discordant results, as partly due to the variability of nanoparticle production and purification methods, to the presence of contaminants or surface functionalization. This implies that the blood compatibility of CNs should be thoroughly assessed on a case-by-case basis. An in-depth knowledge of the biological events occurring as a result of CN exposure to the blood.	(Fedel, 2020)
graphene	toxicity	Moghimian and Nazarpour reviewed the studies on a 6 to10-layer graphene powder. The graphene has shown no adverse effect to animal skin and lung. No gene mutation or DNA damage were observed for graphene in the *in vivo* or *in vitro* genotoxicity tests via inhalation.	(Moghimian and Nazarpour, 2020)

graphene	toxicity, ecotoxicity	Patel *et al.* reviewed the toxic effects of graphene family nanomaterials in various biosystems (*in vitro*, *in vivo*, and in microbial, molecular and environmental systems). Graphene toxicity largely depends on its size, dose, shape, and morphology, route of administration, time of exposure and method of nanomaterial synthesis. All these parameters can influence its cellular uptake, interaction with biomolecules and micronutrients with a damaging impact on cell milieu and membrane destabilization. Besides controlling other parameters of GFNs, surface coating of graphene can be suggested as a way of relieving its toxic effects.	(Patel *et al.*, 2020)
graphene oxide, reduced graphene oxide	toxicity	Chen *et al.* systematically the literature on toxicity of GO toxicity *in vitro* and *in vivo* discussing the mechanisms leading to toxicity. The reported studies using GO mainly included inhalation toxicity, ingestion toxicity, dermal toxicity and haemocompatibility. The toxicity was evaluated using zebrafish, C. elegans and drosophila. The conclusions stated that there is still a long journey to comprehensively study the health risks of graphene-family nanomaterials.	(Chen *et al.*, 2020)
graphene, graphene oxide, reduced graphene oxide	toxicity	Xiaoli *et al.* summarized GFN toxicity and identified the deficiencies and challenges. Significant evidence supports that GFNs accumulate in a number of tissues and organs through different exposure pathways, causing toxicity manifested as lesions or functional impairment. Moreover, GFNs can be internalized by varied types of cells and induce cytoskeletal disorders, organelle dysfunction, and interact directly with biological macromolecules such as DNA, mRNA and proteins, ultimately resulting in greater rates of cell apoptosis, necrosis and autophagic cell death. The toxicological effects of GFN are related to its lateral size, surface structure, functionalization, and propensity to adsorb proteins.	(Xiaoli *et al.*, 2020)
carbon nitride	toxicity	Liao *et al.* reviewed toxicological effects of graphitic carbon nitride. The research on the biosafety of g-C_3N_4 is still very limited. Only preliminary *in vitro* and *in vivo* assessments have been performed. Although these g-C_3N_4-based materials have displayed promising biosafety under the investigated doses, the evaluations on the biodistribution, tolerant threshold, degradation and clearance have been not systematically and deeply studied yet.	(Liao *et al.*, 2020)

graphene, graphene oxide	ecotoxicity	Yang *et al.* reviewed the studies between graphene related materials and soil–plant organisms, mainly discussing the detrimental influences on terrestrial biology from the perspectives of physiology, biochemistry, and gene expression. GO is relatively water soluble and has relatively high biocompatibility. Its nanosheet structures can enter cell walls and reach cortices via the uptake of water-soluble nutrients, and react with cell membranes, affecting cellular enzyme activity, respiration, and growth hormone secretion among other processes. In addition, its micron-level basic structures can be deposited on plant roots through root secretions, thereby clogging pores in cell walls, blocking the upward transport of water-soluble nutrients and organic matter, affecting normal metabolism and inhibiting seedling development.	(Yang *et al.*, 2020)
graphene oxide	ecotoxicity	Malhotra *et al.* focused on the toxic effects of graphene and GO caused on aquatic invertebrates and fish (cell line and organisms). The data demonstrated a lot of gaps that does not allow establishing a concrete statement regarding the toxicity criteria guidelines. It is also difficult to compare the toxicological effects of graphene and GO between different studies due to diversity in size, shape, surface modification, synthetization techniques, and model organisms.	(Malhotra *et al.*, 2020)
graphene nanoplatelets, graphene oxide, reduced graphene oxide	toxicity	Ramal-Sanchez et al reviewed all studies focused on the use of graphene and graphene-based materials in the reproductive field, highlighting the consequences and effects reported to date from experiments performed *in vivo* and *in vitro* and in different animal species (from Archea to mammals). Special attention was given to GO, which has been investigated for its ability to increase *in vitro* fertilization. What is emerging is a very interesting perspective presenting two sides of the same coin: on the one hand the different types of graphene could be harmful materials, with possible toxic effects on this delicate and important function; on the other hand, it is possible to speculate that GO in particular could represent a possible way to fight the problem of infertility by manipulating spermatozoa in a safer and better way.	(Ramal-Sanchez *et al.*, 2021)

graphene, graphene oxide, reduced graphene oxide	toxicity	Borandeh *et al.* reviewed ocular applications of GFNs and covered *in vitro* and *in vivo* ocular toxicity, and the possible toxicity mechanisms, and provided some perspectives on the potential risks of GFNs in material development and biomedical applications. Since the literature about ocular toxicity of GFNs is limited, it was hard to conclude the potential ocular hazards. Some works suggested that GFNs were biocompatible while other reports evidenced unfavorable biological responses and cytotoxicity. These inconsistent results might have been caused by differences in the experimental models (cells or animals), and physicochemical characterizations of GFNs and their compositions. Ocular toxicity of GFNs depends on their physicochemical properties.	(Borandeh *et al.*, 2021)
graphene nanoribbons	toxicity	Zakharova *et al.* reviewed the prospects of using GNRs in the field of biomedicine, particularly in creating nanodevices for the detection of biomolecules and single-molecular techniques. It is pointed out that the toxicity of nanoribbons is higher than chemical analogues and several studies highlighted the possible mechanisms of toxicity including, induction of ROS production and autophagy; inhibition of proliferation; induction of apoptosis; DNA fragmentation and chromosomal aberrations. It is concluded that the chemical modification of the surface of GNRs with hydrophilic groups can significantly increase their bioavailability and the biocompatibility.	(Zakharova *et al.*, 2021)
few layer graphene, graphene oxide, reduced graphene oxide	toxicity	Achawi *et al.* performed a systematic review about the structure−activity relationship of GBMs considering 93 papers. FLGs demonstrated relationships between median size and oxidative stress, between lateral size and both cytotoxicity and oxidative stress, and between thickness and cytotoxicity. However, it appears difficult to highlight clear structure−activity relationships for most physicochemical characteristics (PCC) and biological end points because despite a large amount of available data, the GBMs are often too poorly characterised in terms of PCC descriptors and the biological end points investigation is not standardized enough.	(Achawi *et al.*, 2021)
graphene, graphene oxide, reduced graphene oxide, transition metal dichalcogenide	toxicity	Lin *et al.* reviewed the potential impact and toxicity of 2D materials in macrophages, focusing on the different types of graphene including few-layer graphene. Graphene family materials were found to affect inflammatory cytokines in macrophages. FLG was found to induce apoptosis, damage cell membrane and decrease viability of this type of immune cells.	(Lin, Song and Bianco, 2021)

graphene, graphene oxide, reduced graphene oxide	toxicity	Guo at al. reported a review focusing on the surface functionalization of GBMs, including those intentionally designed for specific applications (*e.g.*, protein corona formation) and for the control of nanotoxicity and the design of safe materials. It is pointed out that no exact conclusion regarding the safety and toxicity of GBMs can be drawn from the data available in the current literature, due to the variability of the materials and cellular or biological systems used as well as the range of approaches for their production and functionalization. It is recognized that changes in expression of genes, proteins, and metabolites from omics studies would be helpful to uncover the AOPs for GBMs with the aim to correlated adverse effects with physicochemical properties.	(Guo *et al.*, 2021)
graphene oxide	toxicity	Rhazouani *et al.* provided a review focusing on the synthesis and toxicity of GO nanoparticles. It is pointed out that studies conducted so far indicate that the toxicity of GO depend on its size, synthesis methods, route of administration, and exposure time. ROS-mediated cellular damage has been postulated as a primary mechanism of GO cytotoxicity. Moreover, it was remarked that the available GO toxicity studies were mainly limited to evaluating acute toxicity, while chronic toxicological studies lack.	(Rhazouani *et al.*, 2021)
Mxene	toxicity	Lim *et al.* reviewed the types of MXene applied in biomedical sciences, covering cytotoxicity and strategies for its mitigation, giving a future outlook. The cytotoxicity of MXene is dependent on the functional groups (-F, –OH, and =O), size (1–100 nm), oxidative state, preparation methods, type and dose administered, and exposure times (24–48 h). The cytotoxicity mechanisms of MXene were attributed to oxidative stress leading to apoptosis. However, the disruption mechanisms of MXene nanoparticles to cells were only superficially explained, and specific signalling pathways need to be elucidated more carefully.	(Lim *et al.*, 2021)
graphene	toxicity, ecotoxicity	Devasena *et al.* reviewed the toxicity of graphene and related materials in cell lines, animals, plants, and microbes. In addition, simulation studies related to graphene toxicity were also mentioned. It was pointed out that, though there are massive numbers of studies on the *in vitro* and *in vivo* toxicity of graphene, the influence of size, surface function, and route of administration on the toxicity, reticuloendothelial uptake profile, and excretion requires further study. Moreover, it was reported that graphene and its family members were also considered less toxic if properly functionalized.	(Devasena, Francis and Ramaprabhu, 2021)

graphene	ecotoxicity	Zhao J. *et al.* reviewed the safety of engineered nanomaterials in the environment by analysing four materials including graphene. It is pointed out that nanomaterials did not pose a high risk by comparing environmental concentrations and the predicted no effect concentrations. It was also concluded that nanotoxicity can be further lowered under environmental conditions for most scenarios. Anyhow, despite the environmental risk of nanomaterials is considered of low concern, the risk in specific scenarios (*e.g.* surface water and soil near the point sources) should be considered carefully.	(Zhao *et al.*, 2021)
graphene oxide	ecotoxicity	Zhao Y. *et al.* summarized the studies on the transformation of GO in the aquatic environment. The composition of GO on the surface varies a lot depending on the methods of preparation, making great differences in the results estimating the environmental transformation of GO. It is pointed out that there are still challenges in evaluating the environmental transformation of GO, *e.g.* the difficulty in environmental transformation of GO is limited by the various technique of synthesis, the short-term evaluation and the complicated constituents in natural waters.	(Zhao *et al.*, 2021)
graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Zhang *et al.* scrutinized the advancements in the usage of CNMs for wastewater treatment and the subsequent aquatic toxicity investigations from the perspective of the major characteristics of each dimensional CNMs. Current research focusing on CNMs- associated aquatic toxicity is discussed thoroughly, mainly demonstrating: 1) the adverse effects on aquatic organisms should not be overlooked prior to large-scale CNMs application; 2) divergent consequences can be further reduced if the ecological niche of aquatic organisms is emphasized; and 3) further investigations on joint toxicity can provide greater beneficial insight into realistic exposure scenarios.	(Zhang, Chen and Ho, 2021)
graphene, graphene oxide, reduced graphene oxide, graphene quantum dots	toxicity	Wu *et al.* reviewed the interactions between DNA and GFNs and summarized the mechanisms of genotoxicity induced by GFNs. The genotoxicity of GFNs on DNA is still widely unknown. It has been concluded that, given the important role of genotoxicity in GFNs exposure risk assessment, research should focus on: (1) the development of reliable testing methods to assess genotoxic effects; (2) a thorough elucidation of the response mechanisms associated with genotoxicity; and (3) the increase of the evaluation database regarding the type of GFNs, applied dosages, and exposure times.	(Wu, Zhou and Ouyang, 2021)

7. Potential impact of graphene, graphene oxide and other 2D materials on health and the environment

7.1 Graphene, few layer graphene, graphene nanosheets and graphene nanoflakes

7.1.1 Toxicity

Toxicological effects of graphene have been extensively reviewed in the last 10 years (Dinadayalane, Leszczynska and Leszczynski, 2013; Hu and Zhou, 2013; Lalwani *et al.*, 2016; Fadeel *et al.*, 2018; Devasena, Francis and Ramaprabhu, 2021) and the full list has been reported in Table 9 above. These reviews cover the toxicity of graphene and graphene related materials in cell lines, animals, plants, and microorganisms. Here below we provide a series of representative research papers covering aspect of toxicity of graphene and few layer graphene.

In an early work by Zhang *et al.*, the authors evaluated the *in vitro* toxicity of graphene by using neuronal PC12 cells compared to single wall carbon nanotubes (SWCNTs) in similar conditions. Both graphene and SWCNTs induced cytotoxic effects, and these effects were concentration- and shape-dependent. Interestingly, at low concentrations (0.01−10 μg/mL), graphene induced stronger metabolic activity than SWCNTs. Reactive oxygen species were generated in a concentration- and time-dependent manner after exposure to graphene, indicating an oxidative stress mechanism. Furthermore, time-dependent caspase 3 activation after exposure to graphene (10 μg/mL) showed evidence of apoptosis. Altogether this study suggests different biological activities of the graphitic nanomaterials, with the shape playing a primary role.(Zhang *et al.*, 2010)

Later, Yuan *et al.* applied the iTRAQ-coupled 2D LC–MS/MS approach to analyse the protein profile change of human hepatoma HepG2 cells treated with graphene and SWCNTs. Results showed that only moderate variation of protein levels for the cells treated with graphene was observed, which indicated graphene was less toxic as compared to SWCNTs.(Yuan, Gao and Ching, 2011)

Hondroulis *et al.* described tested the graphene toxicity against an *in vitro* model of the blood brain barrier (BBB) by measuring trans-endothelial-electrical resistance (TEER). A new approach in terms of electrical impedance sensing was also utilized to kinetically analyze the cytotoxicity of graphene nanomaterials towards the BBB model's individual components, rat astrocytes (CRL-2006) and mouse endothelial cells (CRL-2583), in real time by measuring the impedimetric response. Graphene showed little or no toxicity toward both individual cell types as the resistance measurements were similar to those of the control and further, graphene did not interrupt the integrity of the BBB model as a whole showing the biocompatibility of graphene and the broad potential of using these new nanomaterials for biomedical applications.(Hondroulis *et al.*, 2012)

Ma-Hock *et al.* used male Wistar rats to perform an inhalation toxicity study. Animals were exposed head-nose to atmospheres of 0.1, 0.5, or 2.5 mg/m^3 for multi-walled carbon nanotubes and 0.5, 2.5, or 10 mg/m^3 for graphene, graphite nanoplatelets and low-surface carbon black for 6 hours per day on 5 consecutive days. No adverse effects were observed after inhalation exposure to 10 mg/m^3 graphite nanoplatelets or relatively low specific surface area carbon black. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m^3 for multi-walled carbon nanotubes and 10 mg/m^3 for graphene.(Ma-Hock *et al.*, 2013)

Wang *et al.* compared the immune response resulting from graphene nanosheets (GNS) and MWCNT exposure. To verify this hypothesis, C57BL/6 mice were intravenously injected via the tail vein a single dose of carbon nanomaterial at 1 mg/kg body weight. Mice were sacrificed at day 1 or 7 post injection to follow the development of systemic immune responses. The authors concluded that the use of GNS or MWCNTs as nanocarriers for drug delivery may result in Th2 immune responses that are mediated through the IL-33/ST2 axis and therefore may promote adverse allergic reactions.(Wang *et al.*, 2013)

Yoon *et al.* applied a mini-cell culture system (HeLa cells) integrated with a nanocomposite electrochemical transducer to study the toxicity of graphene nanoflakes under size-, concentration- and time-dependent influences. Cytotoxicity measurements were carried out at 6 h intervals for 1 day upon exposure to graphene solutions of 30 and 100 μg/mL. The results showed that the increased tendency in measured H_2O_2 concentration as the concentration of the GF nanoflakes increased was consistent with the results of the toxicity data. Also, the authors confirmed an increased cytotoxicity for smaller GF size at the same concentration.(Yoon *et al.*, 2013)

Yoon *et al.* analysed the toxicity of graphene nanoflake using a cell-based electrochemical impedance biosensing with interdigitated indium tin oxide (ITO) electrodes installed in a custom-built mini- incubator positioned on an inverted optical microscope. The increased toxicity of smaller graphene nanoflakes (30 nm) as measured by electrochemical impedance sensing and optical monitoring of treated cells was consistent with the biological assay results.(Yoon *et al.*, 2014)

In 2014, Zhou and Gao reviewed the advances in cytotoxicity of graphene, including its interaction and disruption on the structure and function of proteins, DNAs, and cell membranes, with an emphasis on the molecular level understanding of its interactions with biological systems. Both experimental and theoretical approaches reported in the paper showed that graphene can significantly disrupt protein and DNA structures due to the strong π–π stacking interactions and damage the integrity of cell membranes (both bacteria and human cells). Two types of molecular mechanisms for the graphene-induced degradation of cell membranes have been identified, one by severe insertion and cutting, and the other by destructive extraction of lipid molecules.(Zhou and Gao, 2014)

Sasidharan *et al.* presented the results of a comprehensive 3-months study on the acute and chronic toxicity of intravenously administered (20 mg/kg) few-layer graphene (FLG), its carboxylated (FLG-COOH) and PEGylated (FLG-PEG) derivatives in Swiss albino mice. FLG and FLG-COOH accumulated within organs induced significant cellular and structural damages to lungs, liver, spleen, and kidney, ranging from mild congestion to necrosis, fibrosis and glomerular filtration dysfunction, without appreciable clearance.(Sasidharan *et al.*, 2015)

Defteralı *et al.* studied the biocompatibility of thermally reduced graphene (TRG) with neurons and glia, as well as with the generation of new neurons in the adult brain *in vivo*. To achieve this, 9-week-old wild-type C57BL/6N mice were injected into the core of the olfactory bulb with 4 μL TRG (0.004 μg/μL). TRG injected in the brain did not alter de novo neurogenesis, neuronal and astrocyte survival nor did it produce a microglial response. These findings indicate that TRG may be a biocompatible material with neuronal and glial cells *in vivo* and support its use in studies of brain repair and function.(Defteralı *et al.*, 2016)

Liu *et al.* studied the potential effects of graphene on different representative cell lines, including HepG2, A549, MCF-7, and HeLa cells. For cell viability studies, cells were exposed to graphene of 1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg/mL for 24 h. Results showed no influences on cell apoptosis in graphene-treated cells when compared to the negative controls, proving the low cytotoxicity of this emerging nanomaterial.(Liu *et al.*, 2016)

Sasidharan *et al.* used human primary umbilical vein endothelial cells as model of vascular transport, to investigate the basic mechanism underlying the biological behavior of graphene. Mechanistic toxicity studies revealed that FLG exerted cellular toxicity employing an oxidative stress paradigm in HUVEC cells, which severely altered critical cell parameters including cytoskeletal dysfunction, reduction in metabolic activity, compromised plasma membrane integrity, elevated levels of intracellular ROS, lipid peroxidation, oxidized glutathione, ionized calcium and depolarization of mitochondrial membrane potential.(Sasidharan *et al.*, 2016)

In another study from Xie *et al.*, four-week-old male ICR mice were exposed to graphene at doses of 1 μg/d, 10 μg/d and 100 μg/d by gavage everyday for 4 weeks. High-throughput sequencing was applied to characterize the changes in microbial community and antibiotic

resistance genes (ARGs) in mouse gut. Graphene exposure increased the gut microbial diversity of mouse. 1 μg/d graphene had higher influence on gut microbiota than 10 and 100 μg/d graphene, which might be due to aggregation phenomena at higher concentrations. Graphene increased abundances of oxidative stress and membrane-damage related genes. Gram-negative bacteria were more tolerant to graphene than Gram-positive bacteria. Graphene exposure increased types and abundances of antibiotic resistance genes.(Y. Xie *et al.*, 2016)

Pattammattel *et al.* used liquid phase exfoliated graphite in six different animal sera, evaluating its toxicity. The toxicity study of this graphene fully dispersed to human embryonic kidney cells, human lung cancer cells, and nematodes (Caenorhabditis elegans) showed no acute toxicity for up to 7 days at various doses (50–500 μg/mL), but prolonged exposure at higher doses (300–500 μg/mL, 10–15 days) showed cytotoxicity to cells (~95% death) and reproductive toxicity to C. elegans (5–10% reduction in brood size). The origin of toxicity was found to be due to the highly fragmented smaller graphene sheets (<200 nm), while the larger sheets were nontoxic (50–300 μg/mL dose).(Pattammattel *et al.*, 2017)

In another work by Petibone *et al.*, the cytotoxic and genotoxic potential for functionalized graphene (f-G) was found to be dependent on p53 tumor suppressor protein status. f-G exposure (0.025, 0.05, 0.1, 0.3 and 0.5 μg/mL) presented a lower cytotoxic and genotoxic hazard to B-lymphoblastoid cells with functional p53 than the hazard it presented to p53-deficeint B-lymphoblastoid cells. Although, f-G exposures did not increase chromatin loss, or gene mutation, and induced chromosome damage only at the highest dose tested.(Petibone *et al.*, 2017)

Tsai *et al.* evaluated the toxicity effects of graphene on the airway epithelial cell line BEAS-2B, which represents the first barrier of the human body to interact with airborne graphene particles. Results showed that graphene can induce the cellular Ca^{2+} by phospholipase C associated pathway by activating epidermal growth factor receptor, and that graphene exposure may exacerbate lung function and other related diseases such as lung cancer, chronic obstructive pulmonary disease, and cardiovascular diseases.(Tsai *et al.*, 2017)

Li *et al.* compared the effect of corannulene and graphene on embryonic development and sleep/wake behaviors of larval zebrafish. Standard egg water (wild type), corannulene, or graphene at 1, 10, and 50 μg/mL with 0.3% DMSO dissolved in standard egg water was microinjected into the yolk of each embryo ($n > 80$) at a volume of 4 nL/embryo. Graphene showed no significant locomotion alterations and did not disturb the sleep behavior and gene expression patterns.(Li *et al.*, 2017)

Demir and Marcos aimed at determining the effects of graphene or MWCNTs on cytotoxicity, intracellular levels of reactive oxygen species, apoptosis, gene expression changes, and gene mutation induction in L5178Y/Tk+/−3.7.2C mouse lymphoma cell line. Although some adverse effects were observed at concentrations of 350 and 450 μg/mL, which are excessive and therefore not likely to happen, no marked effects were detected at concentrations of 250 μg/mL and lower.(Demir and Marcos, 2018)

The work by Di Cristo *et al.* aimed at providing some elucidations on the specific molecular signalling induced by low doses of a well characterized FLG material in macrophages. For viability experiments cells were exposed to increasing doses (2.5, 5, 10, 20, 40 $\mu g/cm^2$) of FLG. Exposure to low doses of FLG resulted in no significant decrease of macrophage viability. Nevertheless, it elicited a marked oxidative stress. The latter triggered significant inflammatory responses, increasing TNF-α and IL-6 secretion as well as NO production, leading to autophagy via endoplasmic reticulum (ER) stress. This work highlights an interplay between oxidative stress and ER stress-mediated autophagy. It also suggests that such a pathway could protect the cells from over-inflammation.(Di Cristo *et al.*, 2018)

Fernandes *et al.* evaluated the mechanism of graphene toxicity in different tissues of zebrafish, considering different parameters of stress. Animals were injected intraperitoneally with 10 μL of suspensions containing different graphene concentrations (5 and 50 mg/L). The results showed pathological effects in all tissues, excluding the intestine, after exposure to both concentrations.

Overall, these results indicate that graphene induces different grades of toxicological effects that are dependent on the analysed organ, with distinct pathological effects on some and oxidative effects on others. However, the brain and gills seem to be the primary target organs for graphene toxicity.(Fernandes *et al.*, 2018)

Manjunatha *et al.* evaluated the toxicity of graphene using developing zebrafish embryos. To determine this, 4-hpf embryos were exposed to different concentrations of GR (1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/L) and different early life-stage parameters were observed at 24, 48, 72, and 96 hpf. Through embryogenesis, GR was observed to induce significant embryonic mortality, delayed hatching, heartbeat, several morphological defects, pericardial toxicity, and bradycardia. Yolk sac edema and pericardial edema were induced by GR in developing embryos. These outcomes would provide new insights into the adverse effects of GR on the developing embryonic cardiac defects in vertebrates and highlight the probable natural environment and health hazards of GR flakes.(Manjunatha *et al.*, 2018)

In a recent study by Murera *et al.*, the authors studied the impact of FLG on primary murine lymphocytes. Results from the study pointed out that FLG at concentrations up to 100 µg/mL neither impacts the viability and activation of T and B cells nor their autophagic activity.(Murera *et al.*, 2019)

Lahiani *et al.* reported that graphene has dose-dependent effects on the growth of bacteria from intestinal microbiota. Bacterial growth after exposure to graphene (0, 1, 10, and 100 µg/mL) was measured. Results demonstrated that demonstrates that the toxicity of graphene to the intestinal microbiome is time and dose dependent. (Lahiani *et al.*, 2019)

Frontiñan-Rubio *et al.* described the effect of sub-lethal doses of small FLG on the biology of human HaCaT keratinocytes. A powerful methodology is described to detect changes that are not evident with other classical techniques. It was observed that FLG generates a whole series of changes in human skin cells, even on applying sub-lethal doses. In a multi-experimental approach, a remodeling of the cellular energetic metabolism was observed along with alterations in the calcium and redox homeostasis. This metabolic reshaping shares some characteristics with classic tumor cell metabolism.(Frontiñan-Rubio *et al.*, 2020)

Lin *et al.* reported that primary human macrophage viability and activation were unaffected by 24 h treatment with FLG at doses up to 50 µg/mL and, in another study by Ruiz *et al.*, no *in vivo* haematotoxicity at 300 µg/mouse up to 30 days was evidenced after intravenous injection (Lin *et al.*, 2020),(Ruiz *et al.*, 2020).

Burgum *et al.* sought to assess the inhalation hazard of industrially relevant FLG engineered with: (i) no surface functional groups (neutral), (ii) amine, and (iii) carboxyl group functionalization. The findings of the study have demonstrated the capability of neutral-FLG and amine-FLG to induce genotoxicity in human bronchial epithelial cell line 16HBE14o through primary indirect mechanisms, suggesting a possible role for carboxyl groups in scavenging radicals produced via oxidative stress.(Burgum *et al.*, 2021)

7.1.2 Ecotoxicity

Ecotoxicological effects of graphene have been extensively reviewed (Arvidsson, Molander and Sanden, 2013; Hu and Zhou, 2013; Lalwani *et al.*, 2016; Fadeel *et al.*, 2018; Devasena, Francis and Ramaprabhu, 2021). See also Table 9 for additional relevant reviews. Here below we provide an overview of a series of representative research papers covering aspect of ecotoxicity of graphene and few layer graphene.

Begurn *et al.* studied the effects of graphene on root and shoot growth, biomass, shape, cell death, and ROS of cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*), red spinach (*Amaranthus dubius*), and lettuce (*Lactuca sativa*), using a concentration range from 500 to 2000 mg/L. The results of the combined morphological and physiological analyses indicate that after 20 days of exposure under the experimental conditions, graphene significantly inhibited plant growth and biomass compared to a control.(Begurn, Ikhtiari and Fugetsu, 2011)

Begum and Fugetsu in 2013 studied the potential influence of graphene on T87 cells. The authors observed fragmented nuclei, membrane damage, mitochondrial dysfunction and increased ROS. ROS are key mediators in the cell death signalling pathway. Translocation of graphene into cells and an endocytosis-like structure was observed.(Begum and Fugetsu, 2013)

Liu et at. elucidated the effects of graphene on the germination and growth of rice seeds were studied. Seeds were treated with graphene solutions at different concentrations. Significant inhibitions on the stem length and fresh weight of over ground part were observed at concentration of 50 mg/L. In addition, all the indexes were inhibited at concentrations of 100 mg/L and 200 mg/L, indicating that graphene certainly inhibit the morphogenesis of rice seedlings.(Liu *et al.*, 2015)

Ren *et al.* evaluated the impact of graphene on the structure, abundance and function of the soil bacterial community based on quantitative real-time polymerase chain reaction (qPCR), pyrosequencing and soil enzyme activities. Graphene transiently promoted the enzyme activities and soil bacterial biomass. A transiently significant shift in soil bacterial community structure was caused. The effect of graphene on soil bacterial community was time dependent. Graphene significantly suppressed *Nitrospira* and *Planctomyces*. Graphene promoted the growth of some bacteria populations degrading organic pollutants.(Ren *et al.*, 2015)

The study by Fan *et al.* assessed the acute and chronic toxicity of graphene nanomaterial (GNM) to *Daphnia magna* by comparing the toxic effects of GNM with those of three other typical carbon nanomaterials (CNMs), C60, SWCNTs, and MWCNTs. The toxicity of GNM was significantly higher than that of the other three CNMs, although GNM bioaccumulation in D. magna was relatively lower.(Fan *et al.*, 2016)

Muzi *et al.* tested multi-layer graphene (MLG) composed of 2–20 graphene layers on two mammalian cell models and on Xenopus laevis as an important *in vivo* and environmental model organism. MLG showed to be substantially not toxic towards their cellular models and X. larvae.(Muzi *et al.*, 2016)

Ge at al. exposed dry grassland soil for 1 year to 1 mg/g of either natural nanostructured material (biochar), industrial carbon black, three types of MWCNTs, or graphene. After a 1-year exposure, compared to the no amendment control, some treatments reduced soil DNA (*e.g.*, biochar, all three MWCNT types, and graphene) and altered bacterial communities (*e.g.*, biochar, carbon black, narrow MWCNTs, and graphene); however, there were no significant differences across the amended treatments. These findings suggest that these materials may moderately affect dry soil microbial communities, but that the effects are similar to those from natural and industrial carbonaceous materials, even after 1-year exposure.(Ge *et al.*, 2016)

Zhang *et al.* studied the effects of graphene on plant roots and shoots (*Triticum aestivum*) after 48 h or 30 days of hydroponic culture and determined the phytotoxicity. Results showed that although exposure to graphene (250, 500, 1000 and 1500 mg/L) significantly improved root elongation, root hair production was impaired. The authors conclude that graphene has growth-limiting effects on plants, including root hair reduction, oxidative burst, photosynthesis inhibition, and nutritional disorder.(Zhang *et al.*, 2016)

Fernandes *et al.* studied the toxicity of FLG in different tissues of the shrimp *Litopenaeus vannamei* following exposure to FLG through a diet for four weeks. Results demonstrate that exposure to FLG through the diet induces alterations in the redox state of cells, leading to a subsequent oxidative stress situation. It is therefore clear that nanomaterials presenting these physicochemical characteristics may be harmful to aquatic biota.(Fernandes *et al.*, 2017)

In another study, Garacci *et al.* it was found that multilayer graphene can induce growth inhibition to the aquatic benthic diatom *Nitzschia palea* only in the first hours of contamination. The results could be explained by direct contact with the diatoms and to the shading effect. The extracellular polymeric substances, mainly composed of polysaccharides and proteins naturally secreted by diatoms, displayed a strong interaction with graphene, leading to growth recovery.

The latter study implies that the presence of an eco-corona (a biomolecular coating formed when nanomaterials enter the environment and may include proteins and other biomolecules such as metabolites from cellular activity and/or natural organic matter) may impact on the ecotoxicity of graphene materials in analogy with the presence of a biocorona in the human body.(Garacci *et al.*, 2017)

He *et al.* studied the effects of graphene (at 0, 5, 50, 100, and 200 mg/L concentrations) on antioxidant enzyme activity, chlorophyll content and malondialdehyde content in rice seedlings. Results indicated the potential toxicity of graphene in rice seedlings and suggested the possible utility for 5 mg/L graphene to enhance growth in rice seedlings.(He *et al.*, 2017)

Ge et al. studied how carbonaceous nanomaterials (CNMs) can affect agricultural soil prokaryotic communities and how the effects vary with the crop growth stage. To investigate this, soybean plants were cultivated in soils amended with 0, 0.1, 100, or 1000 mg/kg of carbon black, MWCNTs, or graphene. None of the three CNMs affected rhizosphere soil prokaryotic communities at day 0, but they caused mild effects at the vegetative stage (only the low dose of MWCNTs) and relatively stronger effects at the reproductive stage (almost all treatments except the high dose of MWCNTs). Overall, low doses of specific CNMs may have a high impact on soil rhizosphere prokaryotic communities and their associated functions at the soybean reproductive stage, a crucial stage of soybean development for seed yield.(Ge *et al.*, 2018)

Zanelli *et al.* studied the effects of FLG and a similarly layered phyllosilicate, muscovite mica on the *in vivo* reproductive structures, *i.e.*, pollen and stigma, of *Cucurbita pepo L.* ssp. pepo 'greyzini' (summer squash, zucchini). Results showed that FLG is safe under the tested conditions. (Zanelli *et al.*, 2020)

Zhang *et al.* used matrix assisted laser desorption/ionization mass spectrometry imaging -based untargeted metabolomics to investigate the metabolic response of juvenile earthworms (*Eisenia fetida*) to graphene exposure in soil tests for the first time. The results revealed that graphene-exposure significantly disturbs earthworm metabolome, and graphene toxicity on earthworm showed non-concentration-dependent effect.(Zhang *et al.*, 2020)

Li *et al.* studied the combined toxic effects of co-exposure to graphene and triphenyl phosphate (TPP) in *Mytilus galloprovincialis*. The work revealed that TPP could be adsorbed on the surface of graphene. Transcriptomic responses suggested that graphene plus TPP could reduce oxidative stress by modulating the glutathione metabolism pathway and bring down the energy consumption by modulating the glycolysis/gluconeogenesis and TCA cycle pathways.(F. Li *et al.*, 2021)

Demir used *Drosophila melanogaster* as a model organism to identify the potential risks of exposure to graphene and different types of MWCNTs (pure, modified by amino or carboxyl groups) at concentrations ranging from 0.1 to 250 µg/mL. Significant effects were observed at two highest doses (100 and 250 µg/ml) of graphene or MWCNTs. This study reports findings on cellular immune response against hematopoiesis and parasitoids, genotoxicity, phenotypic variations, and locomotor behavior in *Drosophila melanogaster* .(Demir, 2021)

Urban-Malinga *et al.* studied the impact of the short- and long-term exposure (36 h and 24 days) of the marine benthic polychaete *Hediste diversicolor* to various concentrations (36 h: 0.4, 4, 40 and 400 mg L^{-1}; 24 days: 4 and 40 mg L^{-1}) of the multi layer graphene (of thickness 8–12 nm). Experiments revealed a limited toxic effect of graphene on H. diversicolor. Moreover, no neurotoxic effect expressed by inhibition of acetylcholinesterase (AChE) activity was observed. Substantial inter-individual variability in the activities of some biomarkers at the end of the long-term experiment was found.(Urban-Malinga *et al.*, 2021)

In the study by Chen *et al.*, Bara 310 SC (Bara, tolerant genotype) and Gold Empress (Gold, susceptible genotype) seeds were used to investigate how the leaves of alfalfa interpret the physiological responses to graphene stress based on metabolome and transcriptome characterizations. Graphene at different concentrations (0, 1% and 2%, w/w) were selected as

the analytes. Physiological results showed antioxidant defense system and photosynthesis was significantly disturbed under high environmental concentration of graphene. The most important metabolites, which were accumulated under graphene stress included amino acids, flavonoids, organic acids and sugars. Transcriptomic analysis reveals 1125 of core graphene-responsive genes in alfalfa that was robustly differently expressed in both genotypes. Gold seeds were more disturbed by graphene stress at both transcriptional and metabolic levels, since more stress-responsive genes/metabolites were identified.(Chen *et al.*, 2022)

7.2 Graphene oxide

Different reviews highlighted that the toxicity of GO in cells is due to several factors, including dose, lateral size, and surface charge (Tan *et al.*, 2019; Guo *et al.*, 2021; Rhazouani *et al.*, 2021). To date, many studies carried out on the cytotoxicity of GO are contradictory. Some studies have shown that GO has no toxic effects on cellular behaviour, while others have reported that this nanomaterial can induce cellular damage.

Because GO is by far the most studied 2D material, we aim to provide below the most significant results from primary research articles related to GO divided by area, thus, whenever possible, with identification of the target system or the organ affected or studied.

7.2.1 Immunotoxicity

Chen *et al.* reported that small GO (350 nm) induced the formation of small vacuoles in RAW264.7 macrophages without causing apparent cell death. Increasing the GO concentration triggered the formation of more vacuoles and significant cell death (G.-Y. Chen *et al.*, 2012).

Another report by Russier *et al.* showed that GO of small lateral size (0.13 µm) was internalized to a higher extent in comparison to large GO (1.34 µm), leading to significant effects on cell viability and cell activation.(Russier *et al.*, 2013)

A following study by Ma *et al.* showed instead that GO with large lateral size (750 to 1300 nm) was able to generate inflammatory responses significantly higher than those of small GO (350 to 700 nm) *in vitro* and *in vivo*. To study inflammatory responses, 7 weeks old BALB/c male mice were administrated with GO into the peritoneal cavity, lung, or bloodstream. For the intra peritoneal administration, GO was administrated at 5000 µg/kg body weight divided by two injections in 3 days. For lung exposure, mice were instilled at the position of the back tongue with GO at a dose of 2500 µg/kg body weight. For tail vein injection, 5000 µg/kg body weight small GO or large GO was administrated.(Ma *et al.*, 2015)

Duan *et al.* studied graphene oxide nanosheets and the induced cytotoxicity in RAW 264.7 cells, a macrophage cell line, by decreasing mitochondrial membrane potential, causing the accumulation of intracellular ROS, and triggering apoptosis through activation of the mitochondrial pathway. Graphene nanosheets produced holes in the membranes of RAW 264.7, reducing cell viability. This was due to strong interactions between graphene and membrane phospholipid tails.(Duan *et al.*, 2017)

Another study by Mukherjee *et al.* on highly purified GO with different lateral size showed that small (50–300 nm) and large (10–40 µm) GO sheets were readily internalized by macrophages without any toxicity and GO did not trigger the production of pro-inflammatory TNF-α. (Mukherjee, Kostarelos and Fadeel, 2018)

Li *et al.* examined a panel of GO materials comprising GO, rGO, and hydrated GO (hGO) in which quantitative assessment of the hydroxyl, carboxyl, epoxy, and carbon radical contents was used to study the impact on epithelial cells and macrophages and in the murine lung. For animal studies, eight-week-old male C57Bl/6 mice being held in a vertical position, were instilled at the back of the tongue for pulmonary aspiration with 50 µL aliquots, containing 2 mg/kg of the GO suspensions. The mice were sacrificed after 40 h exposure. The authors could show that hGO, which exhibited the highest carbon radical density, triggered cell death in THP-1 and BEAS-2B cells with associated lipid peroxidation of cell membrane, albeit at relatively high concentrations

(up to 200 µg/mL). The authors also demonstrated that hGO was more prone than the other materials to trigger lung inflammation, accompanied by lipid peroxidation in alveolar macrophages. Thus, carbon radical content plays an important role for toxicity of GO.(Li *et al.*, 2018).

7.2.2 Ocular toxicity

Wu *et al.* studied the eye drop stimulation induced by GO using Sprague–Dawley rats administered with increasing concentration of GO (12.5, 25, 50 or 100 µg/mL), demonstrating temporal and dose-dependent ocular injury including corneal epithelial necrosis shedding and corneal stroma exposure (Wu *et al.*, 2016). However, previous studies observed that exposure to graphene and GO did not result in significant ocular toxicity. (Lin *et al.*, 2015)(Yan *et al.*, 2012)

In another study, Chen *et al.* found that GO (thickness: 0.8–1 nm; diameter: 101 - 258 nm) adhered to and enveloped the chorion of zebrafish embryos mainly via hydroxyl group interactions, blocked the pore canals of the chorionic membrane, and caused marked hypoxia and hatching delay. GO spontaneously penetrated the chorion, entered the embryo via endocytosis, damaged the mitochondria and primarily translocated to the eye, heart and yolk sac regions, which are involved in the circulatory system of zebrafish. In these organs, GO induced high generation of reactive oxygen species and increased oxidative stress, DNA damage and apoptosis. GO induced developmental malformation of the eye, cardiac/yolk sac edema, tail flexure and heart rate reduction. The adverse effects of GO on heart rate and tail/spinal cord flexure decreased as the GO concentration increased.(Chen *et al.*, 2016).

In another study, An *et al.* evaluated the ocular toxicity of GO and rGO *in vivo* and *in vitro*. For animal studies, the right conjunctival sacs of eight-week-old Kunming mice (female) were exposed to 10 µL doses of the rGO and GO suspensions (25, 50 and 100 µg/mL) once per day for a total of 7 d. The findings show that short-term repeated GO exposure can cause intraocular inflammation, an incrassated corneal stromal layer, cell apoptosis in the cornea, iris neovascularization and significant cytotoxicity on rat corneal epithelial cells, while rGO causes no significant ocular toxicity in mice.(An *et al.*, 2018)

7.2.3 Pulmonary toxicity

Duch *et al.* investigated the *in vivo* pulmonary effects of GO in water (thickness: 0.5−2 nm) in comparison to graphene nanoplatelets (GNPs) in water (thickness: 1.2−5 nm) or in 2% pluronic F108 (thickness: 1.2− 5 nm. GO-treated animals exhibited severe pulmonary inflammation but no signs of fibrosis. In contrast, GNPs were less inflammogenic, and this was further minimized when the GNPs were well-dispersed using pluronic F108. The authors suggested that oxidation of graphene is a major contributor to its pulmonary toxicity. For animal studies, 8–12 week old male C57BL/6 mice were intratracheally administered with equal weights and volumes of nanomaterials or control vehicles (50 µg/mouse in a total volume of 50 µL/mouse) (Duch *et al.*, 2011)

In contrast, Ali-Boucetta *et al.* reported that, highly pure, colloidally stable, and evenly dispersed GO (lateral dimension: <500 nm) injected intraperitoneally into C57BL6 mice with 0.5 mL containing 50 µg of GO had no signs of inflammation or granuloma formation onto the peritoneal mesothelium as compared to GO prepared by conventional Hummers method, underlying the importance of GO preparation and purification methods on biological results. (Ali-Boucetta *et al.*, 2013)

Li *et al.* studied GO (lateral dimension: 10−800 nm, thickness: 1−2 layers) with intratracheal instillation in C57BL/6 mice at a concentration of 1, 5 or 10 mg/kg using 0.2, 1 and 2 mg/mL suspension solutions, respectively, and their impact on lungs was assessed at various time points from 1 day up to 3 months. Inflammation appeared already at day 1, with a dose-dependent recruitment of immune cells including neutrophils, along with signs of acute lung injury. The peak of the response was observed at 2 days, returning to levels close to, but still above, control levels by day 7. Macrophages started to appear in lung sections by day 7 and were still present

in the lungs at 3 months, though a decrease in the blackness of the lungs from day 1 to day 90 suggested an ongoing clearance process. (Li *et al.*, 2013)

Wang *et al.* compared large (lateral dimension: 1676 nm) versus small (lateral dimension: 179 nm) GO and BSA-dispersed (lateral dimension: 640 nm) versus pluronic F108 dispersed (latealr dimension: 45 nm) GNPs. All materials, except GNPs dispersed in pluronic F108, induced collagen deposition fibrosis 21 days after oropharyngeal aspiration of 2 mg/kg of each of the graphene materials in C57BL/6 mice. F108-dispersed GNPs were less inflammogenic and not fibrogenic compared to BSA-dispersed GNPs, which were both inflammogenic and fibrogenic, whereas both small and large GO were inflammogenic and fibrogenic, while large GO induced more pronounced effects than small GO. (Wang, Duch, *et al.*, 2015)

Bengtson *et al.* studied single intratracheal administration of either GO (lateral dimension: 2−3 μm, thickness: 1−2 nm) or rGO (lateral dimension: 1−2 μm, thickness: 1−2 nm) in mice. GO sheets induced a strong neutrophil influx at 18, 54, and 162 μg/mouse, 1 and 3 days' post-exposure. This was paralleled by a pulmonary acute phase response. In contrast, rGO induced significantly less neutrophil influx, and neutrophil influx was only statistically significantly increased at 162 μg/mouse. However, all three dose levels induced statistically significantly increased neutrophil influx at 90 days post-exposure. DNA damage in bronchoalveolar lavage fluid was found for both GO and rGO. Pulmonary exposure to GO and rGO induced inflammation, acute phase response and genotoxicity but no fibrosis. C57BL/6J female mice, 7-weeks old were used. The applied doses were 18 μg, 54 μg and 162 μg/mouse. Mice were euthanized at 1, 3, 28 or 90 days post exposure.(Bengtson *et al.*, 2017)

Two complementary studies by Vranic *et al.* and Rodriguez *et al.* revealed similar size-dependent responses in the lungs of female C57BL/6 mice exposed via the intranasal route to a single administration of small (total instilled dose 50 μg; lateral dimension: 170 nm, thickness: 1 nm) or large (lateral dimension: 1723 nm, thickness: 1 nm) GO. The large GO induced more immune cell infiltration (primarily neutrophils) in the lungs at day 1 when compared to small GO, leading to the formation of granuloma by day 7, which increased by day 28. However, no fibrosis was observed after 28 days despite the presence of granulomas in the lungs of animals treated with large GO. (Vranic *et al.*, 2018)(Rodrigues *et al.*, 2020)

Li *et al.* reported the effect of the degree of surface oxidation of GO on pulmonary toxicity. GO (lateral dimension: 334.1 nm, 1 layer) was compared to rGO sheets (lateral dimension: 549.2 nm, 3 layers) or hydrated GO sheets (lateral dimension: 329.8 nm, 3 layers). For animal studies, eight-week-old male C57Bl/6 mice being held in a vertical position, were instilled at the back of the tongue for pulmonary aspiration with 50 μL aliquots, containing 2 mg/kg of the GO suspensions. The mice were sacrificed after 40 h exposure. The hydrated GO produced the highest amount of carbon radicals and induced the highest production of ROS. The pulmonary impact was evaluated in mice 40 h after a single pharyngeal aspiration exposure at 2 mg/kg for each of the three materials. Materials with the highest pro-oxidative potential (*e.g.*, hydrated GO) caused more lung damage. (Li *et al.*, 2018)

Drasler *et al.* aerosolized GO and GNPs onto the lung epithelial tissue surface in a 3D human lung model. Exposure to these materials at two different concentrations (~300 and ~1000 ng/cm^2) did not elicit any adverse effects in this model. (Drasler *et al.*, 2018)

Zhang *et al.* determined the role of GO in lung injury, oxidative stress, inflammation and autophagy. The results revealed that lower concentrations of GO (5 and 10 mg/kg) did not cause significant lung injury, but the administration of GO at higher concentrations (50 and 100 mg/kg) induced lung edema, and increased lung permeability and histopathological lung changes. High GO concentrations also induced oxidative injury and inflammatory reactions in the lung. The findings indicated that GO causes lung injury in a dose-dependent manner by inducing autophagy. For animal studies, male Sprague-Dawley rats received 5, 10, 50 and 100 mg/kg GO injections, respectively. GO was injected into the tail vein once a day for 7 consecutive days. (L. Zhang *et al.*, 2021)

7.2.4 Cardiovascular toxicity

Singh *et al.* reported that GO sheets (lateral dimension: 0.2−5 μm) induced strong aggregation of platelets with activation of Src kinases and release of calcium from intracellular stores. Intravenous injection of this GO (250 μg/kg) into 8–12-week-old Swiss male mice was found to induce extensive pulmonary thromboembolism in mice 15 min after administration of the material. For comparison, rGO was significantly less effective in aggregating platelets in the lung vasculature. The variations in surface properties of GO may be responsible for the observed differences between the two materials. (Singh *et al.*, 2011)

In another study, Qu *et al.* reported instead that GO did not cause thromboembolism in the lungs of mice following intravenous injection. For *in vivo* experiments, male BALB/C mice (6–8 weeks old) were intravenously injected with a single dose of 1000 μg/mL GO in a volume of 200 μL. Mice were sacrificed 24 hr post the GO administration.(Qu *et al.*, 2013)

Bengtson *et al.* measured acute phase response proteins, biomarkers for risk of cardiovascular disease following a single intratracheal instillation of GO (lateral dimension: 2−3 μm, thickness: 2−3 layers) in comparison to rGO (lateral dimension: 1−2 μm, thickness: 2−3 layers). Unlike rGO, GO sheets clearly induced a transient acute response, with significant increase of biomarkers of cardiovascular risks at day 1 and day 3, however disappearing by day 28 or day 90. C57BL/6J female mice, 7-weeks old were used. The applied doses were 18 μg, 54 μg and 162 μg/mouse. Mice were euthanized at 1, 3, 28 or 90 days post exposure. (Bengtson *et al.*, 2017)

In the work by Arbo *et al.*, the cytotoxicity and the oxidative process of GO was evaluated in rat cardiomyoblast cell line H9c2. GO caused cardiotoxicity in this *in vitro* model, disturbing mitochondria, generating ROS and interacting with DNA. (Arbo *et al.*, 2019)

Zhang *et al.* studied the cardiotoxicity of GO and rGO *in vitro* and *in vivo*. It was found that rGO showed higher cardiotoxicity than GO. Both GO and rGO exhibited cardiotoxicity by mediating lipid peroxidation, oxidative stress, and by inducing mitochondrial dysfunction. Animal studies were perfomed as follows: healthy C57BL/6 male mice were injected with 4 mg/kg GO and rGO via tail vein and euthanized after 14 days of injection.(J. Zhang *et al.*, 2021)

7.2.5 Gastrointestinal toxicity

Nguyen *et al.* exposed Caco-2 cells to GO at different concentration (10−500 μg/mL) showing that only mild cytotoxic effects at higher concentrations. (Nguyen, Lin and Mustapha, 2015)

In another study by Kucki *et al.*, the uptake of GO and GNPs was shown to be strongly dependent on the differentiation state of the cells: non-differentiated Caco-2 cells were able to incorporate GO and GNPs of several micrometers in size in a dose-dependent manner, whereas differentiated Caco-2 cells displayed repellent properties toward GO and GNPs due to the presence of densely packed microvilli. This suggests that the choice of *in vitro* models is crucial for the outcome. (Kucki *et al.*, 2017)

Guarnieri *et al.* reported that the treatment of GO and FLG with digestive juices to simulate oral ingestion did not induce structural changes or degradation of the materials, and chronic exposure to the digested GO did not affect the intestinal Caco-2 barrier despite long-term repeated exposure (1 and 5 μg/mL; 2 h every 2 days up to 9 days). (Guarnieri *et al.*, 2018)

In the study by Jia *et al.*, the effects of chronic exposure (25 days) of GO were investigated looking to the composition of the intestinal microbiota and the immune response in female and male zebrafish at different concentrations (0.05, 0.5, and 5 mg/L). Chronic exposure to GO disturbed the diversity and richness of intestinal microbes, increased the pathogenic bacterial community in zebrafish, induced damage to the gut tissues, and activated the inflammation response. (Jia *et al.*, 2019)

In another study, Wu *et al.* reported that, even though no significant exhibition of overall

hepatotoxicity was observed in mice under relatively low dose of GO, remarkable disruption of liver functional zonation patterns was evidenced, associated with detrimental changes in cellular components and signalling transduction. GO induced deep changes at the transcriptional and epigenetic levels despite minimal changes in the liver functions. *in vivo* experiments were conducted on BALB/c male mice (7–8 weeks old), intravenously injected with GO suspensions at concentrations of 0.1, 0.5, 2 and 5 mg/Kg. Mice were sacrificed at days 1, 7 and 30 after treatment.(Wu *et al.*, 2020)

7.2.6 Reproductive and developmental toxicity

In the works by Liang *et al.* and Skovmand *et al.*, it was found that male fertility and reproduction was not affected after intravenous and intraperitoneal injection of small or large GO in mice (Liang *et al.*, 2015) nor by pulmonary exposure to GO. (Skovmand *et al.*, 2018). In, addition Qu *et al.* and Sasidharan *et al.* reported that no damage to testis tissue was observed in male after intravenous injection of GO and FLG. (Qu *et al.*, 2013), (Sasidharan *et al.*, 2015)

The work by Fu *et al.* showed a reduced growth of the offspring when maternal mice were given drinking water containing GO (at 0.5 mg/mL). (Fu *et al.*, 2015)

Chatterjee *et al.* have found some negative effects in the reproductive potential of worms influenced by surface functionalization and number of layers of the different graphene materials. Single-layer GO, few-layers GO, GNPs, amidated GNPs and carboxylated GNPs at different concentrations (5, 10, 20, 50 μg/mL) were tested for 72 h. The results, based on the number of offspring at all stages beyond the egg, showed some toxic effects varying with the graphene type, surface functionalization, number of layers, dose and time exposure, with no toxicity for the lowest concentrations (5 and 10 μg/mL). (Chatterjee *et al.*, 2015)

Hashemi *et al.* found some cytotoxic and genotoxic effects in mice spermatozoa when exposed to GO and rGO. After incubating for 2 h with different doses (0.1, 1, 10, 100 and 400 μg/mL), a reduced viability, motility and progressive motility were found. Some kinetic parameters, for concentrations >1 μg/mL, were influenced in a dose-dependent manner. (Akhavan *et al.*, 2015)

Akhavan *et al.* confirmed the uptake of GO by the testis of (Balb)/C male mice after intravenous injection of the material to both female and male mice *in vivo* every week for 8 weeks at concentrations of 2, 20, 200 and 2000 μg/mL. The analysis of the spermatozoa evidenced a reduced viability, motility and progressive motility and a decrease in some kinetic parameters, as well as a higher ROS production and DNA fragmentation when exposed to the highest GO concentrations. In the case of the female mice a decreased fertility, gestation rates and hormone concentrations when using the highest concentrations. (Akhavan *et al.*, 2015)

Mesarič *et al.* used the Mediterranean purple sea urchin (Paracentrotus lividus) to study the sperm toxicity after the exposure to commercial carbon black (CB) and GO with a mean size ranging between 0.5–5.0 μm and containing 20% weight of oxygen. After 1 h of sperm exposure to different concentrations (0.0001, 0.001, 0.01, 0.1 and 1 mg/L), egg fertilization was significantly affected with a reduction of around 50% in the case of CB while there was almost no effect in the case of GO. (Mesarič *et al.*, 2015)

In another work, Liang *et al.* used two sizes of GO (large: 200-300 nm; small <100 nm) that were injected at different concentrations (120 and 300 mg/kg for small GO and 120 mg/kg for large GO, administered every 24 h for 5 days) into the tail veins or abdominal cavities of male and female ICR-strain mice. After 30 days, the health of the spermatozoa, the activities of some epididymal enzymes and the sperm function were analyzed. Findings revealed an absence of toxicity even when using the highest concentrations (up to 300 mg/kg), which was explained with the presence of blood-testis and epidydimal barriers in the male mice that prevent the entry of big-size molecules/materials into the testis. (Liang *et al.*, 2015) (Liang *et al.*, 2016)

Zhao *et al.* added GO at different doses (1, 10, 100, 1000 μg/mL) to the living medium of worms until their young adult's stage. The concentrations of GO between 10 and 100 μg/mL induced the production of more germ cell corpses (thus inducing germ line apoptosis), a slightly

decreased brood size and number of oocytes, an inhibition of the egg injection rate and an increased embryo mortality. An epigenetic protection mechanism probably activated by GO, hypothesizing a novel self-protection mechanism against toxicity was also hypothesized. (Zhao, Wu and Wang, 2016)

In another work, Chatterjee *et al.* further investigated and compared the effects of few-layers GO and rGO at doses of 20, 50 and 100 µg/mL for 72 h, demonstrating a lower reproductive capacity of GO than rGO. (Chatterjee *et al.*, 2017)

D'Amora *et al.* compared the toxicity of GO, oxidized carbon nano-onions and oxidized carbon nanohorns in zebrafish. The results clearly demonstrated a toxic effect of GO in a dose-dependent manner on hatching and development. Above 50 µg/mL, embryos and larvae treated with GO presented a survival rate under 85%, hatching rate/time disturbance, a developmental delay with different malformations and a decrease of spontaneous movements. (d'Amora *et al.*, 2017)

Nirmal *et al.* used Wistar rats to evaluate the toxicity of GO (lateral dimension: 5–10 µm) injected intraperitoneally at doses of 0.4, 2 and 10 mg/kg for 7, 15 or 30 days (injected on alternate days). The findings evidenced a dose-dependent reduction in the number of spermatozoa, spermatogonia and spermatids, a decreased sperm motility, and some morphological abnormalities in the groups that were treated with the highest concentrations. Some oxidative stress was confirmed with the increased activity of antioxidant enzymes, without causing damage to the testis or reducing the fertility potential. These observations were confirmed with the healthy offspring derived from the matching with female rats. (Nirmal, Awasthi and John, 2017)

Kucki *et al.* studied the impact of four GO materials on human BeWo cells that did not reveal evident cytotoxicity after 48 h of exposure at concentrations up to 40 µg/mL despite internalization of GO. Exposure to GO induced a transient opening of the cell barrier as evidenced by a temporary increase in the translocation of a fluorescent dye and a slight decrease in human chorio-gonadotropin secretion. These observations highlight the need for further studies on the long-term consequences of GO and other graphene materials on placenta functionality and maternal fetal health. (Kucki *et al.*, 2018)

The study by Kim *et al.* showed that young adult C. elegans exposed to GO for 72 h, at a concentration of 10 µg/mL, displayed GO accumulation in the reproductive organs, leading to a reduced sperm number and a damage of the spermatogenesis. (Kim *et al.*, 2018)

In another work, Bernabo *et al.* explained the reduction in the sperm motility and kinetic parameters (*in vitro*) by the attachment of the spermatozoa to the GO and rGO, intercepting the sperm free movement but without interfering with the sperm fertilizing ability. (Bernabo *et al.*, 2018)

Martins *et al.* supplied with the diet GO and oxidized MWCNTs to the insect Spodoptera frugiperda at different doses (10, 100, 1000 µg/g of food). Both carbon nanomaterials affected the reproductive performance when they were used at the highest concentrations. (Martins *et al.*, 2019)

In another study, Bernabò *et al.* studied the addition of 0.5 µg/mL GO to a sperm suspension before performing *in vitro* fertilization. It was found an increased number of fertilized oocytes and the birth rate after embryo transplantation in foster mothers in a more effective way than methyl-β-cyclodextrin, the gold standard in promoting *in vitro* fertility of mice spermatozoa. It was also demonstrated that GO exerts its positive effect by extracting cholesterol from the membranes, without affecting the integrity of the membrane microdomains and thus preserving the sperm function. (Bernabò *et al.*, 2020)

Liu *et al.* found that orally administrated GO daily during gestational day (GD) 7–16 caused dose-dependent pregnant complications of mice at the endpoint (GD 19), including decreased

weight of dam and live fetus, high rate of resorbed embryos and dead fetus, and skeletal development retardation. The damages to GO exposure on the placenta barrier and pregnancy were dose-dependent, and GO exposure was responsible for gut microbiome dysbiosis in mice with pregnant complications. These findings suggest evaluating reproductive risks of GO to mammals. For animal studies, sexually mature ICR mice were treated with GO dispersed in sterile water at concentration gradients (0.1 mg/mL, 0.5 mg/mL and 2 mg/mL). The oral administration volume was 0.2 mL/10 g body weight daily during organogenesis period (GD7-GD16). (Liu *et al.*, 2021)

Zhao *et al.* investigated the toxic effects of GO on Eisenia fetida by exposing the earthworm to different doses of GO (0, 5, 10, 20, and 30 g/kg) for 7, 14, 21, and 28 days. Findings suggest that GO induces oxidative stress and genotoxicity, resulting in lipid peroxidation, decreased lysosomal membrane stability and DNA damage. (Zhao, Wang and Duo, 2021).

Jin *et al.* employed C. elegans to explore the multi-generational toxicity effects of GO. After continuous exposure (0.01, 0.10, 1.00 and 10.00 μg/L) for several generations, worms grew smaller and lived shorter. The locomotion behaviors were reduced across the filial generations and these reduced trends were following the impairments of locomotion-related neurons. The extended defecation cycles from the third filial generation were in consistency with the relative size reduction of the defecation related neuron. Simultaneously, the fertility function of the nematode was impaired under consecutive exposure as reduced brood sizes and oocytes numbers, increased apoptosis of germline, and aberrant expression of reproductive related genes were detected in exposed worms. Continuous multi-generational exposure to GO caused damage to the neuron development and the reproductive system in nematodes. (Jin *et al.*, 2022)

In the study by Duo *et al.*, the acute and chronic toxicity of GO on Eisenia fetida was again evaluated. Individual and histological endpoints of earthworms, including growth, reproduction, and histopathological changes in the intestine and skin, were assessed. The growth rate and reproduction rate of earthworms showed a significant GO concentration-related decrease. GO induced serious skin and intestine toxicity at increasing concentrations. (Duo, Wang and Zhao, 2022)

7.2.7 Central nervous system toxicity

Rauti *et al.* evaluated the effect of GO of different lateral dimensions on cells belonging to relevant structures of the central nervous system (CNS). Neurons and glial cells from dissociated rat hippocampus or cortex were cultured in the presence of 10 μg/mL dispersions of large and small GO (10 - 30 μm and 200 nm - 1 μm, respectively). Small GO were shown to alter synapses and induce glial cell reaction. After 6−8 days of incubation, it appears that large GO induced unequivocal neuroglial and neuronal loss. When cells were treated with the same concentration of FLG, no reduction in cell density or viability was observed in both neuronal and glial populations, thus demonstrating that CNS cells survival *in vitro* seems dependent on the graphene sheet dimensions as well as its chemical composition.(Rauti *et al.*, 2016)

Bramini *et al.* conducted proteomic and lipidomic analyses on primary neuron and astrocyte cultures exposed to GO and, in the case of astrocytes, to GO or FLG. It was found calcium signalling along with several Ca^{2+}-binding and buffering proteins were up- or down-regulated. The lipidomics analysis revealed that the exposed neurons were characterized by an up-regulation of phosphatidylethanolamine and down-regulation of phosphatidylserine. Cholesterol was found to be one of the most altered lipids in astrocytes exposed to the materials. Regarding the functionality of cells, a closer analysis of Ca^{2+} dynamics revealed alterations in both neurons and astrocytes consisting of reduced number of spontaneously oscillating cells, reduced basal cytoplasmic Ca^{2+} concentration, and altered responses to external stimuli. These effects were elicited only by chronic GO exposure, whereas acute exposure to FLG and GO did not cause any functional alterations in both cultures. (Bramini *et al.*, 2016)

The study by Yang *et al.* was focused on the zebrafish and RAW264.7 cell line as *in vivo* and *in vitro* models to assess the potential developmental neurotoxicity and immunotoxicity of GO. No obvious acute developmental toxicity was observed upon treatments with 0.01, 0.1, and 1 μg/mL

GO for five consecutive days. Decreased hatching rate, increased malformation rate, heartbeat rate and hypoactivity of locomotor behaviour were detected when exposed to 10 μg/mL GO. (X. Yang *et al.*, 2019)

In the study by Kim *et al.*, the neurotoxic potential of GO and its underlying molecular and cellular mechanism were investigated using C. elegans. The study suggested the GO, administered at 10 mg/L of GO for 24 h, possesses neurotoxic potential, especially on neurotransmitters and AFD neuron. The AFD neurons of C. elegans provide a single neuron system in which to explore the molecular basis of thermosensation and plasticity of behaviors linked to thermosensation. (Kim *et al.*, 2020)

In the work by Cao *et al.* exposure of zebrafish embryos to 10, 50 and 100 mg/L of carboxylated GO induced neurodevelopmental abnormalities and altered tendency of locomotor in larval fish. Carboxylated GO exposure led to increase of AchE and ATPase activities and oxidative stress up-regulation, and it altered the expression of genes involved in neurodevelopment and neurotransmitter pathway. (Cao *et al.*, 2021)

7.2.8 Ecotoxicity

In the study by Begurn *et al.*, the leaves of cabbage, spinach, and tomato exhibited a decrease in size after exposure to GO and a decrease in number due to oxidative stress-mediated cell death by necrosis. (Begurn, Ikhtiari and Fugetsu, 2011)

Zhang *et al.* studied GO and GO modified with PEGylated poly-L-lysine using *Caenorhabditis elegans* and proposed a mechanism of toxicity under stress conditions involving the over-production of hydroxyl radicals and the formation of oxidizing cytochrome c intermediates. (Zhang *et al.*, 2012)

Chen *et al.* studied zebrafish embryos and found that GO can be integrated into the chorion causing hypoxia and a significant delay in hatching. A slight inhibition of cell growth (without significant induction of apoptosis) and a slight hatching delay after exposure to GO were also observed. (L. Q. Chen *et al.*, 2012)

Mesaric *et al.* studied larvae of marine crustacean *Amphibalanus amphitrite* that showed inhibition of mobility, as well as mortality, after exposure to GO (0.01, 0.1, 0.5 mg/mL). (Mesaric *et al.*, 2013)

Pretti *et al.* studied t *Artemia salina* that was exposed to GO resulting in no acute toxicity even when GO aggregated in the intestine. (Pretti *et al.*, 2014)

Anjum *et al.* found that the absorption of GO by the roots in Vicia faba has both beneficial and toxic effects depending on the concentration. Increased V. faba sensitivity at the highest doses was apparently due to an increased oxidative stress and a concomitant impairment of glutathione metabolism, whereas lower concentrations showed positive effects. (Anjum *et al.*, 2014)

Liu *et al.* studied rice seeds noticing that delayed germination rates were observed with increasing GO concentrations (50 μg/mL and above), and the growth of radicle and plumule was inhibited. (Liu *et al.*, 2015)

Tang *et al.* investigated the freshwater Microcystis aeruginosa testing combined exposures to GO and Cd^{2+} (concentrations between 1−50 μg/mL and 0.2−0.7 μg/mL, respectively). GO alone at low concentrations had no significant toxicity, even if the material easily adhered to and entered the algal cells. However, mortality and induction of oxidative stress due to Cd^{2+} uptake were both increased by the presence of GO. (Tang *et al.*, 2015)

In studies by Wang *et al.* and Zhao *et al.*, 2-week-old seedlings of *Arabidopsis thaliana* cultured with GO for 2 further weeks showed accumulation of GO in the root system but not in the leaf cells, implying that the plant copes with GO translocation from root to stem or leaves, although GO was found in all the compartments of the cotyledon cells. (Wang *et al.*, 2014),(Zhao *et al.*,

2015)

Tang *et al.* reported that the toxic amplification of other toxicants or pollutants by graphene materials is poorly investigated. It is underlined that it is fundamental to understand how graphene materials interact with other pollutants co-occurring in the environment in terms of adsorption, transport, bioavailability, and the subsequent effects upon pollutant toxicity and biodegradability. GO can apparently amplify phytotoxicity of arsenic in wheat, Triticum aestivum, and phytotoxicy of cadmium in the freshwater cyanobacterium, Microcystis aeruginosa. (Tang *et al.*, 2015)

Hu *et al.* reported on the toxicity of GO toward the protozoa Euglena gracilis and it was evidenced the inhibition of growth and the enhancement of malondialdehyde content and antioxidant enzyme activities. (Hu *et al.*, 2015)

Dziewięcka *et al.* reported that in the insect Acheta domesticus (adult males), oxidative stress was observed after injection of pure and manganese-contaminated GO at a dose of 0.1 μL of a 0.4 mg/μL GO solution per 100 mg of insect's body weight injected into the body cavity by piercing the intersegmental membrane between the sternites on the abdomen. (Dziewięcka *et al.*, 2016)

In a study by Xie *et al.* in which Phanerochaete chrysosporium was exposed for 14 days to GO (0−4 mg/mL) it is showed that GO stimulated growth at low concentrations, whereas inhibitory effects were seen at the highest concentrations, with a complete loss of decomposition activity due either to growth inhibition and/or defective enzyme excretion. (J. Xie *et al.*, 2016)

In the work by Zhao *et al.* GO, rGO, and multi-layer graphene exhibited much higher toxicity than other carbonaceous materials (*i.e.*, carbon nanotubes and graphite) to Chlorella pyrenoidosa. The shading effect was incriminated in the growth inhibition by GO due to its higher dispersibility and transformation, whereas the other graphene materials did not show such effects. (Zhao *et al.*, 2017)

Chen *et al.* studied how GO inhibited wheat germination of seeds at high concentrations and was observed to accumulate in the root, with a limited translocation to stem and leaves, inducing oxidative stress. (Chen *et al.*, 2017)

Ren *et al.* showed activation of a series of antimicrobial proteins in the C. elegans after exposure to GO. (Ren *et al.*, 2017)

De Marchi *et al.* reported that GO impact on the regenerative capacity of the polychaete, Diopatra neapolitana exposed to different GO concentrations, *e.g.* 0.00; 0.01; 0.10 and 1.00 mg/L. It was shown that at higher concentrations, less segments were regenerated, taking longer periods to complete regeneration and altering energy-related responses. (De Marchi *et al.*, 2017)

Zhang *et al.* showed that in the oligochaete, Tubifex tubifex, no mortality was observed following GO exposure, whereas burrowing activity was significantly reduced. (P. Zhang *et al.*, 2017)

Souza *et al.* showed that in adult zebrafish, GO exposure caused an increase in the number of apoptotic and necrotic gill cells, but genotoxicity was not observed. (Souza *et al.*, 2017)

Zhang *et al.* reported that the development of zebrafish embryos exposed to trace concentrations (1−100 μg/L) of GO was impaired because of DNA modification, protein carbonylation, and excessive ROS generation. The authors noted skeletal and cardiac malformations, while transcriptomics analysis revealed dysregulation of collagen and matrix metalloproteinase-related genes following exposure to 100 μg/L of GO. (X. Zhang *et al.*, 2017)

Another study by Lagier *et al.* compared the effects of GO, FLG, nanodiamonds, carbon nanotubes, and oxidized carbon nanotubes on the growth inhibition in Xenopus larvae. These effects are governed by surface area, whereas mass concentration is a poor descriptor of toxicity

for these different types of carbon forms. The available data suggest that growth inhibition observed in amphibians is related to physical blockage of the gills and/or digestive tract, limiting the exchange surfaces between the gills and/or gut lumen and the internal wall, leading to a decrease in absorption of nutrients and/or gas (anoxia). (Lagier *et al.*, 2017)

In the study by Montagner *et al.*, aeroterrestrial green microalgae were not affected by short (30 and 60 min) and long (4 weeks) exposures to GO and FLG. Potential oxidative effects of the same materials were also studied through the analysis of quantum yield of primary photochemistry in the dark-adapted state and changes of gene expression of genes encoding antioxidant enzymes and stress-related proteins in the lichen photobiont Trebouxia gelatinosa. GO was found to be inert, while FLG caused the downregulation of HSP70 gene, although this did not correspond to a decrease in the expression of HSP70 protein. The impact on Trebouxia was considered negligible due to their constitutive adaptation to extreme environments and the lack of internalization of GO or FLG by these microalgae characterized by a thick cell wall. (Montagner *et al.*, 2017)

De Marchi et at. compiled information on properties, applications and characterization methods of graphene family materials in aquatic environments and identified biological toxic impacts of these NMs, with special focus on GO. (De Marchi *et al.*, 2018)

In a study by Carniel *et al.*, *in vitro* experiments in the Nicotiana tabacum and in Corylus avellana showed that pollen germination and tube elongation were affected at GO concentrations ≥50 µg/mL, decreasing by 20% and 19% in N. tabacum and by 68% and 58% in C. avellana, respectively. Other experiments on C. avellana demonstrated that the main factor influencing pollen performances was the acidic property of GO. FLG also showed a minimal negative effect on pollen tube elongation, probably due to physical interactions with the pectin-rich wall of the pollen tube and/or Ca2+ sequestration, whereas pollen germination and pollen tube growth were not affected by rGO. (Carniel *et al.*, 2018)

Souza *et al.* evaluated graphene oxide (GO) effects on the freshwater cladoceran Ceriodaphnia dubia through acute and chronic toxicity, feeding rates, and ROS generation. The mean effective concentration (EC50) estimated during acute exposure was 1.25 mg L^{-1} of GO. The chronic exposure resulted in significant decrease in the number of neonates. This study provides useful information on GO concentrations that might impair the aquatic biota. (Souza *et al.*, 2018)

Castro *et al.* evaluated the effect of GO on aquatic ecosystems considering the interaction with humic acid on nine different organisms: Raphidocelis subcapitata (green algae), Lemna minor (aquatic plant), Lactuca sativa (lettuce), Daphnia magna (planktonic microcrustacea), Artemia salina (brine shrimp), Chironomus sancticaroli (chironomidae), Hydra attenuata (freshwater polyp), and C. elegans and Panagrolaimus sp. (nematodes). GO showed low acute toxicity for these aquatic organisms included. The presence of humic acid in the medium increased GO colloidal stability and caused an increase in the toxicity of GO to microcrustaceans (growth rate) and to C. elegans (fertility and reproduction). The results of this study could be useful for predicting ecologically safe GO concentrations. (Castro *et al.*, 2018)

Paital *et al.* investigated the toxic impacts of sub-lethal doses of GO on selected oxidative stress physiology markers, protein and nucleic acid content along with haematological parameters in A. testudineus. The results indicated that GO can induce oxidative stress in cell and mitochondria in fish. (Paital *et al.*, 2019)

Zheng *et al.* fed zebrafish with diets containing monolayer graphene, GO and rGO, for 21 days. Only graphene markedly reduced the diversity of gut microbiota. All materials caused instead critical taxa alternation. The three materials led to additional damage in intestines by generating more vacuolation and goblet cells. Graphene-family nanomaterials (GFNs) pose potential health risks to aquatic organisms through alteration of gut microbiota. (Zheng *et al.*, 2019)

Mendonca *et al.* performed experiments with the soil invertebrate Enchytraeus crypticus (E. crypticus) to evaluate the effects of commercial GO and rGO at concentrations of 250 and 1000

mg/Kg of dry soil. By evaluating the life cycle of the soil, reduced hatching, survival and reproduction rates after the treatment with the highest concentrations were observed. This occurred in a dose-dependent way for GO suggesting that this effect is connected to the oxidation degree of graphene. (Mendonca *et al.*, 2019)

Zanelli *et al.* verified the effect of GO interaction with the pollen−stigma system on the entire reproduction process of the model plant, Cucurbita pepo. The stigmatic surface integrity was not compromised by GO. However, pollen adhesion and germination over the stigma decreased, fruit development was altered, and seed production was completely suppressed. The similar effect of GO and highly purified GO supported the hypothesis that the physical interposition of planar GO between pollen and stigma compromised the reproduction process by lowering the pollen load and affecting pollen-stigma signalling. The necrotic effect of GO on fruits also suggested a chemical interaction of this material with the plant tissues, similar to other reactive substances. (Zanelli, Carniel and Tretiach, 2021)

Tsai *et al.* assessed the environmental levels of GO leading to adverse effects on C. elegans. Prolonged exposure to the low doses of GO led to disruption of reproduction and locomotion, attenuation of longevity, and induction of oxidative stress. (Tsai *et al.*, 2021)

Another study by Pires *et al.* assessed the effects of different GO nanosheet concentrations (0, 0.01, 0.1, 1 and 10 mg/L) on the behaviour, feeding activity, mucus production, regenerative capacity, biochemical damage and metabolism of H. diversicolor. Results revealed that the presence of GO, even at the lower levels tested, impaired behavioural, physiological, and biochemical traits in polychaetes, suggesting that the increase of GO in the environment can disturb these benthic organisms. (Pires *et al.*, 2022)

Malina *et al.* studied the interaction of three differently oxidized GO with aquatic plant Lemna minor. Although none of the three GO caused lethal phytotoxicity to Lemna after 7 days, the mechanism of action was dependent on the GO surface oxidation. Based on the amount of functional surface groups, the GO was able to directly interact with the Lemna's root through its edges. In contrast to algae and crustaceans, the interaction did not lead to a mechanical damage. GO can be considered not hazardous to Lemna minor even at very high concentrations (up to 25 mg/L), because the root barrier proved to be strong enough to prevent GO penetration and preventing a possible toxicity. (Malina *et al.*, 2022)

7.3 Reduced graphene oxide

7.3.1 Toxicity

Akhavan *et al.* used human mesenchymal stem cells isolated from umbilical cord blood to investigate for the first time the size-dependent cytotoxic and genotoxic effects of the rGO. rGO showed genotoxic effects on these cells through DNA fragmentations and chromosomal aberrations, even at low concentration of 0.1 µg/mL. (Akhavan, Ghaderi and Akhavan, 2012)

Wilczek *et al.* demonstrated that rGO was an attractive material for modifying scaffolds to create bioprosthetic heart valves. Results showed that rGO coating did not affect platelet adhesion to the surface of the scaffold or platelet activation, confirming that rGO was hemocompatible and biocompatible. (Wilczek *et al.*, 2015)

Xu *et al.* examined the effect of the same small and large rGO at different doses (6.25, 12.5, 25 mg/kg mouse) by intravenous injection into female ICR-strain mice to rGO influence on reproductive ability and offspring development. The experiment was performed at day 1 or day 30 prior to the mating or during the pregnancy (early and late). The results showed that the oestrogen levels, the mating behaviour and the pups (until the third litter of offspring) were unaffected. Some adverse consequences were found when the treatment was done during the pregnancy, based on the number of abortions or deformed foetuses, however not triggering secondary effects to the later generations. (Xu, Zhang and Chu, 2015).

In a work by Zhang *et al.* the short- and long-term (up to 60 days) effects of orally administered

rGO (oral gavage every 24 h for 5 consecutive days; total: 300 mg/kg per mouse) on male C57black/6 mice, including general locomotor activity level, balance and neuromuscular coordination, exploratory and anxiety behaviour, and learning and memory abilities were explored. The results demonstrated that oral exposure to a high dose of rGO caused a short-term decrease in locomotor activity and neuromuscular coordination but did not affect anxiety-like, exploratory, or spatial learning and memory behavior. (Zhang *et al.*, 2015)

Asghar *et al.* compared rGO to functionalized oxidized SWCNTs at concentrations of 1–25 µg/mL on human sperm viability. SWCNTs generated significant reactive superoxide species at the highest concentration, while rGO did not. Exposure to these materials did not hinder the sperm sorting process. Microfluidic sorting systems can select the sperm that show low oxidative stress post-exposure. (Asghar *et al.*, 2016)

Mendonca *et al.* aimed to study whether the rGO inside the hippocampus triggered toxic alterations in this brain region and in target organs (*e.g.*, blood, liver and kidney) of rats at various time points (15 min, 1, 3 h and 7 days). Animals (Rattus norvegicus) received a single tail vein injection of rGO (7 mg/kg dose; concentration of 1 mg/mL). The toxic effects seemed to be peripheral and transitory in the short-term analysis after systemic administration of rGO. The effects were self-limited and non-significant even at 7 days post-rGO administration. (Mendonca *et al.*, 2016)

In another study by Mendonça *et al.*, the effects of rGO and rGO functionalized with PEG were analysed on astrocytes and endothelial cells, *in vitro* and *in vivo* in rats. The *in vitro* studies demonstrated a concentration-dependent toxicity. The highest concentration (100 µg/mL) of rGO had a lower toxic influence on cell viability in primary cultures of astrocytes and rat brain endothelial cells as respect to PEGylated rGO. *in vivo* studies were conducted on male Wistar rats (Rattus norvegicus, 6-week-old, 180 ± 40 g) receiving a single i.v. injection of rGO-PEG (7 mg/kg; concentration 1 mg/mL). Results from *in vivo* evaluation showed a significant and long-lasting downregulation of BBB-associated proteins induced by PEGylated rGO, which implies impaired BBB function and probably a homeostatic disturbance of the hippocampal milieu. In contrast, non-PEGylated rGO induced a transient and slighter down-regulation of BBB-associated proteins, which was resolved 7 days post-rGO exposure.(Mendonça *et al.*, 2016)

Reshma *et al.* elucidated the interactions of rGO and PEGylated rGO with lung alveolar epithelial cells (A549). The results evidenced possible toxic effects of both rGO upon their inhalation and persistence in the lungs. (Reshma, Syama and Mohanan, 2016)

Contreras-Torres *et al.* reported a study where myocardial H9c2 cells were exposed to rGO (lateral dimension: 150 nm) and GO (lateral dimension: 380 nm). Cytotoxicity was dose-dependent above 10 µg/mL, and rGO was found to be more toxic than GO, and was internalized to a greater extent than GO. (Contreras-Torres *et al.*, 2017)

In the work my Liao *et al.*, epithelial-mesenchymal transition (EMT, a process involved in pulmonary fibrosis) effect of rGO was studied on A549 cells. rGO penetrated through the membrane of A549 cells into the cytosol by endocytosis and located in late endosome and/or lysosomes. rGO was well tolerated by the cells. rGO promoted cell migration and invasion capacities at low doses (below 10 µg/mL), but significantly inhibited these capacities at 20 µg/mL. rGO-induced EMT were evidenced by decreased expression of epithelial marker and increased expression of mesenchymal markers. Based on these findings, it is supposed that rGO can effectively induce EMT through altering epithelial-mesenchymal transition markers in A549 cells. (Liao *et al.*, 2018)

Pérez *et al.* analysed the genome-wide and global DNA methylation dynamics associated with the medium-term exposure of human lung epithelial cells to rGO at concentrations of 1 and 10 µg/mL. The results show no effects associated with each condition tested. (Pérez *et al.*, 2020)

In the work by Zambrano-Andazol *et al.*, rGO membranes (rGOM) were tested as substrates for ocular regenerative medicine. *In vitro* and *in vivo* biocompatibility and genotoxicity with different

types of human ocular cells were evaluated. rGO membranes allowed the growth of different ocular cells without inducing *in vitro* or *in vivo* toxicity or genotoxicity in the short-term. For *in vivo* biocompatibility studies, Wistar rats were divided in two groups (7 and 21 days) and subdivided in two subgroups: rats with or without rGOM (control group). A 1 cm incision was made on the back of the rats; then, rGOM (Ø = 0.5 cm) were implanted into subcutaneous tissue. The wounds were closed with 5-0 nylon non-absorbable sutures. Biocompatibility was also evaluated in 4 New Zealand white rabbits. Rabbits were divided in two groups. The first group were transplanted with intrastromal rGOM and the second group were transplanted with intrascleral rGOM. (Zambrano-Andazol *et al.*, 2020)

In the work by Guo *et al.*, the effects of rGO, carboxylated, hydroxylated and aminated graphene were evaluated on human neuroblastoma cells (SK-N-SH). All graphene materials inhibited cellular growth at concentrations of 0.1–10 mg/L after 24 h exposure. The toxicity was attenuated over longer exposure times, with the exception of aminated graphene. (Guo *et al.*, 2020)

In the work by Lee *et al.*, the interaction between rGO, carbon dots, carbon nanotubes and mesoporous carbon nanoparticles and amyloid-beta (Aβ) protein was studied. This protein could either activate or interrupt neuronal functions, depending on the dimension of the carbon material. Chronic exposure to carbon nanomaterials could induce neuronal cell death and accelerated the development of degenerative nerve diseases. It was found that the dimension of carbon nanoparticles was a critical factor controlling interactions between neuron cells and Aβ assembled structures, being carbon nanotubes and mesoporous carbon nanoparticles the most toxic among the series. (Lee *et al.*, 2021)

7.3.2 Ecotoxicity

Du *et al.* evaluated the impact of rGO on the microalgae Scenedesmus obliquus to determine its phytotoxicity. rGO treatment for 72 impaired the cellular morphology, inhibited algal growth and reduced chlorophyll a and chlorophyll b levels, due to increased oxidative stress. rGO significantly downregulated photosystem II activity due to the coating of the rGO on the algal cell surface. (Du *et al.*, 2016)

Hao *et al.* compared the toxicity of rGO, fullerene and MWCNTs on a mini ecosystem of rice grown in a loamy potted soil. The results indicate that different carbon nanomaterials resulted in environmental toxicity to rice and soil bacterial community in the rhizosphere. The results suggest that these materials and their incorporated products should be scrutinized to control their release/discharge into the environment to prevent their toxic effects on living organisms and the potential risks to food safety. (Hao *et al.*, 2018)

Yang *et al.* studied the influence of rGO on growth, structure and decomposition activity of white-rot fungus, whose decomposition function is vital for carbon cycle. rGO slightly stimulated the fresh weight and dry weight gains of Phanerochaete chrysosporium. rGO had low toxicity to white-rot fungus and was relatively safe for the carbon cycle. (Yang *et al.*, 2018)

Chen *et al.* investigated the translocation of two ^{13}C-labelled graphene materials in pea plants (Pisum sativum L.) and their effects on photosynthesis in leaves. After the chemical reduction of GO, rGO was translocated from roots into leaves and directly inhibited the activity of photosystem II by damaging the oxygen-evolving-complex on the donor side, due to oxidative stress. Chemical reduction of GO seems to play an important role in the translocation of graphene materials in plants, supporting potential risks of such materials to disrupt vitalmetabolic processes in carbon cycle. (L. Y. Chen *et al.*, 2019)

Forstner *et al.* investigated the effects of rGO, GO and CNTs at three time points (7, 14 and 30 days) and over a range of concentrations (1 ng, 1 µg and 1 mg/kg dry soil) on soil bacterial diversity. This study highlighted that these carbon materials can induce changes in soil bacterial diversity, at doses that are environmentally realistic. (Forstner *et al.*, 2019)

Yang *et al.* incubated rGO in the white rot fungus Phanerochaete chrysosporium culture system

for 4 weeks and investigated the transformation of rGO by multiple techniques. Results showed the efficient oxidation of rGO in P. chrysosporium culture systems. The fungal transformation of rGO was mainly due to the enzymatic oxidation and evidenced as the addition of oxygen atoms to the graphene skeleton. rGO wrapped in the fungal balls had higher transformation than those precipitated in the culture medium. (H. Yang *et al.*, 2019)

Hao *et al.* investigated the impact on the composition of the rice endophyte community, rice seedlings were exposed to rGO, MWCNTs and fullerene at 10–250 mg/L for 20 days. The results suggested that fungal endophytes in rice were sensitive to exposure to these carbon materials even at 10 mg/L. (Hao *et al.*, 2020)

Xu *et al.* studied the morphology-dependent toxicity of rGO, CB and SWCNTs in earthworms. The aspect ratio and hydrodynamic size of these three materials dictated the adverse effects on the body weights, antioxidant systems, coelomocytes, and non-targeted metabolomic profiles of the worms. In particular, it was observed that the effect sequence on multiple indicators could be ordered as SWCNTs ≥ rGO > CB, indicating that the morphology, including the aspect ratio and size, determined the carbon nanomaterial impact on the earthworms. No acute toxicity, but a significant weight loss, was found in the rGO and SWCNT treatments, perhaps due to metabolism interference. (Xu *et al.*, 2021)

The study of Ouyang *et al.* reported the stimulating effects of rGO on nitrogen-fixing bacterium Azotobacter chroococcum. rGO stimulated the cell growth at 10–500 μg/mL. rGO wrapped over A. chroococcum cells without inducing ultrastructural changes. rGO decreased the leakage of cell membrane, but slight oxidative stress was observed. rGO promoted the nitrogen fixation activity of A. chroococcum at 500 μg/mL. About 30% increase of organic nitrogen occurred at this dose of rGO. rGO might possibly benefit the plant growth through enhancing the indoleacetic acid production in A. chroococcum. These results highlighted the positive environmental effects of rGO to nitrogen-fixing bacteria in nitrogen cycle. (Ouyang *et al.*, 2022)

7.4 Graphene nanoplatelets and graphene nanoparticles

7.4.1 Toxicity

In the study of Singh *et al.* GNPs were chemically modified and compared to GO. GNPs functionalized with amine groups did not activate isolated human platelets and did not induce pulmonary thromboembolism in mice after intravenous administration. These amine-bearing GNPs did not cause hemolysis of isolated human red blood cells (RBCs) up to 10 μg/mL, whereas GO sheets caused RBC membrane rupture even at the lowest concentration (2 μg/mL). (Singh *et al.*, 2012)

Similarly, Sasidharan *et al.* reported a study where GNPs and acid-oxidized GNPs induced neither haemolysis nor activation and aggregation of platelets. (Sasidharan *et al.*, 2012)

Zanni *et al.* incubated C. elegans with large GNPs (average size 9 μm and 9 nm thickness), for 3 h at doses of 50, 100 and 250 μg/mL showing that their reproductive capability was unaffected, measuring the viability and the brood size. (Zanni *et al.*, 2012)

In the work of Schinwald *et al.* large GNPs (1−10 μm, 10 layers) were oropharyngeal instilled in female C57BL/6 strain mice to assess their pulmonary effects. At 1-day, large numbers of polymorphonuclear leucocytes, mainly neutrophils and eosinophils, were recruited into the lungs, and cytokine levels increased. Exposure to GNPs showed signs of frustrated phagocytosis and induced the formation of large granuloma (indicative of inflammation) at the surface of the pleural mesothelium and non-adhesive rosette-like cell/particle agglomerates in the pleural cavity. These results suggested that nanoplatelets could pose novel hazard concerns.(Schinwald *et al.*, 2012)

In another study, Schinwald *et al.*, using the same materials, observed no inflammation at 7 days or 6 weeks post-exposure following instead pharyngeal aspiration (50 μg per female C57BL/6 strain mouse). At these time points, there were no longer signs of inflammation in the

lungs. Similarly, there was no fibrosis despite the obvious persistence of large amounts of GNPs in the airways. Importantly, there was no sign of the materials in the pleural cavity (neither after 1 day nor after 6 weeks). These results indicated that GNPs were not able to translocate into the pleural space, thus preventing them from inducing granuloma on the pleural mesothelium, a hallmark of asbestos pathogenicity.(Schinwald *et al.*, 2014)

Kim *et al.* studied the pulmonary effects of GNPs in animals exposed via inhalation. In a first study, rats were exposed for 6 h per day for 5 days at 0.68 or 3.86 mg/m^3 of material (lateral size: 550 nm, thickness: 8 nm), resulting in deposited doses of 18 or 102 µg, respectively. Despite the observation of recruitment of macrophages, no effects on BAL cell composition or lactate dehydrogenase (LDH) release (indicative of lung damage) were seen at 1, 3, 7, or 28 days post-exposure. (Shin *et al.*, 2015) In a second study, rats were exposed to GNPs (lateral sizes up to 2 µm, 20−30 layers) for 6 h per day, 5 days per week, for 4 weeks, at 0.12, 0.47, and 1.88 mg/m^3, leading to an estimated deposited dose of 12, 50, and 198 µg, respectively. The animals were assessed at 1, 28, and 90 days after exposure. Inhaled materials were found in macrophages, but no signs of inflammation were noticed at any time points, regardless of the doses applied. Inhaled materials were also found in the mediastinal lymph nodes, suggesting translocation of materials from the airways to the lymphatic system. These results suggest that the 5-day repeated exposure to GNPs only had a minimal toxic effect at the concentrations and time points set in this study (Kim *et al.*, 2016)

Kucki *et al.* elucidated the influence of the physicochemical properties of different graphene materials (four GO and one GNP) on intestinal epithelial barrier, Caco-2 cell model was studied. All four GOs were noncytotoxic, while GNP displayed a low level of acute toxicity at high concentrations. It was also observed that aggregation, number of layers, or C/O ratio had a more pronounced effect on cell viability than the lateral dimensions. (Kucki *et al.*, 2016)

In another study, Park *et al.* observed the persistence of GNPs (average dimension: 325 nm; thickness: 3−4 nm) in the lungs of six-week-old male ICR mice up to 28 days after single bolus exposure, delivered using a 24-gauge catheter at a dose of 2.5 and 5 mg/kg by intratracheal instillation. (Park *et al.*, 2015) Despite the persistence of the materials, no lung lesions (*e.g.*, granuloma or fibrosis) were observed. In a follow-up study, five-week-old male ICR mice were intratracheally instilled with GNP (1.25, 2.5 or 5 mg/kg, 140–150 µL/mouse, 16 mice per group) using a 24-gauge catheter and assessed at day 90 after a single bolus exposure. There was an increase of the percentage of lymphocytes in bronchoalveolar lavage (BAL) fluid of the animals treated with the lowest dose (1.25 mg/kg) and an increase in the total number of apoptotic cells in the BAL fluid of animals treated with the highest dose (5 mg/kg). Elevated levels of cytokines and chemokines were also found 90 days after exposure in the high-dose-treated animals. Long persistence of GNPs in the lung may cause adverse effects by affecting immunological and physiological homeostasis. (Park *et al.*, 2017)

Lee *et al.* observed the translocation to mediastinal lymph nodes was observed after exposing animals to various types of pristine or functionalized GNPs by intratracheal instillation. GNPs were dispersed in saline, and 500 µL was instilled into the lungs of Wistar rats by intratracheal instillation at concentrations of 0.3, 1, and 3 mg/rat. Early inflammatory responses with recruitment of neutrophils were observed, more pronounced for the amino-functionalized GNPs.(Lee *et al.*, 2017)

Krajnak *et al.* examined how different forms of graphene could affect peripheral vascular functions, generate ROS and change gene expression, that may be indicative of cardiovascular and/or renal dysfunction. Three different sizes of GNPs and two oxidized forms of graphene were used *in vivo* and compared to CB as reference material. GNPs had particle dimensions of 1, 5 or 20 µm lateral size and thickness of 1–2, 7, and 7 nm, respectively. GO and rGO were derived from GNPs with 5 µm later size. Upon anesthesia, the mouth of male C57BL/6J mice was opened and tongue moved aside. A single 50 µL aliquot of media containing 40 µg of the non-oxidized particles (1, 5 or 20 µm graphite nanoplatelets) or CB was pipetted onto the base of the tongue. Alteration of gene expression were observed in the heart for GNPs and CB that may lead to cardiovascular dysfunction. (Krajnak *et al.*, 2019)

Svadlakova *et al.* compared two types of GNPs (different lateral size: 80-300 nm and 250-400 nm, respectively) and commercial MWCNTs to determine their proinflammatory potential. Neither GPNs nor MWCNTs induced a significant release of LDH even after 72 h incubation, while a slight decrease of mitochondrial potential was observed for all materials at concentrations above 30 µg/mL. This study demonstrates a possible proinflammatory potential of GNPs through activation of inflammasome. (Svadlakova *et al.*, 2020)

In another study, Malkova *et al.* exposed THP-1 cell line to three concentrations of two different GNPs over 40 h. The cytotoxicity was assessed by the measurement of LDH and ROS. The cytostatic, genotoxic potential and immunotoxicity were assessed by the measuring micronuclei and IL-6, IL-10 and TNF-α cytokines. Genotoxicity increased during the short-term *in vitro* exposure of THP-1 cells to two GNPs, while no increase in cytotoxicity, immunotoxicity, or cytostasis were observed. (Malkova *et al.*, 2021)

In the study by Bi *et al.*, the authors investigated the long-term pulmonary exposure model of graphene nanoplates and CB and discovered that long-term pulmonary exposure of the materials led to lung cancer metastasis and progression. Their findings elucidate the how the pulmonary cell deaths induced by GNPs reshaped the tumor microenvironment by releasing damage-associated molecular patterns and promoting tumor metastasis. *in vivo* studies were performed on female C57BL/6 or BALB/c mice that were exposed to two nanomaterials using a nose-only exposure system at a concentration of 18.36 mg/m^3 for 28 days.(Bi *et al.*, 2021)

7.4.2 Ecotoxicity

Baysal *et al.* evaluated the behavior of GNPs and MWCNTs in environmental media (sea water, soil, and airborne fine particulate) by looking at the influence of nanomaterial physicochemical properties (size, zeta potential, surface chemistry, morphology and sedimentation) on the viability of Gram-positive and Gram-negative bacteria. The results indicated that the toxicity depended on the type of environmental media, the concentration of the materials, and their physicochemical properties. (Baysal *et al.* 2018)

Rodd *et al.* evaluated how GNPs and CB nanoparticles influenced particle morphology and surface properties on adsorption and bioavailability of benzo(a)pyrene, a model aromatic hydrocarbon, to brine shrimp Artemia franciscana and PLHC-1 fish liver cells. Acellular adsorption studies showed that GNPs adsorbed more benzo(a)pyrene than CB with comparable surface area. The graphene-based materials mitigated benzo(a)pyrene uptake into Artemia franciscana without causing damage to the gut lining or altering benzo(a)pyrene distribution within larvae. In the PLHC-1 fish liver cell line, GNPs significantly reduced the expression of Cyp1a, a biomarker of the cellular response to benzo(a)pyrene exposure. (Rodd *et al.*, 2018)

7.5 Graphene nanoribbons

Zakharova *et al.* reviewed the studies on GNRs, as components of devices and therapeutic agents. The limiting factors for the use of GNRs in biomedicine are their high hydrophobicity and insufficiently data on toxicity. There is a lack of information on the effect of GNRs on the whole organism using *in vivo* experiments, as well as on environmental toxicity, accumulation, migration, and destruction within ecosystems. There is evidence of good biocompatibility of GNRs on human cell lines, but toxic effects, including cytotoxicity and genotoxicity were evidenced in some studies. The possible mechanisms of toxicity are attributed to the tendency of GNRs to damage the cell membrane mechanically, stimulate ROS production, autophagy, and inhibition of proliferation, as well as apoptosis induction, DNA fragmentation, and the formation of chromosomal aberrations.(Zakharova *et al.*, 2021)

It is worth to note that no studies on the environmental effects of GNRs have been reported so far.

7.6 MXenes

7.6.1 Toxicity

Lim *et al.* reviewed the types of MXene applied in biomedical sciences, covering cytotoxicity and strategies for its mitigation, and giving a future outlook. (Gim Pao Lim *et al.*, 2021)

Jastrzębska *et al.* covered the *in vitro* toxicity of 2D sheets of Ti_3C_2 MXene where the biological activity of this MXene was determined on two normal (MRC-5 and HaCaT) and two cancerous (A549 and A375) cell lines. The results indicated that the reduction of cell viability was higher in cancerous cells compared to normal ones. Results also indicated oxidative stress phenomena as the potential mechanisms of toxicity. (Jastrzębska *et al.*, 2017)

Scheibe *et al.* investigated a series of multi-, few-, and single-layered $Ti_3C_2T_x$, TiC, Ti_2AlC, and Ti_3AlC_2 in terms of cytotoxicity, membrane permeability, reactive oxygen stress, and mechanical stress, using human fibroblasts (MSU1.1) and cervical cancer cells (HeLa). The analyses revealed that incubation with high concentrations (≥400 µg/mL) of TiC, Ti_2AlC, and Ti_3AlC2_2 particles with the sizes <44 µm induced a significant cytotoxic effect via oxidative and mechanical stress. All the Ti_3C2T_x forms were safe for MSU1.1 cells with only slight cytotoxic behaviour at the highest concentration. The cytotoxic behaviour was also cell-type dependent, with higher cytotoxicity observed for cancer cells. (Scheibe *et al.*, 2019)

Szuplewska *et al.* evaluated the biocompatibility of Ti_2NT_x MXene on human skin malignant melanoma cells, human keratinocytes, human breast cancer cells, and normal human mammary epithelial cells. The multilayered 2D sheets of Ti_2NT_x show higher toxicity towards cancer cell lines (MCF-7and A365) in comparison to normal ones (MCF-10A and HaCaT). The decrease of cell viability was dose dependent. The identified mechanisms of toxicity were the generation of ROS following cellular internalization of the materials. (Szuplewska *et al.*, 2019)

The study by Wu *et al.* evaluated the cytotoxicity of Ti_3C_2 using primary neural stem cells (NSCs) and NSCs-derived differentiated cells. Ti_3C_2 induced a dose-dependent cytotoxicity to both type of cell lines with no observed effects at 12.5 µg/mL Ti_3C_2. However, results showed that Ti_3C_2 at a concentration of 25 µg/mL induced significant level of apoptosis and cell membrane disruption. (W. Wu *et al.*, 2020)

Alhussain *et al.* aimed to assess the potential toxicity of Ti_3C2T_x MXene nanosheets on the early stage of the embryo as well as angiogenesis. Avian embryos at 3 and 5 days of incubation were used. The study results revealed that $Ti_3C_2T_x$ MXene may produce adverse effect on the early stage of embryogenesis as ~46% of MXene-exposed embryos died during 1–5 days after exposure. The authors also found that MXene at tested concentration inhibits angiogenesis of the chorioallantoic membrane of the embryo after 5 days of incubation. More significantly, RT-PCR analysis of seven genes, which are key regulators of cell proliferation, survival, cell death and angiogenesis, revealed that these genes were deregulated in brain, heart and liver tissues from MXene-treated embryos in comparison with controls. Overall, this study suggests that Ti_3C2T_x MXene at studied concentration might induce a toxic effect on the early stage of embryogenesis. (Alhussain *et al.*, 2020).

In another study, Lim *et al.* studied the cytotoxicity of Ti_2CT_x MXene on HeLa cells as 3D spheroids in comparison to 2D cell culture system. The biological results with both cell systems indicated that the Ti_2CT_x MXene was moderately cytotoxic in a dose dependent manner. (G P Lim *et al.*, 2021)

A study from Jastrzębska *et al.* focused on the oxidation-state-related *in vitro* cytotoxicity of V2CTz MXene onto immortalized keratinocytes (HaCaT) and malignant melanoma (A375) human cell lines. The oxidation of V2CTz highly increased their cytotoxicity on both cell lines, also in a time and dose dependency. The identified mode of cytotoxic action related to the cell cycle and cellular membrane disintegration through direct physicochemical interactions. (Jastrzębska *et al.*, 2021)

7.6.2 Ecotoxicity

Nasrallah *et al.* investigated the biocompatibility of Ti_3C2T_x by analysing its potential toxicity in zebrafish embryos and the aggregation patterns of Ti_3C2T_x suspensions in seawater. The acute toxicity of Ti_3C2T_x was tested at concentrations of 25, 50, 100 and 200 µg/mL. After 96 h treatment, the concentration of of Ti_3C2T_x killing the 50% of embryos (LC50) was calculated to be 257.46 µg/mL and the highest no observed effect concentration (NOEC; <20% mortality) was 50 µg/mL. The lowest observed effect concentration (LOEC) (≥20% mortality) of Ti_3C2T_x was measured to be 100 µg/mL, as this concentration showed a slight increase in mortality (21%). No significant teratogenic effects were observed on zebrafish embryos at 100 µg/mL. This absence of toxic effects was confirmed by locomotion and neurotoxicity assays, as 50 µg/mL of Ti_3C2T_x showed no harmful effects on neuromuscular activities. Because the LC50 of Ti_3C2T_x was greater than 100 µg/mL, this material can be classified within the "practically nontoxic" group according to the Acute Toxicity Rating Scale by the Fish and Wildlife Service. (Nasrallah *et al.*, 2018).

7.7 2D boron nitride

Liu *et al.* systematically investigated the cytotoxicity of boron nitride (BN) on human hepatoma HepG2 cells at different toxic end points. Results showed that BN decreased cell viability at 30 µg/mL and induced adverse effects on intracellular ROS generation (≥2 µg/mL), mitochondrial depolarization (≥4 µg/mL), and membrane integrity (≥2 µg/mL for BN). (Liu *et al.*, 2017)

Lin *et al.* reviewed the impact of 2D materials including hexagonal boron nitride (hBN) on macrophages. Few studies involving boron compounds have been conducted on this type of cells. These studies have evidenced that boron was nontoxic, likely due to its suppression of phagocytosis, although it was shown to induce inflammatory responses, which could be indirectly linked to its inhibitory effects on cellular uptake. The review suggests extending the investigation of the effects of 2D hBN on macrophages and other immune cells, as its use is rapidly increasing. (Lin, Song and Bianco, 2021).

Another study by Kar *et al.* investigated the dose-dependent biological effect of hBN nanoparticles *in vivo*. Male Wistar albino rats were injected intravenously from the jugular vein with hBN nanoparticles at concentrations of 50, 100, 200, 400, 800, 1600, 3200 µg/kg body weight. Results from this study showed no significant changes in the hematological and biochemical parameters for low doses, except for the 1.6 and 3.2 mg/kg b.w. doses. Histological analyses indicated that these two concentrations of hBN could induce significant damage in the liver, kidney, heart, spleen and pancreas. (Kar, Hacioglu, *et al.*, 2021)

In another study by Kar *et al.*, hBN at concentrations varying between 0.05–3.2 mg/kg were administered by intravenous injection to Wistar albino rats. Blood and tissue samples were taken after 24 h. The results revealed that hBN induced oxidative stress in a dose-dependent manner by modulating sulphur homeostasis at the highest concentrations. (Kar, Söğüt, *et al.*, 2021)

Lucherelli *et al.* studied the cytotoxic effect of two types of hBN, one with sharp cornered edges and one made of nanosheets characterized by a round form, on epithelial lung cells. Molecular dynamic simulations revealed that the sharp hBN could penetrate a lipid bilayer and form a cross-membrane water channel along its exposed polar edges, while the round hBN sheet did not. This water channel could facilitate molecule and ion cross-membrane transport, leading to lysosomal membrane permeabilization, with release of cathepsin B and generation of radical oxygen species. Following this behaviour, the cornered hBN edges provoked a dose-dependent apoptosis (up to 80 µg/mL tested doses) on the epithelial cells, whereas cytotoxic effects of round hBN were negligible. (Lucherelli *et al.*, 2021)

It is worth to report that no studies on the environmental effects of BN have been reported so far.

7.8 Transition metal dichalcogenides

7.8.1 Toxicity

In one study by Teo *et al.* on A549 cells, MoS_2 and WS_2 exhibited low cytotoxicity to this type of cells, even at the highest concentration (400 µg/mL). WSe_2 nanosheets showed instead dose-dependent cytotoxic effects, with reduced cell viability to 31.8% at the highest concentration likely due to the type of chalcogen. Compared to graphene oxides and halogenated graphenes, MoS_2 and WS_2 were much less toxic, whereas WSe_2 exhibited a similar degree of cytotoxicity to graphene materials (Teo *et al.*, 2014).

In another work by Chng *et al.*, it was found that the number of layers of exfoliated MoS_2 have an influence on its toxicity. It was demonstrated that MoS_2 that was exfoliated to a greater extent had increased cytotoxicity, possibly be due to an increase in surface area and active edge sites. (Chng, Sofer and Pumera, 2014)

In another work by Wang *et al.*, aggregated MoS_2 was shown to induce strong pro-inflammatory responses (increasing IL-8, TNF and IL-1b in THP-1 cells) and pro-fibrogenic responses, suggesting that exfoliation would decrease its toxicity (Wang, Mansukhani, *et al.*, 2015)

In the study by Corazzari *et al.*, MoS_2 did not exert any significant cytotoxic effect on A549 cells at low and intermediate doses (10 and 25 µg/cm^2 of cell culture), inducing a significant increase in the extracellular lactate dehydrogenase activity (indicative of membrane permeabilization) only at a high dose (50 µg/cm^2 of cell culture). (Corazzari *et al.*, 2014)

Shah *et al.* studied the cytotoxicity of the MoS_2 nanosheets on rat pheochromocytoma cells (PC12) and rat adrenal medulla endothelial cells (RAMEC). The results indicated that the MoS2 nanosheets were safe to these cells. (Shah *et al.*, 2015)

In the study of Wu *et al.*, metabolomics analysis was applied to explore the effects of different concentrations of MoS_2 nanosheets on Escherichia coli. The results showed that increasing the concentrations of this material, the survival rate of these bacteria decreased. (N. Wu *et al.*, 2016)

Liu *et al.* investigated the cytotoxicity of MoS_2 on human hepatoma HepG2 cells analysing different toxic end points. The results showed that MoS_2 nanomaterial decreased cell viability at 30 µg/mL and induced adverse effects on intracellular ROS generation (≥2 µg/mL), mitochondrial depolarization (≥4 µg/mL), and membrane integrity (≥8 µg/mL). (Liu *et al.*, 2017)

In another study by Chen *et al.*, MoS_2 were prepared via direct sulfurization of molybdenum thin film on quartz plate, avoiding possible contamination deriving from other chemicals and allowing a direct assessment of their toxicity. Six different types of cells, including normal, cancer, and immortalized cells, were cultured with dispersed MoS_2 microparticles, evaluating their viability level. Allergy tests on skin of guinea pigs were also conducted. The results on co-cultured cells using different concentrations of dispersed MoS_2 microparticles showed that these microparticles induced no toxic effects at concentrations up to of 16 µg/mL. The patch testing on guinea pigs further confirmed that there was little adverse effect of MoS_2 thin film and microparticles on the skin of these animals. (W. B. Chen *et al.*, 2018)

In the study by Kaur *et al.*, few-layered and defect-free MoS_2 nanosheets, exfoliated and dispersed in pure water, were found to be stable up to three weeks. The impact of this material on two cancer cells (breast cancer, MCF7, and leukemia U937), and normal epithelial cells (HaCaT) was assessed. It was observed that MoS_2 exhibited significant reduction of cell viability in the tumor cell lines, but no effects were observed in normal cells. (Kaur *et al.*, 2018)

In the study by Wu *et al.*, C57BL/6 mice were feed with nano-MoS_2 and micro-MoS_2 (nano-MoS_2: diameter: 0.02–1 µm; thickness: 1–10 nm; micro-MoS_2: size: ~1.5 µm) via food premixed with 15 and 150 mg of each MoS_2 per kg of meal for 90 days. The results showed that nano- MoS_2 and micro- MoS_2 exposure induced Mo accumulation in the organs of the mice, especially in the

small and in the large intestine. Additionally, both types of MoS_2 changed the intestinal microbiota, especially in the large intestine. The results of this study led to the conclusion that micro-MoS2 changed the metabolic profiles of the intestine by changing the microbial community, while the changes caused by nano- MoS_2 were due to a combination of change in the microbial community and direct toxicity. (Wu *et al.*, 2019)

In the study by Lin *et al.*, viability and activation of M1 and M2 macrophage were not affected by MoS_2 at doses up to 50 µg/mL. MoS_2 was little toxic in human macrophages even though it was found to trigger cell stress and inflammatory responses. (Lin *et al.*, 2020)

In the study by Li *et al.* the interactions between 1T and 2H crystal phases of monolayer MoS2 and zebrafish embryos were studied upon exposure to nanosheets at concentrations of 0.1, 0.01, and 0.001 mg/L. Compared to 1 T- MoS_2, 2H- MoS_2 penetrated embryos easier, and caused higher developmental toxicity. Metabolic pathways related to amino acid and protein biosynthesis and energy metabolism were affected by the nanomaterial surface atomic arrangements. (K. Li *et al.*, 2021)

7.8.2 Ecotoxicity

Yuan *et al.* prepared chemically exfoliated WS2 (Ce-WS2, mainly present in 1T phase) and annealed exfoliated WS2 (Ae-WS2, 2H phase). Ce-WS2 showed higher levels of cellular uptake, oxidative stress, lipid peroxidation, membrane damage, and inhibition of photosynthesis than Ae-WS2 in Chlorella vulgaris. Metabolomics analysis revealed that Ce-WS2 induced more alterations in metabolites and metabolic pathways than Ae-WS2. These alterations correlated with cell membrane damage, oxidative stress and photosynthesis inhibition. (Yuan, Zhou and Hu, 2018)

In another study, Chen *et al.* evaluated the biological effects of MoS2 on a N2-fixation cyanobacteria by monitoring growth and metabolome changes. MoS2 did not exert evident toxicity to these bacteria at the two tested doses (0.1 and 1 mg/L). On the contrary, the intrinsic enzyme-like activities and semiconducting properties of MoS2 promoted bacterial metabolic processes, including enhancing CO_2-fixation-related Calvin cycle metabolic pathway. (Chen *et al.*, 2021)

Domi *et al.* analyzed the cytotoxicity effects of commercially available aqueous dispersions of monolayer WS_2. A549 cells and the ecotoxicology model Saccharomyces cerevisiae were selected. While WS_2 suspensions showed very low toxicity towards A549 cells, a comparable concentration (160 mg/L^{-1}) reduced the viability of yeast. The toxicity of a nanosized WS_2 commercialized in dry form from the same provider was also assessed, showing the ability to reduce again the viability of yeast. (Domi *et al.*, 2021)

In the study by Luo *et al.*, the effect of MoS2 on the physiological index, subcellular morphology, and transcriptomic profile of the marine microalgae Dunaliella salina was investigated. Exposure to MoS_2 (50 mg/mL) caused improved the cell activity, the chlorophyll content, the primary metabolites, and the activity of antioxidant enzymes, without remarkable alteration of the subcellular morphology. ROS level was augmented within MoS_2-exposed algae simultaneously, but the activity of antioxidant enzymes precluded impaired cell growth and photosynthesis. This "doping-like" effects of MoS_2 on microalgae might influence rudimentary microalgae composition of marine ecosystems or facilitate the outbreaks of red tide associated with burgeoning MoS2 emissions in marine environments. (Luo *et al.*, 2021)

In the study by Sharma *et al.* the uptake, bioaccumulation, and impact of α-MoO_3 and MoS_2 were studied in rice (Oryza sativa L) cv. HUR 3022. Seedlings were exposed to 100, 500, and 1000 ppm concentrations for 10 days in the growth medium. The results indicated that a 100 ppm of MoS_2 had low translocation level and less accumulation with no significant impact on growth of rice cv. HUR 3022 seedlings and appeared to be environmentally safe for future applications. (Sharma, Raghubanshi and Shah, 2021)

In another study by Zeng *et al.*, the effects of single-layer MoS_2 in a nano-colloidal form were

compared to the treatment of the same material with humic acid on environmental transformation and ecotoxicity. MoS_2 nanocolloids induced serious damage (cell distortion and deformation), MoS_2 internalization, and metabolic perturbation on C. vulgaris. In contrast, the addition of humic acid induced the growth promotion and lower ROS level, inhibited the internalization of MoS_2, and mitigated metabolic perturbation on C. vulgaris. (Zeng *et al.*, 2021)

In the work by Zhao *et al.* studied the DNA cleavage activity induced by Ce-MoS_2 nanosheets. Results showed that Ce-MoS_2 can induce DNA cleavage in an aqueous solution also in the dark environment, making it different from graphene-based nanomaterials. The authors showed that DNA cleavage was enhanced under higher pH values due to more amount of ROS generation. The study provided insights into the potential environmental risk posed by ce-MoS_2 in the aquatic environment through the induction of DNA cleavage. (Zhao, Xu and Jiang, 2021)

The work by Zou *et al.* revealed that extracellular polymeric substances (EPS) of freshwater algae can significantly change the properties and toxicity of MoS_2 to aquatic fish. The morphological and structural alterations after EPS binding to MoS_2 alleviated its toxicity (*e.g.*, malformation and oxidative stress) to infantile zebrafish. (W Zou *et al.*, 2021)

In their work Zou *et al.* discovered that surface vacancies decreased the colloidal stability and promoted free radical generation and dissolution of single layer 2H-MoS_2 in aqueous solution. In addition, surface vacancies provided specific edge sites of MoS2 for molecular binding. Compared to the pristine form, surface-vacant 2H-MoS_2 exhibited stronger affinity to proteins containing thiol groups, which play key roles in the life process of algae. Importantly, the increased capacity of free radical generation and specific interactions with proteins after surface vacancy formation aggravated algal responses by 2H-MoS_2. (Wei Zou *et al.*, 2021)

In their work, Zhao *et al.* pointed out that 1T phase MoS_2 is unstable in the environment and is easily oxidized leading to release of Mo ions. The high dissolution rate resulted in greater bioavailability for exposed plants. The bioaccumulated Mo in rice did not induce over phytotoxicity regardless of phase or dose. From the perspective of metabolism, Mo has a positive effect on the growth, particularly stress resistance, as Mo triggered up-regulation of a number of metabolites associated with stress tolerance. In addition, 2H phase of MoS_2 significantly increased the relative abundance of N2-fixation cyanobacteria and plant growth-promoting rhizobacteria Bacillus. (Zhao *et al.*, 2022)

7.9 Black phosphorus

7.9.1 Toxicity

In the study by Song *et al*., the cytotoxic effects of layered BP on cell metabolic and membrane integrity were investigated. The dose- and time-dependent cytotoxicity of BP were assessed against L-929 fibroblasts. The findings indicate that the cytotoxicity of BP is proportionally dependent on their concentration and exposure time, which is affected by the oxidative stress-mediated enzyme activity reduction and membrane disruption.(Song *et al.*, 2018)

In the work by Tan *et al.* toxicological effects of BP were found to be dose-dependent on human lung carcinoma epithelial cells. Using two different assays, a 48% and 34% reduction in cell viability was observed at a concentration of 50 µg/mL. However, below 4 µg/mL of BP with average lateral size of at 920 nm, L-929 fibroblasts maintained viability of 82% after a 24 h treatment. (Tan *et al.*, 2019)

BP was also found to have size- and cell-line-dependent effects. Zhang *et al.* investigated layered BP of lateral sizes of approximately 884 nm, 426 nm, 209 and 92 nm, 27 nm and thickness of 17 nm. It was found that the largest flakes were the most cytotoxic, being 293T cells the most sensitive to treatment, followed by NIH3T3 and HCoEpiC. The main causes of toxicity were attributed to ROS generation and loss of cell membrane integrity. (X. J. Zhang *et al.*, 2017; Tan *et al.*, 2019)

Latiff *et al.* studied the toxicity of different types of BPs. Black phosphorus was more toxic than

the red and violet forms. The degree of exfoliation and oxidation extent turned out to be more significant than the synthesis process in affecting cell viability. However, thinner sheets produced by vapor growth were more toxic than thicker sheets by high pressure conversion and higher toxicity was evidenced when BPs were more oxidized. (Latiff *et al.*, 2018; Tan *et al.*, 2019)

7.9.2 Ecotoxicity

In their work, Xiong *et al.* evaluated the bacterial toxicity of exfoliated BP against Gram-negative E. coli and Gram-positive B. subtilis. Time- and concentration-dependent bacterial toxicity profile was observed. Bacterial toxicity against E. coli decreased over time due to membrane self-healing. ROS generation and membrane damage were the main bactericidal mechanisms. This study indicates the potential environmental risk of BP. (Xiong *et al.*, 2018)

Li *et al.* carried out a series of toxicity tests on BP using Chlorella vulgaris. After 120 h exposure, BP at 1 mg/L promoted the growth of C. vulgaris, while BP at higher concentrations (5 and 10 mg/L) inhibited its growth .(Li *et al.*, 2020)

7.10 Graphitic carbon nitride

The research on the biosafety of graphitic carbon nitride (g-C_3N_4) based materials is still at a very early stage. Only preliminary *in vitro* and *in vivo* assessments have been performed so far, therefore requiring additional and extensive evaluation for assessing their safety profile. Toxicological effects of graphitic carbon nitride have been reviewed by Liao *et al.* (Liao *et al.*, 2020)

In the study by Ramezani *et al.*, the effects of sulfur-doped g-C_3N_4 on cognitive function and histopathology of hippocampus were investigated in mice. Male NMRI mice were orally treated by g-C_3N_4 at doses 50, 150 or 500 mg/kg for one week. Histological evaluations showed an increased level of neuronal loss and glial activation in the hippocampus of g-C_3N_4 treated mice at doses of 150 and 500 mg/kg. The data indicated that g-C_3N_4 induced the cognitive impairment that was partly mediated via its exacerbating impacts on neuronal loss and glial activation.(Ramezani *et al.*, 2021)

It is worth to note that no studies on the environmental effects of g-C_3N_4 has been reported so far.

8. Conclusions and recommendations

Some of the following conclusions and recommendations can be applied to all types of graphene and 2D materials, while others can be considered specific to a single class of materials.

All 2D materials

The ever-increasing library of 2D materials being discovered and potentially applied in a myriad of ways and applications needs to be systematically assessed for their impact on health and environment. Therefore, it is advisable that a proper standardization of the synthesis, a thorough characterization of the physicochemical properties of the materials, and a proper testing of materials are performed, to enable appropriate comparison across the literature, as well as protocols to be applied for risk assessment to identify potential hazards under probable contexts of exposure.

It should be noted that many documents and reports have a lot of flaws in term of material definitions and characterizations. It is recommended to look if the biological studies provide a precise description of the material tested as the biological results are relevant only if they can be correlated to precise physicochemical properties of the tested materials.

The studies on the health and the environmental impact involving 2D materials beyond graphene are still very limited. Some of these materials have not been tested in an environmental context yet. On the basis of the available results, often the parameters to measure cell viability are not homogeneous and therefore it is difficult to compare the results. A lack of systematic and detailed material characterizations, consistent design of toxicological and ecotoxicological assays, and mechanistic understanding of the toxic responses, lead to contradictory results in terms of environmental, animal and human impact of many 2D materials. The difficulties of comparing the results stem from the diversity of the models used for the experiments and the readouts. Increasing the number of studies and following recommended OECD (Organisation for Economic Co-operation and Development) test guidelines and ISO (International Organization for Standardization) standards for nanomaterials will allow to cover the current gaps. More studies on ecotoxicity are strongly recommended.

Graphene-based materials

Although the toxicity of graphene-based materials has been studied for more than ten years, and as a consequence a sound number of reports is available in literature, it is worth to note that it is still difficult to compare the toxicological effects between the different studies due to diversity in size, shape, surface chemistry and modification, preparation protocols and definition of the concentration descriptors (e.g. mass/volume, specific area/volume, etc.), cell models and organisms tested. It is evident that the toxicity of graphene oxide (GO) depends on its size, synthesis methods, routes of administration, dose and time of exposure. More studies on both toxicity and ecotoxicity using validated OECD test guidelines and ISO documentary standards are needed.

In vitro and *in vivo* studies on toxicity of graphene materials have revealed that the toxic effects are induced by the extent of material aggregation; the mode of interaction of graphene with the cells; the density of graphene (*i.e.*, graphene nanoplatelets are more toxic); the size, particulate state, oxygen content, or surface charge of graphene; the nature of cells (*i.e.* the same dose of GO is more toxic to fibroblasts than to epithelial cells).

ROS-mediated cellular damage has been postulated as a primary mechanism of GO cytotoxicity. GO can be taken up by immune competent cells like macrophages, leading to reduced cell viability, and affecting cytokine expression, all these effects being influenced again by factors, such as lateral size, surface charge, surface radicals, dispersibility. Additional studies on macrophage polarization (leading to distinct functional phenotypes) will allow to better understand the possible acute or chronic inflammatory risks of GO and other graphene materials.

The studies focused on lungs have evidenced that the extent of pulmonary impact is directly correlated to the specific physicochemical properties of the tested GO. Dimensions (e.g. lateral sizes above several microns) seem once again to be an essential driver of the biological response. Only few studies have reported the induction of fibrosis, a hallmark of lung damage, after pulmonary exposure to GO and graphene nanoplatelets (GNPs). The lack of pulmonary fibrosis is an important difference when comparing GO and GNPs with pathogenic multi-walled carbon nanotubes (*i.e.*, those classified by IARC as potential human carcinogens). However, further systematic investigations looking at long-term impact of graphene-based materials including subacute, subchronic and chronic repeated-dose toxicity studies are warranted to fully address this issue.

It should be noted that, most often, all toxic effects are dose dependent. Considering the available results, when health risks are identified and reported for a specific graphene material, doses and exposure scenarios should be taken into consideration during their manipulation and when it is envisaged for a targeted use.

Studies on the environmental impact of graphene-based materials should pay attention to the risks in specific scenarios such as surface water and soil near the point sources. Adverse effects on aquatic organisms should not be overlooked prior to large-scale applications of graphene-based materials, and further investigations on joint (human/eco) toxicity can provide greater beneficial insight into realistic exposure scenarios. Remaining gaps include classifying the hazards associated with human/environmental exposure, fully assessing the life cycle of graphene materials, and developing a consensus on biosafety in compliance with the regulatory guidelines.

In summary, the following conclusions and recommendations have been formulated:

CONCLUSION 1.
It is mandatory to provide in any study a thorough characterization of each type of graphene and 2D material in terms of chemical composition, structure, lateral size, number of layers, in order to link any identified concern during use and disposal with the particular characteristic of the material. Not all graphene materials are graphene alike.

RECOMMENDATION 1. The application of the definitions and available documentary standards should allow to clearly identify the type of graphene and 2D materials used for the different applications and to evidence potential toxicity issues and risks.

CONCLUSION 2.
There is a strong need for specific and multiple analytical and spectroscopic methods for the detection and quantification of graphene and related carbon nanomaterials in biological and environmental matrices.

RECOMMENDATION 2. Multiple characterization techniques should be applied to clearly identify and quantify graphene materials in cells, tissues, organs and the environment.

CONCLUSION 3. Evidenced human and environmental toxic effects of graphene and 2D materials depend on their physicochemical characteristics.

RECOMMENDATION 3. Conclusions on toxicity and ecotoxicity should not be generalized and need to be associated to a precise description of the material used in the tests.

CONCLUSION 4. Cytotoxic effects have been identified for specific graphene and 2D materials both on health and environment, mainly dose dependent.

RECOMMENDATION 4. When health and environmental risks are reported or identified for a specific graphene or 2D material, doses and exposure scenarios should be considered for their manipulation and use.

CONCLUSION 5. Long-term/chronic studies are still limited, particularly for *in vivo* and repeated-dose administrations.

RECOMMENDATION 5. To assess chronic toxicity of graphene and 2D materials protocols for repeated-dose studies should be considered.

CONCLUSION 6. Studies using immune depressed or diseased models are still lacking, particularly for *in vivo* and repeated-dose administrations.

RECOMMENDATION 6. To assess potential toxicity of graphene and 2D materials relevant immune suppressed or diseased animal models should be considered.

CONCLUSION 7. Studies on genotoxicity of graphene and 2D materials are still very limited.

RECOMMENDATION 7.
To assess the potential genotoxic risks, reliable testing methods should be developed; response mechanisms associated with genotoxicity should be evaluated in depth; appropriate description of the type of graphene and 2D material tested should be reported; and different dosages and exposure times should be applied.

CONCLUSION 8. Toxicity studies of chemically exfoliated graphene and 2D materials lack of appropriate controls.

RECOMMENDATION 8. The solvents and the molecules used to exfoliate bulk materials into single- or few-layer graphene or 2D materials might remain as residues in the end-product, likely affecting the (eco)toxicity results. It is recommended to consider and include these potential impurities in the tests to exclude their implication and responsibility on (eco)toxicity.

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Appendix 1. Full list of relevant documents and main conclusions

2D material	Endpoint	Main Conclusions (from abstract or conclusion sections)	Reference
graphene oxide	ecotoxicity	The 96-hour median effective concentration (EC50) of GO and GOQDs were determined to be 49.32 and 22.46 mg/L, respectively. Both GO and GOQDs were internalized by heteroagglomeration and envelopment processes, with GOQDs inducing stronger upregulation of cell permeability, plasmolysis and lipid bodies than GO. Cracking of thylakoid layers, disappearance of nucleoid, and disintegration of cell infrastructure were observed at higher concentrations. In comparison to GO, GOQDs induced higher reactive oxygen species (ROS) and malondialdehyde (MDA) and disrupted antioxidant enzymes, leading to the inhibition of cellular contents such as chlorophyll a and proteins. Furthermore, both GO and GOQDs adsorbed nutrients from the algal medium, resulting in nutrient depletion-induced indirect toxicity, with GOQDs depleting more nutrients than GO. The current study provides new understanding of nanotoxicity of GO and GOQD and aids in the potential risks of nanomaterials in aquatic environments.	Yan, Z., Yang, X., Lynch, I. & Cui, F. Comparative evaluation of the mechanisms of toxicity of graphene oxide and graphene oxide quantum dots to blue-green algae Microcystis aeruginosa in the aquatic environment. *Journal of hazardous materials* 425, 127898–127898 (2022).
MoS2 nanosheets	ecotoxicity	The concentration of added MoS2 nanosheets (100 mg kg−1 in soil) is likely significantly greater than under realistic environmental exposure. This study provides important insight on the physiochemical properties, dissolution, phytoaccumulation, and phytotoxicity of MoS2 nanomaterials. metallic 1T phase MoS2 nanosheets are unstable in the environment and are easily oxidized and release Mo ions into the environment. The high dissolution rate result in greater bioavailability for exposed plants. the bioaccumulated Mo in rice did not induce over phytotoxicity regardless of phase or dose. From the perspective of metabolism, Mo has a positive effect on the growth, particularly stress resistance, as Mo triggered up-regulation of a number of metabolites associated with stress tolerance, *e.g.*, proline, GABA. Last, 2H phase of MoS2 nanosheets significantly increased relative abundance of N2-fixtion cyanobacteria and Bacillus (PGPR).	Zhao, L. *et al.* Environmental implications of MoS2 nanosheets on rice and associated soil microbial communities. *Chemosphere* 291, 133004–133004 (2022).

graphene oxide	ecotoxicity	This work investigated 120-day interaction between GO (500 and 5000 μg/L) and constructed wetlands (CWs) planted with Iris pseudacorus. CWs showed the effective retention for GO via mature biofilm but less biodegradation. GO significantly induced enzyme activities, which was attributed to increases in ecological association and enzyme abundance. GO decreased microbial biomass on day 30, but it had no impacts on day 120. The microbial community showed gradual self-adaption with time due to protection of antioxidant defense system (L-ascorbate oxidase, superoxide reductase, and glutathione related enzyme). The antioxidant enzymes and lipid peroxidation of Iris pseudacorus were increased by GO, accompanied by reduction on chlorophyll biosynthesis. Overall, the separate effects of GO on micro-regions and individual bodies in CWs were obvious, but it was acceptable that variations in pollutant removal were not evident due to synergetic role of plant-substrate-microbe. Organic matter and phosphorus removals reached to above 93%, and ammonia and total nitrogen removals in GO groups were reduced by 7–8% and 9–13%, respectively.	Yan, C. N. *et al.* Response of constructed wetland for wastewater treatment to graphene oxide: Perspectives on plant and microbe. *JOURNAL OF HAZARDOUS MATERIALS* 422, (2022).
multi-layer graphenes	toxicity	The main purpose of this study was to assess the acute toxicity, histopathological and behavioural changes caused by the exposure of ZnO NPs and multi-layer graphenes (MLGs), alone and combined, to the blackfish Capoeta fusca. The estimated mean 96 h-LC50 for ZnO NPs was 4.9 mg L−1 and 68.4 mg L−1 for MLGs. In combination, MLGs increased the acute toxicity of the ZnO NPs. The effects of the different NPs on the gills included hyperplasia, aneurisms, and fusion of the lamellae. In the intestine, exposure to the NPs resulted in an increase in the number and swelling of goblet cells and tissue degeneration. Loss of balance, restlessness, erratic and abnormal swimming patterns were the most common behavioural changes seen in the ZnO NPs' exposed blackfish. In contrast with the acute toxicity findings, MLGs decreased the histopathological and behavioural effects of the ZnO NPs on both gills and intestinal tissues as well as fish behaviour.	Sayadi, M. H. *et al.* Co-exposure of zinc oxide nanoparticles and multi-layer graphenes in blackfish (Capoeta fusca): evaluation of lethal, behavioural, and histopathological effects. Ecotoxicology (London, England) (2022) doi:10.1007/s10646-022-02521-x.
graphene oxide nanosheets	ecotoxicity	This study revealed that the presence of GO nanosheets, even at the lower levels tested, impaired behavioural, physiological, and biochemical traits in polychaetes, suggesting that the increase of this engineered nanomaterial in the environment can disturb these benthic organisms, affecting the H. diversicolor population. Moreover, given the important role of this group of organisms in coastal and estuarine food webs, the biogeochemical cycle of nutrients, and sediment oxygenation, there is a real possibility for repercussions into the estuarine community.	Pires, A., Figueira, E., Silva, M. S. S., Sá, C. & Marques, P. A. A. P. Effects of graphene oxide nanosheets in the polychaete Hediste diversicolor: Behavioural, physiological and biochemical responses. Environmental pollution (Barking, Essex : 1987) 299, 118869–118869 (2022).

reduced graphene oxide	ecotoxicity	In this study, the stimulating effects of reduced graphene oxide (RGO) to nitrogen-fixing bacterium Azotobacter chroococcum were reported. RGO stimulated the cell growth of A. chroococcum at 0.010–0.500 mg/mL according to the growth curves and the colony-forming unit (CFU) increases. RGO wrapped over the A. chroococcum cells without inducing ultrastructural changes. RGO decreased the leakage of cell membrane, but slight oxidative stress was observed in A. chroococcum. RGO promoted the nitrogen fixation activity of A. chroococcum at 0.5 mg/mL according to both isotope dilution method and acetylene reduction activity measurements. Consequently, the increases of soil nitrogen contents were evidenced, in particular about 30% increase of organic nitrogen occurred at 0.5 mg/mL of RGO. In addition, RGO might possibly benefit the plant growth through enhancing the indoleacetic acid production of A. chroococcum. These results highlighted the positive environmental effects of graphene materials to nitrogen-fixing bacteria in nitrogen cycle.	Ouyang, P. *et al.* Stimulating effects of reduced graphene oxide on the growth and nitrogen fixation activity of nitrogen-fixing bacterium Azotobacter chroococcum. *Chemosphere* 294, 133702–133702 (2022).
graphene oxide	ecotoxicity	The authors studied the interaction of three differently oxidized GO systems with model aquatic plant Lemna minor. They found that although none of the three GOs caused lethal phytotoxicity to Lemna after 7 days, the mechanism of action was dependent on the GO's surface oxidation. Based on the amount of functional surface groups, the GO was able to directly interact with the Lemna's root through its edges. However, in this case in contrast to algae and crustaceans, the interaction did not lead to a mechanical damage. Therefore, these results showed that GO is not hazardous to Lemna minor even at very high concentrations (up to 25 mg/L), because the root barrier proved to be strong enough to prevent GO's penetration and its consequent toxicity.	Malina, T., Lamaczová, A., Maršálková, E., Zbořil, R. & Maršálek, B. Graphene oxide interaction with Lemna minor: Root barrier strong enough to prevent nanoblade-morphology-induced toxicity. *Chemosphere* 291, 132739–132739 (2022).
nanographene oxide	toxicity	In this study it is demonstrated that electrochemically derived NGO has good dispersion stability and biocompatibility. NGO significantly enhanced angiogenesis in calvarial bone defect areas *in vivo*, providing a good microenvironment for bone regeneration. NGO activated endothelial tip cells by coupling with lysophosphatidic acid (LPA) in serum via strong hydrogen bonding interactions.NGO-coupled LPA activates LPAR6 and facilitates the formation of migratory tip cells via Hippo/Yes-associated protein (YAP) independent of reactive oxygen species (ROS) stimulation or additional complex modifications.	Liu, W. *et al.* Electrochemically derived nanographene oxide activates endothelial tip cells and promotes angiogenesis by binding endogenous lysophosphatidic acid. *Bioactive materials* 9, 92–104 (2022).

graphene oxide	ecotoxicity	The morphological, physiological, and transcriptomic changes of buckwheat in response to GO were analyzed in the present study. High concentrations of GO significantly inhibited seedlings growth and induced ROS production, and GO can penetrate into root and stem. The root and stem of the seedlings under GO treatment were selected for RNA-Seq analysis. In total 2039 GO-responsive DEGs were identified. Of these DEGs, 36 genes involved in ROS detoxification showed response to GO, which implied that GO may affect plant growth via regulating ROS detoxification. Root and stem exhibit distinct transcriptomic responses to GO, and some GO-responsive DEGs in stem involved in cell cycle and epigenetic regulation were found. 40 genes involved in biosynthesis and signaling of plant hormones were significantly regulated by GO. Additionally, 97 SSPs encoding genes were found to be involved in GO response and the network mediated by TFs-SSPs-RLKs signaling modules in regulating GO response was proposed. This study provides useful information for investigating the molecular mechanisms underlying the response of buckwheat to GO and exploring the interactions between GO and plants.	Liu, C Y, L Sun, Y X Sun, X Q You, Y Wan, X Y Wu, M L Tan, *et al.* «Integrating transcriptome and physiological analyses to elucidate the molecular responses of buckwheat to graphene oxide». JOURNAL OF HAZARDOUS MATERIALS 424 (2022).
graphene oxide, carbon nanofiber	toxicity	Isolated hepatocytes from rainbow trout (Oncorhynchus mykiss) were exposed to ranges of concentrations of different forms of GRM, two graphene oxides (GO) of sheet-like structure and one tubular-shaped carbon nanofiber (CNF) in the presence or absence of fetal bovine serum (FBS) for 24 and 72 h. In the presence of FBS, GO affected metabolic activity and cell membrane integrity more than CNF, whilst absence of serum further reduced cell viability in GRM-exposed cells. GRM did not alter lysosomal function nor did it induce ROS formation or EROD activity. Intracellular uptake was observed only in the case of CNF when incubated without FBS. Results emphasize the role of serum proteins in the toxicological responses following exposure to GRM with important implications for the environmental risk assessment of these nanomaterials.	Kalman, Judit, Fernando Torrent, e José M Navas. Cytotoxicity of Three Graphene-Related Materials in Rainbow Trout Primary Hepatocytes Is Not Associated to Cellular Internalization. Ecotoxicology and Environmental Safety 231 (gennaio 2022): 113227–113227.
graphene oxide	toxicity	In the present study, Caenorhabditis elegans was employed to explore the multi-generational toxicity effects of graphene oxide and the underlying mechanisms. After continuous exposure for several generations, worms grew smaller and lived shorter. The locomotion behaviors were reduced across the filial generations and these reduced trends were following the impairments of locomotion-related neurons. In addition, the extended defecation cycles from the third filial generation were in consistency with the relative size reduction of the defecation related neuron. Simultaneously, the fertility function of the nematode was impaired under consecutive exposure as reduced brood sizes and oocytes numbers, increased apoptosis of germline, and aberrant expression of reproductive related genes ced-3, ced-4, ced-9, egl-1 and ced-13 were detected in exposed worms. Furthermore, the antioxidant enzyme, SOD-3 was significantly increased in the parent and filial generations. Thus, continuous multi-generational exposure to graphene oxide caused damage to the neuron development and the reproductive system in nematodes. These toxic effects could be reflected by indicators such as growth inhibition, shortened lifespan, and locomotion behavior impairment and induced oxidative response.	Jin, L. *et al.* Sublethal toxicity of graphene oxide in Caenorhabditis elegans under multi-generational exposure. *Ecotoxicology and environmental safety* 229, 113064–113064 (2022).

graphene oxide	toxicity	Herein, W1118 flies were used as a model organism to study GO toxicity at relatively low concentrations. It is found that GO exposure led to remarkable weight loss, delayed development, retarded motion, and shortened lifespan of these flies. On the other hand, the GO influence on their sex ratio and the total number of pupae and adults were insignificant. The toxicological effect of GO was shown to be related to its serious compromise of the nutrient absorption in flies due to the severe damages in midguts. These damages were then attributed to the excessive accumulation of reactive oxygen species (ROS), which triggers the oxidative stress.	Guo, Q. *et al.* Graphene oxide toxicity in W1118 flies. *The Science of the total environment* 805, 150302–150302 (2022).
graphene oxide	toxicity	Silver and graphene nanoparticles have proven to have antimicrobial properties and are used as coating of these facemasks to increase the effectivity of the textile fibres. In the case of graphene, important gaps were detected in the database, especially regarding toxicokinetics, which prevents the derivation of a systemic no effect level. Nevertheless, the qualitative approach suggests that the risk of dermal repeated exposure to graphene is very low, or even negligible. It is estimated that for both nanomaterials, the risk of skin sensitisation and genotoxicity is also negligible.	Estevan, C., Vilanova, E. & Sogorb, M. A. Case study: risk associated to wearing silver or graphene nanoparticle-coated facemasks for protection against COVID-19. *ARCHIVES OF TOXICOLOGY* 96, 105–119 (2022).
graphene oxide	toxicity	The acute and chronic toxicity of graphene oxide on Eisenia fetida was evaluated in this study. Individual and histological endpoints of earthworms, including growth, reproduction, and histopathological changes in the intestine and skin, were assessed. The growth rate and reproduction rate of earthworms showed a significant GO concentration-related decrease. Graphene oxide induced serious skin and intestine toxicity with increasing GO concentrations.	Duo, L., Wang, Y. & Zhao, S. Individual and histopathological responses of the earthworm (Eisenia fetida) to graphene oxide exposure. *Ecotoxicology and environmental safety* 229, 113076–113076 (2022).
graphene	ecotoxicity	In this study, Bara 310 SC (Bara, tolerant genotype) and Gold Empress (Gold, susceptible genotype) were used to investigate how the leaves of alfalfa interpret the physiological responses to graphene stress based on metabolome and transcriptome characterizations. Herein, graphene at different concentrations (0, 1% and 2%, w/w) were selected as the analytes. Physiological results showed antioxidant defence system and photosynthesis was significantly disturbed under high environmental concentration of graphene. The most important metabolites which were accumulated under graphene stress includes amino acids, flavonoids, organic acids and sugars. Transcriptomic analysis reveals 1125 of core graphene-responsive genes in alfalfa that was robustly differently expressed in both genotypes. . Gold was more disturbed by graphene stress at both transcriptional and metabolic levels, since more stress-responsive genes/metabolites were identified in Gold.	Chen, Z. *et al.* Phytotoxic effect and molecular mechanism induced by graphene towards alfalfa (Medicago sativa L.) by integrating transcriptomic and metabolomics analysis. *Chemosphere* 290, 133368–133368 (2022).

graphene oxide	Combined toxicity	Due to different exposure routes, bioavailability and metabolic capacities in the different developmental stages of zebrafish, GO exacerbated the endocrine disruption effect of bisphenol A (BPA) on adult male zebrafish. The joint effects of GO and BPA were relevant to the life stages of organisms. The presence of 250G led to significantly reduced T and FSH levels and less spermatozoa in comparison with 500B. Metabolomics provided valuable information to reveal the molecular mechanisms of the joint interactions between BPA and GO. Relative to BPA alone, the combined mixtures affected three amino acid metabolic pathways and ascorbate and aldarate metabolism, and altered the levels of lactic acid and cholesterol involved in energy metabolism and steroidogenesis respectively. GO led to lower T and FSH levels compared to BPA alone in adult zebrafish. GO enhanced the toxicity of BPA on the testis development in adult zebrafish. Higher metabolic disturbance was caused by GO + BPA in adult zebrafish.	Chen, P. *et al.* Graphene oxide enhanced the endocrine disrupting effects of bisphenol A in adult male zebrafish: Integrated deep learning and metabolomics studies. *The Science of the total environment* 809, 151103–151103 (2022).
graphene oxide	toxicity	Carbon fiber compoistes (CFC) samples reinforced with (CFC + GO) or without GO (CFC) were subjected to simultaneous impact and fire. Soot and residues were characterised and their toxicity was compared to that of virgin GO. Virgin GO was not cytotoxic but induced pro-inflammatory and oxidative stress responses. The toxicity profile of CFC was globally not cytotoxic, inducing a pro-inflammatory response and no oxidative stress. An increased cytotoxicity at the highest concentration was potentially caused by fibres of reduced diameters or fibril bundles, which were observed only in this condition. While the presence of GO in CFC did not alter the cytotoxicity profile, it seemed to drive the pro-inflammatory and oxidative stress response in soot. On the contrary, in CFC + GO residue the biological activity was decreased due to the physicochemical alterations of the materials.	Chapple, R. *et al.* Graphene oxide incorporating carbon fibre-reinforced composites submitted to simultaneous impact and fire: Physicochemical characterisation and toxicology of the by-products. *Journal of hazardous materials* 424, 127544–127544 (2022).
graphene oxide	toxicity	Overall, authors' results on thyroid histopathology and GG cells, did not specifically establish GO as a thyroid endocrine disruptor-chemicals (TDC) in Japanese medaka larvae on 47 dph following exposure on 1st fry stage. The observed effects of GO on thyroid follicles, thyrocytes, and/or GG cells were not concentration-dependent, even though inconsistent effects were observed. However, authors' data indicate significant differences in responses to GO exposure between sexes either as males and females or as XY and XX. These studies indicate that GO nanoparticles have the possibility of agglomeration in tissue fluids; therefore, disrupting effects of TDCs on thyroidal structures and functions are dependent on the ability of GO to reach the target organ and transportation of GO to the target organs/tissues (thyroid follicles).	Asala, T. E., Dasmahapatra, A. K., Myla, A. & Tchounwou, P. B. Histological and Histochemical Evaluation of the Effects of Graphene Oxide on Thyroid Follicles and Gas Gland of Japanese Medaka (Oryzias latipes) Larvae. *Chemosphere* 286, 131719–131719 (2022).
MoS2 nanoflakes	ecotoxicity	This work discovered that surface vacancies (SVs) decreased the colloidal stability and promoted free radical generation and dissolution of single layer 2H-MoS2 in aqueous solution, which is critical in the fate and toxicity of MoS2. In addition, SVs provide specific edge sites of MoS2 for molecular binding. Compared to the pristine form, S-vacant 2H-MoS2 exhibited stronger affinity to proteins containing high relative abundances of -SH groups and thiol amino acids, which play key roles in the life process of algae. Importantly, the increased capacity of free radical generation and specific interactions with proteins after SVs formation aggravated adverse algal responses induced by 2H-MoS2.	Zou, W. *et al.* Sulfur vacancies affect the environmental fate, corona formation, and microalgae toxicity of molybdenum disulfide nanoflakes. *Journal of hazardous materials* 419, 126499–126499 (2021).

MoS2	ecotoxicity	This study revealed that the interplay with extracellular polymeric substances (EPS) of freshwater algae significantly changed the properties and toxicity of MoS2 to aquatic fish. The morphological and structural alterations after EPS binding alleviated the toxicity (*e.g.*, malformation and oxidative stress) of MoS2 to infantile zebrafish.	Zou, W. *et al.* Impact of algal extracellular polymeric substances on the environmental fate and risk of molybdenum disulfide in aqueous media. *WATER RESEARCH* 205, (2021).
MoS2 nanosheets	ecotoxicity	The DNA cleavage activity induced by ce-MoS2 nanosheets was examined for the first time. MoS2 has the ability to induce DNA cleavage in an aqueous solution in the dark environment, making it different from two-dimensional graphene-based nanomaterials. The DNA cleavage was enhanced under higher pH values due to more amount of ROS generation. MoS2 nanosheets can serve as effective photoinduced DNA cleavage agents in the presence of NADH under UV light irradiation, where the cleavage activity is enhanced with the prolongation of UV irradiation time and increase of MoS2 and NADH concentrations.	Zhao, Y., Xu, J. & Jiang, X. DNA Cleavage by Chemically Exfoliated Molybdenum Disulfide Nanosheets. *Environmental science & technology* 55, 4037–4044 (2021).
graphene oxide	toxicity	The toxic effects of graphene oxide (GO) on earthworms (Eisenia fetida) were thoroughly investigated. Exposure to different doses of GO (0, 5, 10, 20, and 30 g kg−1) was conducted for 7, 14, 21, and 28 days. Findings suggest that GO induces oxidative stress and genotoxicity in Eisenia fetida, resulting in lipid peroxidation, decreased lysosomal membrane stability and DNA damage.	Zhao, S., Wang, Y. & Duo, L. Biochemical toxicity, lysosomal membrane stability and DNA damage induced by graphene oxide in earthworms. *Environmental pollution (Barking, Essex : 1987)* 269, 116225–116225 (2021).
graphene oxide	toxicity	The aim of the present study was to determine the role of GO in lung injury induction, as well as its involvement in oxidative stress, inflammation and autophagy. The results revealed that lower concentrations of GO (5 and 10 mg/kg) did not cause significant lung injury, but the administration of GO at higher concentrations (50 and 100 mg/kg) induced lung edema, and increased lung permeability and histopathological lung changes. High GO concentrations also induced oxidative injury and inflammatory reactions in the lung, demonstrated by increased levels of oxidative products [malondialdehyde(MDA) and 8-hydroxydeoxyguanosine (8-OHdG)] and inflammatory factors (TNF-α, IL-6, IL-1β and IL-8). In conclusion, the findings of the present study indicated that GO causes lung injury in a dose-dependent manner by inducing autophagy.	Zhang, L. *et al.* Graphene oxide induces dose-dependent lung injury in rats by regulating autophagy. *Experimental and therapeutic medicine* 21, 462–462 (2021).
graphene oxide, reduced graphene oxide	toxicity	This study aimed to evaluate the cardiotoxicity of graphene oxide (GO) and reduced GO (rGO) *in vitro* and *in vivo*, as well as to investigate the underlying toxicity mechanisms. It is found that rGO could be generated by the reduction of GO following gamma irradiation, which showed higher cardiotoxicity than GO. Both GO and rGO exhibited cardiotoxicity by mediating lipid peroxidation, oxidative stress, and mitochondrial dysfunction.	Zhang, J., Cao, H.-Y., Wang, J.-Q., Wu, G.-D. & Wang, L. Graphene Oxide and Reduced Graphene Oxide Exhibit Cardiotoxicity Through the Regulation of Lipid Peroxidation, Oxidative Stress, and Mitochondrial Dysfunction. *Frontiers in cell and developmental biology* 9, 616888–616888 (2021).

graphene oxide	toxicity	By using melittin (Mel), a representative pore-forming peptide, as a benchmark, this study demonstrates the strong biological influences of mechanical perturbations caused by low-concentrated GO and C60 on a cell membrane, especially their different roles in changing the normal function of proteins or peptides.	Zhang, C. *et al.* Membrane perturbation of fullerene and graphene oxide distinguished by pore-forming peptide melittin. *CARBON* 180, 67–76 (2021).
single-layer MoS2	ecotoxicity	In this study, the effects of natural nanocolloids were compared to humic acid on the environmental transformation and ecotoxicity of single-layer molybdenum disulfide (SLMoS2). SLMoS2-nanocolloids induced serious damage (cell distortion and deformation), SLMoS2 internalization, and metabolic perturbation on Chlorella vulgaris (C. vulgaris). In contrast, the addition of HA induced the growth promotion and lower ROS level, inhibited the internalization of SLMoS2, and mitigated metabolic perturbation on C. vulgaris. This work provides insights into the effect of natural nanocolloids on the behaviors and biological risks of ENMs in aquatic environments	Zeng, H., Hu, X., Ouyang, S. & Zhou, Q. Nanocolloids, but Not Humic Acids, Augment the Phytotoxicity of Single-Layer Molybdenum Disulfide Nanosheets. *Environmental science & technology* 55, 1122–1133 (2021).
graphene oxide	ecotoxicity	In this study, the effect of GO interaction with the pollen−stigma system was verified on the entire reproduction process of the model plant, C. pepo. The stigmatic surface integrity was not compromised by GO; still, pollen adhesion and germination over the stigma decreased, fruit development was altered, and seed production was completely suppressed. The similar effect of GO and GO purified from production residues (PGO) support the hypothesis that the physical interposition of planar nanoparticles between pollen and stigma compromises the reproduction process by lowering the pollen load and affecting pollen-stigma signaling. However, the necrotic effect of GO on fruits also suggests a chemical interaction of this material (and its potential contaminants) with the plant tissues, similar to other reactive substances. This study allowed us to highlight possible effects due to GO depositions on stigmas caused by very localized and abundant releases of these materials, such as sprays of GO-based fungicides or pesticides.	Zanelli, D., Carniel, F. C. & Tretiach, M. The Interaction of Graphene Oxide with the Pollen-Stigma System: *in vivo* Effects on the Sexual Reproduction of Cucurbita pepo L. *APPLIED SCIENCES-BASEL* 11, (2021).
graphene nanoribbons	toxicity, ecotoxicity	In this review, the prospects for the application of graphene ribbons in biomedicine, taking into account safety aspects is analyzed. It is concluded that conclude that graphene nanoribbons, as components of high-precision nanodevices and therapeutic agents, have significant potential for biomedical applications; however, additional studies of their safety are needed. Particular emphasis should be placed on the lack of information about the effect of graphene nanoribbons on the organism as a whole obtained from *in vivo* experiments, as well as about their ecological toxicity, accumulation, migration, and destruction within ecosystems.	Nanomaterials 2021, 11(9), 2425; https://doi.org/10.3390/nano11092425

graphene oxide	toxicity, ecotoxicity	It is reported that the transcript levels of Cd transporters, including OsIRT1, OsIRT2, OsNramp1, OsNramp5, and OsHMA2, were decreased by 56–96% in Cd-stressed rice seedlings with exposure to 400 mg L−1 GO compared with those without GO exposure. The in situ non-invasive microelectrodes test revealed that GO clearly reduced the net Cd influx of rice roots. Thus, GO exposure decreased the level of Cd in rice seedlings by approximately 60%, compared with the GO-free condition. However, the analyses of biomass, chlorophyll fluorescence parameters and Evans blue staining, indicated that GO had adverse effects on the robustness of plants under the Cd co-contaminated condition. Taken together, although GO reduced the accumulation of Cd in rice seedlings, it still negatively affected plant growth.	You, Y. *et al.* Graphene oxide decreases Cd concentration in rice seedlings but intensifies growth restriction. *Journal of hazardous materials* 417, 125958–125958 (2021).
graphene oxide	toxicity	Results revealed that GO injured cells through reactive oxygen species (ROS) induction and induced apoptosis in protein-free microenvironment. However, GO-induced cytotoxicity was weakened remarkably in the presence of protein, which attributed to the reduction of GO internalization and ROS generation. GO induced cell cycle arrest in the G0/G1 phase when the protein concentration is low but had no remarkable cytotoxicity when the protein concentration was high.	Yang, Y. *et al.* Protein corona reduced graphene oxide cytotoxicity by inhibiting endocytosis. *COLLOID AND INTERFACE SCIENCE COMMUNICATIONS* 45, (2021).
Few-Layered Black Phosphorus	toxicity	The developmental toxicity of few-layered BP toward the zebrafish was investigated. After exposure of embryos to 10 mg/L BP, developmental malformations appeared at 96 hpf, especially heart deformities such as pericardial edema and bradycardia, accompanied by severe circulatory system failure. Cardiovascular defects were further characterized by using transgenic zebrafish larvae using cardiac enlargement and impaired cardiac vessels as indicators of damage to the cardiovascular system upon BP exposure. It was performed transcriptomic analysis on zebrafish embryos treated with a lower concentration of 2 mg/L. The results showed disruption in genes associated with muscle development, oxygen involved processes, focal adhesion, and VEGF and MAPK signaling pathways. These alterations also indicated that BP carries a risk of developmental perturbation at lower concentrations.	Yang, X. X. *et al.* Developmental Toxicity of Few-Layered Black Phosphorus toward Zebrafish. *ENVIRONMENTAL SCIENCE & TECHNOLOGY* 55, 1134–1144 (2021).
reduced graphene oxide	ecotoxicity	Observations revealed a morphology-dependent toxicity of carbon black (CB), reduced graphene oxide (RGO), and single-wall carbon nanotube (SWCNT) to earthworms. The aspect ratio and hydrodynamic size of CNs most likely dictated their adverse effects on the body weights, antioxidant systems, coelomocytes, and non-targeted metabolomic profiles of the worms. The results highly suggested that the soil environmental risk of carbon black (CB), reduced graphene oxide (RGO), and single-wall carbon nanotube (SWCNT) was related to their particle morphology.	Xu, K. *et al.* Toxicity of three carbon-based nanomaterials to earthworms: Effect of morphology on biomarkers, cytotoxicity, and metabolomics. *SCIENCE OF THE TOTAL ENVIRONMENT* 777, (2021).

graphene, graphene oxide	toxicity	Graphene can construct covalent and non-covalent bonds with different nucleobases, and graphene oxide is responsible for generation of reactive oxygen species (ROS), corroborating its genotoxicity. On the other hand, non-cytotoxic effect on glioblastoma cells could be demonstrated. The pharmacokinetics analysis showed high plasmatic concentration and clearance. Topical application of 0.1 and 1 mg/kg of graphene nanoparticles on the hamster skinfold preparation did not show inflammatory effect. The cell internalization assay showed that 1-hour post contact with cells, graphene can cross the plasmatic membrane and accumulate in the cytoplasm. Radio labeling with 177Lu is possible and its use as therapeutic nanosystem is viable. Finally, the ecotoxicity analysis showed that A. silina exposed to Graphene showed pronounced uptake and absorption in the nauplii gut and formation of ROS. The data obtained showed that although being formed exclusively of carbon and carbon-oxygen, graphene and graphene oxide respectively generate somewhat contradictory results and more studies should be performed to certify the safety use of this nanoplatform.	Xing, H. X. *et al.* Graphene: Insights on Biological, Radiochemical and Ecotoxicological Aspects. *JOURNAL OF BIOMEDICAL NANOTECHNOLOGY* 17, 131–148 (2021).
graphene oxide	toxicity	Data highlight the importance of the structure-related activity of GO on its biological properties and provide an in-depth understanding of how GO-derived cellular redox signaling induces mitochondrion-related cascades that modulate cell functionality and survival	Xiaoli, F. *et al.* Graphene oxide disrupted mitochondrial homeostasis through inducing intracellular redox deviation and autophagy-lysosomal network dysfunction in SH-SY5Y cells. *JOURNAL OF HAZARDOUS MATERIALS* 416, 126158–126158 (2021).
graphene nanoplatelets	toxicity	intratracheal instillation of each type of surface functionalized GNPs showed significant acute neutrophilic inflammation with resolving over time, but the positively charged GNPs showed the slightly higher inflammogenicity than negatively charged ones. All types of GNPs had similar patterns of extrapulmonary translocation into the mediastinal lymph nodes, and prolonged retention was evident	Lee, J. K. *et al.* The role of surface functionalization on the pulmonary inflammogenicity and translocation into mediastinal lymph nodes of graphene nanoplatelets in rats. *Archives of toxicology* 91, 667–676 (2017).
graphene oxide	toxicity	Even though no significant exhibition of overall hepatotoxicity could be observed in mice under relatively low-dose GO exposure, tremendous disruption of liver functional zonation patterns was uncovered, associated with detrimental changes in cellular components and signaling transduction. The study shows that GO induces profound changes at the transcriptional and epigenetic levels despite minimal changes in the liver function studies.	Wu, Y., Feng, W., Liu, R., Xia, T. & Liu, S. Graphene Oxide Causes Disordered Zonation Due to Differential Intralobular Localization in the Liver. *ACS nano* 14, 877–890 (2020).

graphene oxide	toxicity	Indeed, GO is able to induce oxidative stress and inflammatory response at low concentrations, leading to mutagenic effects *in vivo* in Xenopus laevis tadpoles. At higher concentrations, the toxicity is reflected by disturbances in erythrocytic mitosis, resulting in accumulation of cells in G0/G1 phase.	Evariste, L. *et al.* Thermal Reduction of Graphene Oxide Mitigates Its *in vivo* Genotoxicity Toward Xenopus laevis Tadpoles. *NANOMATERIALS* 9, (2019).
graphene oxide	toxicity	The genotoxicity of GFNs on DNA is still widely unknown. Given the important role of genotoxicity in GFNs exposure risk assessment, research should focus on: (1) the development of reliable testing methods to assess genotoxic effects; (2) a thorough elucidation of the response mechanisms associated with genotoxicity; and (3) the increase of the evaluation database regarding the type of GFNs, applied dosages, and exposure times.	Wu, K., Zhou, Q. & Ouyang, S. Direct and Indirect Genotoxicity of Graphene Family Nanomaterials on DNA-A Review. *Nanomaterials (Basel, Switzerland)* 11, (2021).
graphene oxide	toxicity	The most significant finding in this communication is that the level of oxidative modification of the GO surface as well as the presence of carbon radicals determine the *in vitro* and *in vivo* hazard potential, as reflected by lipid peroxidation of the surface membrane, membrane damage, subcellular processing, cytotoxicity, and the generation of acute pro-inflammatory effects in small airways of the lung. Pristine GO showed moderate effects, while rGO-2 induced low levels of lung inflammation.	Li, R. *et al.* Surface Oxidation of Graphene Oxide Determines Membrane Damage, Lipid Peroxidation, and Cytotoxicity in Macrophages in a Pulmonary Toxicity Model. *ACS nano* 12, 1390–1402 (2018).
reduced graphene oxide	toxicity	The most significant finding in this communication is that the level of oxidative modification of the GO surface as well as the presence of carbon radicals determine the *in vitro* and *in vivo* hazard potential, as reflected by lipid peroxidation of the surface membrane, membrane damage, subcellular processing, cytotoxicity, and the generation of acute pro-inflammatory effects in small airways of the lung. Pristine GO showed moderate effects, while rGO-2 induced low levels of lung inflammation.	Li, R. *et al.* Surface Oxidation of Graphene Oxide Determines Membrane Damage, Lipid Peroxidation, and Cytotoxicity in Macrophages in a Pulmonary Toxicity Model. *ACS nano* 12, 1390–1402 (2018).
graphene oxide	toxicity	It this study it is examined the phenotypic responses and potential mechanism of a lepidopteran insect Asian corn borer (ACB) to graphene oxide (GO). It was demonstrated that GO could significantly promote the growth of ACB. The transcriptomic and proteomic results consistently verified that GO might activate trypsin-like serine protease, glutathione S-transferase, heat shock protein and glycosyltransferase to further influence the development of ACB	Wang, X. *et al.* Phenotypic responses and potential genetic mechanism of lepidopteran insects under exposure to graphene oxide. *Ecotoxicology and environmental safety* 228, 113008–113008 (2021).

graphene oxide	ecotoxicity	In this work it is evaluated the effects of two different GO samples (chemically synthesized GOC and electrochemically synthesized GOE) on Lemna gibba L., after incubation with different concentrations over a period of 21 days. Results demonstrated that the smallest GOE nano sheets were very well dispersed on L. gibba fronds than GOC. This effect could be explained considering the lower GOE functionalization degree and, as a result, a less electrostatic adhesion among nanosheets. The presence of electrically charged functionalities mainly provides electrostatic interactions with plants causing growth inhibition as shown by GOC.	Valentini, F. *et al.* Chemical interactions and ecotoxicity effects between graphene oxide and Lemna gibba. *FULLERENES NANOTUBES AND CARBON NANOSTRUCTURES* 29, 746–753 (2021).
graphene nanoplatelets	toxicity	This pilot study, performed on 12 workers vs.11 controls, demonstrates that BMCyt and fpg-comet assays are the most sensitive biomarkers of early, still reparable, genotoxic and oxidative effects exerted by graphene nanoparticles.	Ursini, C. L. *et al.* Occupational exposure to graphene and silica nanoparticles. Part II: pilot study to identify a panel of sensitive biomarkers of genotoxic, oxidative and inflammatory effects on suitable biological matrices. *Nanotoxicology* 15, 223–237 (2021).
few layer graphene	ecotoxicity	Experiments revealed a limited toxic effect of graphene on H. diversicolor. Although the polychaetes ingested graphene, no impact on their total energy content was found.	Urban-Malinga, B., Jakubowska, M., Hallmann, A. & Dąbrowska, A. Do the graphene nanoflakes pose a potential threat to the polychaete Hediste diversicolor? *Chemosphere* 269, 128685–128685 (2021).
graphene oxide nanoparticles	ecotoxicity	In this study, the environmental levels of GO NPs are addressed to examine whether GO leads to adverse effects on an in-vivo model of Caenorhabditis elegans (C. elegans). Prolonged exposure to the low-dose GO NPs might be associated with disruption of reproduction and locomotion, attenuation of longevity, and induction of oxidative stress in nematodes.	Tsai, M. H. *et al.* Toxicity of Low-dose Graphene Oxide Nanoparticles in an in-vivo Wild Type of Caenorhabditis elegans Model. *AEROSOL AND AIR QUALITY RESEARCH* 21, (2021).
graphene oxide	ecotoxicity	The results showed that the impact of GO concentrations on aerobic granular sludge (AGS) was dose- and time-dependent. The microbial bacteria responded differently to the stimulation of different concentrations of GO.	Tian, Y., Yu, D., Wang, Y. & Chen, G. Performance and responses of aerobic granular sludge at different concentrations of graphene oxide after a single administered dose. *Water environment research : a research publication of the Water Environment Federation* 93, 2210–2222 (2021).

graphene nanoplatelets	toxicity	Authors' study was focused on multi-walled carbon nanotubes (MWCNTs) and two different types of graphene platelets (GPs) and whether their intracellular presence modulates a proinflammatory response from human primary monocytes towards common pathogens. At firstm it is confirmed that all tested C-BNMs caused neither direct cytotoxicity nor the release of tumour necrosis factor α (TNF-α), interleukin (IL)-6 or IL-10. However, such pre-exposed monocytes showed increased responsiveness to additional bacterial stimuli. In response to several types of bacteria, monocytes pre-treated with GP1 produced a significantly higher quantity of TNF-α, IL-6 and IL-10. Monocytes pre-treated with MWCNTs produced increased levels of IL-10. All the tested C-BNMs enhanced monocyte phagocytosis and accelerated their differentiation towards macrophages.	Svadlakova, T. *et al.* Carbon-Based Nanomaterials Increase Reactivity of Primary Monocytes towards Various Bacteria and Modulate Their Differentiation into Macrophages. *NANOMATERIALS* 11, (2021).
2D Ti3C2 nanosheets	toxicity	These findings demonstrated that Ti3C2 nanosheets might disturb respiration without inflammatory responses and pathological lesions, suggesting that these effects may occur by decreasing SP-B-mediated airway resistance. This indicates that organ function maintenance differs from biological safety for Ti3C2 nanosheets.	Sui, B., Liu, X. & Sun, J. Biodistribution, inter-/intra-cellular localization and respiratory dysfunction induced by Ti(3)C(2) nanosheets: Involvement of surfactant protein down-regulation in alveolar epithelial cells. *Journal of hazardous materials* 402, 123562–123562 (2021).
graphene oxide	toxicity	In NHDF (normal human dermal fibroblasts) and A549 (adenocarcinomic human alveolar basal epithelial cells) cell cultures, the answer to ultrapure (GO) and Mn2+-contaminated (GOS) graphene oxide exposure measured with oxidative stress markers was more related to the exposure time than to the type of graphene oxide. Independently, in the same types of cells, the cytokines' levels were related to the exposure time as well as to the GO type. Short-time exposure to Mn2+-contaminated GO induced the stronger response of oxidative stress markers but this increase did not collapse the antioxidative systems of analyzed cells. Increased levels of inflammatory cytokines after GO- and Mn2+-contaminated GO exposure were similar both in NHDF and cancer A549 cells, which shows that cytotoxicity of the studied graphene-derived materials triggers comparable physiological answers in *in vitro* conditions.	Stygar, D. *et al.* Graphene Oxide Normal (GO + Mn(2+)) and Ultrapure: Short-Term Impact on Selected Antioxidant Stress Markers and Cytokines in NHDF and A549 Cell Lines. *Antioxidants (Basel, Switzerland)* 10, (2021).
graphene oxide nanofibers	toxicity	The antimicrobial and biocompatible nature of GO/SI NF was evaluated in comparison with GO NF. The results showed the non-toxic nature of both the nanofibers (GO NF and GO/SI NF) on Gram-negative bacteria (Klebsiella oxytoca) and Gram-positive bacteria (Staphylococcus aureus). Both the nanofibers were hemo-/biocompatible toward human red blood cells and human epithelial cell lines (TH 1)	Sivalingam, S., Kunhilintakath, A., Nagamony, P. & Parthasarathy, V. P. Fabrication, toxicity and biocompatibility of Sesamum indicum infused graphene oxide nanofiber - a novel green composite method. *APPLIED NANOSCIENCE* 11, 679–686 (2021).

graphene oxide	toxicity	Toxicity profiling of GO was also done on the HepG2 cell line using MTT assay after 24 h incubation and found out the biocompatible nature of GO even at 100 µg/ml dose concentration.	Singh, V., Sandhir, R. & Singhal, N. K. Synthesis, characterization and toxicity profiling of graphene oxide-metformin hydrogel as a sustained release system for metformin in-vitro. in *MATERIALS TODAY-PROCEEDINGS* vol. 36 769–774 (2021).
nanographene oxide	toxicity	GOn in amounts superior to those which could permeate the skin were shown not to affect human skin fibroblasts (HFF-1) morphology or viability, after 24 h of incubation	Silva, F. A. L. S. *et al.* Graphene Oxide Topical Administration: Skin Permeability Studies. *Materials (Basel, Switzerland)* 14, (2021).
MoS2 nanosheets	ecotoxicity	A 100 ppm MoS2 NPs concentration has low translocation and less accumulation with no significant impact on growth of rice cv. HUR 3022 seedlings and appears to be environmentally safe for future applications.	Sharma, P. K., Raghubanshi, A. S. & Shah, K. Examining the uptake and bioaccumulation of molybdenum nanoparticles and their effect on antioxidant activities in growing rice seedlings. *Environmental science and pollution research international* 28, 13439–13453 (2021).
graphene oxide	ecotoxicity	This study aimed to identify the physiological responses of house cricket females following short-term exposure to relatively low dietary doses of graphene oxide (GO, 20 µg · g−1 food), silver (Ag, 400 µg · g−1 food) nanoparticles (NPs), or graphene oxide-silver nanoparticle composite (GO-AgNPs, 20: 400 µg · g−1 food). This study confirms the hypothesis that used nanoparticles can change gut functions and deregulate the energy budget in the early stage of exposure. Even low amounts of dietary NPs can provoke an early physiological response that is stimulatory rather than inhibitory and resembles hormesis.	Seyed Alian, R., Dziewięcka, M., Kędziorski, A., Majchrzycki, Ł. & Augustyniak, M. Do nanoparticles cause hormesis? Early physiological compensatory response in house crickets to a dietary admixture of GO, Ag, and GOAg composite. *The Science of the total environment* 788, 147801–147801 (2021).
nanographene oxide	ecotoxicity	nanographene oxide (NGO) exposure at high doses (400 and 800 µg mL^{-1}) prompted oxidative stress and produced a toxic effect on calli physiological regulations under both PEG and non-PEG-treated media through reduction of the antioxidative defense system, which was related to the overexpression of H2O2 level as a cellular injury marker	Samadi, S., Lajayer, B. A., Moghiseh, E. & Rodriguez-Couto, S. Effect of carbon nanomaterials on cell toxicity, biomass production, nutritional and active compound accumulation in plants. *ENVIRONMENTAL TECHNOLOGY & INNOVATION* 21, (2021).

graphene	toxicity	One method of toxicity assessment was based on measurement of the efflux of hemoglobin from suspended red blood cells. At the smallest size, graphene oxide showed the greatest hemolytic activity, whereas aggregated graphene sheets exhibited the lowest hemolytic activity. the compacted graphene sheets are more damaging to mammalian fibroblasts than the less densely packed graphene oxide. Clearly, the toxicity of graphene and graphene oxide depends on the exposure environment (*i.e.*, whether or not aggregation occurs) and mode of interaction with cells (*i.e.*, suspension versus adherent cell types)	Liao, K.-H., Lin, Y.-S., Macosko, C. W. & Haynes, C. L. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *ACS applied materials & interfaces* 3, 2607–2615 (2011).
graphene oxide	toxicity	One method of toxicity assessment was based on measurement of the efflux of hemoglobin from suspended red blood cells. At the smallest size, graphene oxide showed the greatest hemolytic activity, whereas aggregated graphene sheets exhibited the lowest hemolytic activity. the compacted graphene sheets are more damaging to mammalian fibroblasts than the less densely packed graphene oxide. Clearly, the toxicity of graphene and graphene oxide depends on the exposure environment (*i.e.*, whether or not aggregation occurs) and mode of interaction with cells (*i.e.*, suspension versus adherent cell types)	Liao, K.-H., Lin, Y.-S., Macosko, C. W. & Haynes, C. L. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *ACS applied materials & interfaces* 3, 2607–2615 (2011).
graphene oxide	toxicity	In this study it is reported the effects of graphene oxides on human fibroblast cells and mice with the aim of investigating graphene oxides' biocompatibility. graphene oxides exhibit dose-dependent toxicity to cells and animals, such as inducing cell apoptosis and lung granuloma formation, and cannot be cleaned by kidney	Wang, K. *et al.* Biocompatibility of Graphene Oxide. *Nanoscale research letters* 6, 8–8 (2011).
graphene oxide	toxicity	in comparison to its smaller counterpart, larger GO showed a stronger adsorption onto the plasma membrane with less phagocytosis, which elicited more robust interaction with toll-like receptors and more potent activation of NF-κB pathways. authors' study delineated the size-dependent M1 induction of macrophages and pro-inflammatory responses of GO *in vitro* and *in vivo*.	Ma, J. *et al.* Crucial Role of Lateral Size for Graphene Oxide in Activating Macrophages and Stimulating Pro-inflammatoryResponses in Cells and Animals. *ACS nano* 9, 10498–10515 (2015).
graphene oxide	toxicity	The results suggest that GO does not enter A549 cell and has no obvious cytotoxicity. But GO can cause a dose-dependent oxidative stress in cell and induce a slight loss of cell viability at high concentration. These effects are dose and size related, and should be considered in the development of bio-applications of GO.	Chang, Y. *et al. In vitro* toxicity evaluation of graphene oxide on A549 cells. *Toxicology letters* 200, 201–210 (2011).

graphene oxide	toxicity	In this study it is reported there was no significant decrease in viability of cells (MCF-7, HUVEC, KMBC/71 cells) after exposure to the non-cytotoxic dose of GO and GQDs, but it was observed significant alterations in the expression level of miR-21, miR-29a, Bax, Bcl2 and PTEN genes after treatment in all three cells. In addition to molecular changes, it is observed alteration in mitochondrial activity at cellular level.	Hashemi, M. S., Gharbi, S., Jafarinejad-Farsangi, S., Ansari-Asl, Z. & Dezfuli, A. S. Secondary toxic effect of graphene oxide and graphene quantum dots alters the expression of miR-21 and miR-29a in human cell lines. *Toxicology in vitro : an international journal published in association with BIBRA* 65, 104796–104796 (2020).
graphene oxide nanosheets	toxicity	In this study it is found that cytotoxicity metrics (viability, apoptosis, intracellular reactive oxygen species, and mitochondrial membrane potential) were very similar in ESC-RPE cells and hRPE cells, and those in ARPE19 cells were very different. It is concluded that cell models of GO cytotoxicity derived from ESCs are an excellent alternative to primary human cells, without the limitations of tissue availability.	Hu, L. *et al.* Evaluating the cytotoxicity of graphene oxide using embryonic stem cells-derived cells. *Journal of biomedical materials research. Part A* 108, 1321–1328 (2020).
graphene oxide	toxicity	Regardless of size, GO was cleared from the blood quickly and accumulated mainly in the liver and lungs. The uptake of GO in lungs increased with increasing injection dose and size. The dispersion state (*i.e.*, size of the GO–protein complex in blood) dominated the biodistribution. Conclusion: The size and dose of GO affected its fate *in vivo*. For medical applications, small-sized GO with suitable funtionalization is recommended.	Liu, J. H. *et al.* Effect of size and dose on the biodistribution of graphene oxide in mice. *NANOMEDICINE* 7, 1801–1812 (2012).
graphene oxide	toxicity	The results showed that the adult male mice injected with high dosages of GO (25 mg/kg mouse) via the tail vein exhibited normal sex hormone secretion and retained normal reproductive activity. All untreated female mice mated with the GO-treated male mice could produce healthy pups. There were no significant differences in pup numbers, sex ratio, weights, pup survival rates or pup growth over time between the GO-treated and control groups. Furthermore, these GO-treated male mice could produce a second, third, fourth and even fifth litter of healthy offspring when they lived with the untreated female mice. no damaging effects were seen at high dose rates of GO (total 300 mg/kg male mouse, 60 mg/kg every 24 h for 5 days) via intra-abdominal injection. Thus, GO showed very low or nearly no toxicity for male reproduction.	Liang, S., Xu, S., Zhang, D., He, J. & Chu, M. Reproductive toxicity of nanoscale graphene oxide in male mice. *Nanotoxicology* 9, 92–105 (2015).

graphene oxide	toxicity	The results showed that the adult male mice injected with high dosages of GO (25 mg/kg mouse) via the tail vein exhibited normal sex hormone secretion and retained normal reproductive activity. All untreated female mice mated with the GO-treated male mice could produce healthy pups. There were no significant differences in pup numbers, sex ratio, weights, pup survival rates or pup growth over time between the GO-treated and control groups. Furthermore, these GO-treated male mice could produce a second, third, fourth and even fifth litter of healthy offspring when they lived with the untreated female mice. no damaging effects were seen at high dose rates of GO (total 300 mg/kg male mouse, 60 mg/kg every 24 h for 5 days) via intra-abdominal injection. Thus, GO showed very low or nearly no toxicity for male reproduction.	Liang, S., Xu, S., Zhang, D., He, J. & Chu, M. Reproductive toxicity of nanoscale graphene oxide in male mice (vol 9, pg 92, 2015). *NANOTOXICOLOGY* 10, 1204–1204 (2016).
graphene oxide	toxicity	this study examined the pulmonary effects of graphene oxide using male Sprague-Dawley rats and a single 6-hour nose-only inhalation technique. Following the exposure, the rats were allowed to recover for 1 day, 7 days, or 14 days. these results demonstrate that the single inhalation exposure to graphene oxide induce minimal toxic responses in rat lungs at the concentrations and time points used in the present study.	Han, S. G. *et al.* Pulmonary Responses of Sprague-Dawley Rats in Single Inhalation Exposure to Graphene Oxide Nanomaterials. *BioMed research international* 2015, 376756–376756 (2015).
nanographene oxide	toxicity	In this work, it is presented a systematic study on the *in vivo* distribution and pulmonary toxicity of NGO for up to 3 months after exposure. NGO could result in acute lung injury (ALI) and chronic pulmonary fibrosis. Such NGO-induced ALI was related to oxidative stress and could effectively be relieved with dexamethasone treatment.	Li, B. *et al.* Biodistribution and pulmonary toxicity of intratracheally instilled graphene oxide in mice. *NPG ASIA MATERIALS* 5, (2013).
graphene oxide	toxicity	In this work, it is presented a systematical investigation *in vivo* biodistribution and potential toxicity of as-made GO and a number of polyethylene glycol (PEG) functionalized GO derivatives with different sizes and surface coatings, after oral and intraperitoneal administration at high doses. NO ACCUMULATION OF as-made GO in the reticuloendothelial (RES) system including liver and spleen is observed after i.p. injection. Further investigations based on histological examination of organ slices and hematological analysis discover that although GO and PEGylated GO derivatives would retain in the mouse body over a long period of time after i.p. injection, their toxicity to the treated animals is insignificant.	Yang, K. *et al. in vivo* biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. *Biomaterials* 34, 2787–2795 (2013).

Carboxyl graphene oxide	toxicity	Exposure of zebrafish embryos to 10, 50 and 100 mg/L GO-COOH specifically induced neurodevelopmental abnormalities and altered tendency of locomotor in larval fish. Furthermore, GO-COOH exposure led to increase of AchE and ATPase activities and oxidative stress upregulation, and disrupted the expression of genes involved in neurodevelopment and neurotransmitter pathway. Authors' results suggest that GO-COOH has the potential to induce neurotoxicity and Parkinson's disease-like symptoms in zebrafish larvae. Authors' results suggest that GO-COOH has the potential to induce neurotoxicity and Parkinson's disease-like symptoms in zebrafish larvae.	Cao, Z. *et al.* Carboxyl graphene oxide nanoparticles induce neurodevelopmental defects and locomotor disorders in zebrafish larvae. *Chemosphere* 270, 128611–128611 (2021).
graphene oxide	toxicity	In this study it is explored the effects of graphene oxide (GO) on the growth and behaviours of VSMCs by using a rat aortic smooth muscle cell line, A7r5, as a VSMC model. Results demonstrated that GO had no obvious toxicity to VSMCs.	Ren, J. *et al.* On the biocompatibility of graphene oxide towards vascular smooth muscle cells. *Nanotechnology* 32, 55101–55101 (2021).
carbon nitride	toxicity	In this study, the effects of sulfur-doped g-C3N4 (TCN) on cognitive function and histopathology of hippocampus were investigated in mice. Histological evaluations showed an increased level of neuronal loss and glial activation in the hippocampus of TCN treated mice at doses of 150 and 500 mg/kg. Overall, authors' data indicate that TCN induces the cognitive impairment that is partly mediated via its exacerbating impacts on neuronal loss and glial activation.	Ramezani, F., Ghasemi-Kasman, M., Nosratiyan, N., Ghasemi, S. & Feizi, F. Acute administration of sulfur-doped g-C3N4 induces cognitive deficits and exacerbates the levels of glial activation in mouse hippocampus. *BRAIN RESEARCH BULLETIN* 176, 54–66 (2021).
graphene oxide nanoribbons	toxicity	Plate-culture experiments showed that GORs exerted a significant cytotoxic effect on E. coli in a concentration-dependent manner. The depletion of nutrients in the LB medium showed that GORs adsorbed metal ions and competed with E. coli, which inhibit the growth and reproduction of E. coli.	Qiang, S. *et al.* Cytotoxic Effect of Graphene Oxide Nanoribbons on Escherichia coli. *Nanomaterials (Basel, Switzerland)* 11, (2021).
graphene oxide, reduced graphene oxide	toxicity	Female C57BL/6 mice were exposed by a single intratracheal instillation of 0, 18, 54 or 162 μg/mouse of graphene oxide (GO) or reduced graphene oxide (rGO). GO exposure induced more differentially expressed genes, affected more functions, and perturbed more pathways compared to rGO, both in lung and liver tissues. The largest differences were observed for the pulmonary innate immune response and acute phase response, and for hepatic lipid homeostasis, which were strongly induced after GO exposure. These changes collective indicate a potential for atherosclerotic changes after GO, but not rGO exposure.	Poulsen, S. S. *et al.* A transcriptomic overview of lung and liver changes one day after pulmonary exposure to graphene and graphene oxide. *Toxicology and applied pharmacology* 410, 115343–115343 (2021).

graphene oxide	toxicity	Authors' results indicated that GO coatings on steel and gold were not toxic towards L929 cells in a direct cell adhesion test—on all tested materials. This proves that GO synthesized by authors' group in the form of coating is non-toxic; however, it can show toxicity if detached from the surface. The obtained materials did not show any hemolytic properties.	Poniatowska, A., Trzaskowska, P. A., Trzaskowski, M. & Ciach, T. Physicochemical and Biological Properties of Graphene-Oxide-Coated Metallic Materials. *Materials (Basel, Switzerland)* 14, (2021).
graphene oxide, reduced graphene oxide	toxicity	In this investigation, recent studies on the genotoxicity of most widely used GBMs such as graphene oxide (GO) and the chemically reduced graphene oxide (RGO) toward human retinal pigment epithelium (RPE) cells are presented. Results suggest that both GO and RGOs induced ROS-dependent DNA damage.	Ou, L. *et al.* Oxygen content-related DNA damage of graphene oxide on human retinal pigment epithelium cells. *Journal of materials science. Materials in medicine* 32, 20–20 (2021).
graphene oxide	toxicity	In the present work, GO was administered intraperitoneally to adult Wistar rats in four incremental doses, *i.e.*, 0.0 mg/kg (control), 0.4 mg/kg (low dose), 2.0 mg/kg (mid-dose), and 10.0 mg/kg (high dose) FOR 15 repeated doses over a period of 30 days. Histopathological and morphometric analyses of liver and kidney were also performed. Results demonstrated dose-dependent toxicity of GO. General behavior and liver indices remained unaffected in the study. Serum levels of ALT, ALP, and AST were altered significantly in high-dose treated animals. Changes were found insignificant in the low- and mid-dose groups. Catalase activity in liver tissue homogenates was decreased in the high-dose group. MDA levels were found elevated in treated rats. Unlike control and low dose, mid- and high-dose treated rats exhibited varying degrees of histopathological changes like inflammation around the central vein and portal veins, vacuolations, hepatocytic injury, and near normal to abnormal hepatic sinusoids. These findings show that GO has considerable toxic potential to mammalian liver and thorough toxicity studies are needed before these nanosheets are used in biomedicine.	Nirmal, N. K., Awasthi, K. K. & John, P. J. Hepatotoxicity of graphene oxide in Wistar rats. *Environmental science and pollution research international* 28, 46367–46376 (2021).
graphene oxide	toxicity	Many fish species including Japanese medaka have XX/XY sex determination mechanism, however, sex reversal (SR) can be induced by external and genetic factors. Despite genotypic differences (XY/XX), in the histopathology/histochemistry of liver and kidneys GO effects was found to be minimum. Taken together, present study showed spontaneous induction of SR in some XX genotypes; however, exposure of fasting fries to GO had no apparent EDC effects.	Myla, A., Dasmahapatra, A. K. & Tchounwou, P. B. Sex-reversal and Histopathological Assessment of Potential Endocrine-Disrupting Effects of Graphene Oxide on Japanese medaka (Oryzias latipes) Larvae. *CHEMOSPHERE* 279, (2021).

graphene nanoplatelets, hexagonal boron nitrideflakes	toxicity	The phagotrophic protist Tetrahymena thermophila was exposed to subinhibitory levels (10 mg L−1) of multiwall carbon nanotubes (CNTs), graphene nanoplatelets (GNPs), carbon black (CB), hexagonal boron nitride flakes (hBN) and boron nitride nanotubes (BNNTs). The uptake rates were similar for all three carbonaceous ENMs, but significantly higher and lower, respectively, for hBN and BNNTs compared to carbonaceous ENMs. Uptake appeared to correlate with the tapped densities of the ENMs, *i.e.*, ratio of ENM mass to the volume occupied after tapping to constant volume, assumed to be representative of the ENM "packing" density in protozoan food vacuoles. Depuration was relatively slower for the tubular ENMs (CNTs and BNNTs) compared to planar (GNPs and hBN) and spherical (CB)	Mortimer, M., Kefela, T., Trinh, A. & Holden, P. A. Uptake and depuration of carbon- and boron nitride-based nanomaterials in the protozoa Tetrahymena thermophila. *ENVIRONMENTAL SCIENCE-NANO* 8, 3613–3628 (2021).
graphene oxide	ecotoxicity	The molecular structure plays a secondary role for the GO ecotoxicity, whereas the size of sheets is more decisive. Larger GO sheets, regardless their oxidation degree and presence of carboxylic acid groups, adsorb onto the zebrafish eggs, thereby delaying hatching and causing death of embryos. On the other hand, GO sheets smaller than 200 nm do not interfere on the zebrafish developmental stages, which succeed in the same way as in the control condition (absent of GO).	Moreira, C. C. C. *et al.* Oxidation degree or sheet size: What really matters for the photothermal effect and ecotoxicity of graphene oxide? *FLATCHEM* 26, (2021).
graphene oxide	toxicity	The current study was, therefore, designed to investigate the possible induction of chromosomal and DNA damage by GO nanoparticles and their impact on the tissue architecture in mice. Oral administration of GO nanoparticles for one or five consecutive days at the three dose levels 10, 20 or 40 mg/kg significantly increased the micronuclei and DNA damage levels in a dose-dependent manner in mice bone marrow cells, as well as caused, histological lesions including apoptosis, necrosis, inflammations and cells degeneration in the mice liver and brain tissue sections compared to the normal control mice. Thus, it is concluded that oral administration of GO nanoparticles induced chromosomal and DNA damage in a dose-dependent manner as well as histological injuries in both acute and subacute treatments.	Mohamed, H. R. H., Welson, M., Yaseen, A. E. & El-Ghor, A. Induction of chromosomal and DNA damage and histological alterations by graphene oxide nanoparticles in Swiss mice. *Drug and chemical toxicology* 44, 631–641 (2021).
graphene oxide, reduced graphene oxide	toxicity	This study is aimed to (1) assess the capacity of graphene oxide (GO) to sorb PAHs and (2) to evaluate the toxicity of GO alone and in combination with PAHs on zebrafish embryos and adults. In embryos exposed to different GO nanomaterials alone and with PAHs, no significant mortality was recorded for any treatment. Nevertheless, malformation rate increased significantly in embryos exposed to the highest concentrations (5 or 10 mg/L) of GO and reduced GO (rGO) alone. Results demonstrated the capacity of GO to carry PAHs and to exert sublethal effects in zebrafish.	Martínez-Álvarez, I. *et al.* Uptake and effects of graphene oxide nanomaterials alone and in combination with polycyclic aromatic hydrocarbons in zebrafish. *The Science of the total environment* 775, 145669–145669 (2021).

Pristine graphene, graphene oxide	toxicity	In the present study, the effect of pG and GO with environmental concentrations (0, 5, 10, 15, 20, and 25 μg/L of pG; 0, 0.1, 0.2, 0.3, and 0.4 mg/mL of GO) on multi-organ system in developing zebrafish larvae was experimentally assessed. The pG and GO bioaccumulation leads to multi organ toxicity in zebrafish larvae.	Manjunatha, B., Seo, E., Park, S. H., Kundapur, R. R. & Lee, S. J. Pristine graphene and graphene oxide induce multi-organ defects in zebrafish (Danio rerio) larvae/juvenile: an *in vivo* study. *Environmental science and pollution research international* 28, 34664–34675 (2021).
graphene nanoplatelets	toxicity	The aim of the presented article was to bring new cytotoxic, cytostatic, genotoxic and immunotoxic data concerning *in vitro* exposure of the human THP-1 cell line to two types of GP. the cell membranes' integrity has not been disrupted. It is also found no evidence of the induction of oxidative stress due to GP exposure and it was not observed any significant increase in cytostasis. It wa found a significant dose-dependent increase in DNA damage and changes in the IL-6, IL-10 and TNF-α levels were not significant and indicated the absence of any induction of an immune response and/or the induction of oxidative stress.	Malkova, A. *et al. In vitro* Assessment of the Genotoxic Potential of Pristine Graphene Platelets. *NANOMATERIALS* 11, (2021).
graphene oxide	toxicity	In this study, it was found found that GSH could be oxidized to GSSG by GO, leading to the formation of reduced GO (rGO). GSH depletion affects the intracellular reductive/oxidative balance, provoking the increase of the reactive oxygen species level, sequentially inhibiting cell viability and proliferation. Therefore, the reaction between GO and GSH provides a new perspective to explain the origin of GO cytotoxicity	Ma, B., Guo, S., Nishina, Y. & Bianco, A. Reaction between Graphene Oxide and Intracellular Glutathione Affects Cell Viability and Proliferation. *ACS applied materials & interfaces* 13, 3528–3535 (2021).
graphene oxide	toxicity	the results from this study suggested that impaired lipid droplet biogenesis was involved in GO-induced cytotoxicity in HUVECs, and inducing lipid droplet biogenesis could prevent the cytotoxicity of GO	Luo, Y., Wang, X. & Cao, Y. Transcriptomic analysis suggested the involvement of impaired lipid droplet biogenesis in graphene oxide-induced cytotoxicity in human umbilical vein endothelial cells. *Chemico-biological interactions* 333, 109325–109325 (2021).
MoS2 nanoparticles	ecotoxicity	In this study, the effect of MoS2 NPs on the physiological index, subcellular morphology, transcriptomic profiles of the marine microalgae Dunaliella salina was investigated for the first time. The "doping-like" effects on marine algae suggest that the low concentration of MoS2 NPs might change the rudimentary ecological composition in the ocean.	Luo, S. W. *et al.* Physiological and molecular responses in halotolerant Dunaliella salina exposed to molybdenum disulfide nanoparticles. *JOURNAL OF HAZARDOUS MATERIALS* 404, (2021).

hexagonal boron nitride	toxicity	The cornered hBN with lateral polar edges results in a dose-dependent cytotoxic effect, whereas round hBN does not cause significant toxicity, thus confirming authors' premise.	Lucherelli, M. A. *et al.* Boron Nitride Nanosheets Can Induce Water Channels Across Lipid Bilayers Leading to Lysosomal Permeabilization. *Advanced materials (Deerfield Beach, Fla.)* 33, e2103137–e2103137 (2021).
graphene oxide nanosheets	ecotoxicity	In this study, Rhizobium sp. E20-8 and graphene oxide (GO) nanosheets were applied on container-grown maize seedlings in watered and drought conditions. the effect of GO nanosheets was negligible under the present experimental conditions. However, the main effects of GO nanosheets were in a drought context, with the osmotic and antioxidant protection conferred by GO nanosheets to drought shoots, leading to higher shoot biomass.	Lopes, T. *et al.* A Multifactorial Approach to Untangle Graphene Oxide (GO) Nanosheets Effects on Plants: Plant Growth-Promoting Bacteria Inoculation, Bacterial Survival, and Drought. *Nanomaterials (Basel, Switzerland)* 11, (2021).
graphene oxide	toxicity	In this study it was found that orally administrated GO daily during gestational day (GD) 7–16 caused dose-dependent pregnant complications of mice on the endpoint (GD19), including decreased weight of dam and live fetus, high rate of resorbed embryos and dead fetus, and skeletal development retardation. The damages of GO exposure to placenta barrier and pregnancy were dose-dependent and GO exposure was responsible for gut microbiome dysbiosis in mice with pregnant complications. These findings could provide referable evidence to evaluate reproductive risk of GO to mammals.	Liu, X. *et al.* Altered gut microbiome accompanying with placenta barrier dysfunction programs pregnant complications in mice caused by graphene oxide. *Ecotoxicology and environmental safety* 207, 111143–111143 (2021).
graphene oxide	toxicity	Authors' data demonstrate that GO-induced intestinal epithelial cells (IECs) apoptosis via ROS/AMPK/p53 pathway activation accounts for the exacerbation of colitis *in vivo* and aggravation of inflammation *in vitro*. These findings provide a new insight into the pathogenesis of IBD induced by environmental factors. Furthermore, authors' findings enhance authors' understanding of GO as a potential environmental toxin, which helps delineate the risk of exposure to patients with disturbed intestinal epithelial barrier/inflammatory disorders such as inflammatory bowel disease.	Liu, S. *et al.* Graphene oxide exacerbates dextran sodium sulfate-induced colitis via ROS/AMPK/p53 signali ng to mediate apoptosis. *Journal of nanobiotechnology* 19, 85–85 (2021).
MXene	toxicity	In this study it is presentd a report on the *in vitro* cytotoxicity of Ti2CTx MXene against cervical cancer cell lines (HeLa) in a form of 3D spheroid with comparison to 2D cell culture system. The biological results with 2D and 3D HeLa indicated that the Ti2CTx MXene was moderately cytotoxic to cells and the cytotoxicity was dose dependent.	Lim, G. P. *et al.* Synthesis, characterization and biophysical evaluation of the 2D Ti2CTx MXene using 3D spheroid-type cultures. *CERAMICS INTERNATIONAL* 47, 22567–22577 (2021).

graphene oxide	toxicity	The current study investigated the cellular functions of A549 cells exposed to varying concentrations of GO through a label-free quantitative proteomic analysis. Through comparative proteomics and network analysis, authors' results indicate that the dysregulation of DEPs induced by GO contributes to disturbed spliceosome functions, complement and coagulation cascades, p53 signaling pathway, and transcriptional misregulation in cancer. Moreover, the common DEPs in response to GO exposure mainly contribute to regulation of gene transcription, immune response, cell growth, and apoptosis.	Liao, Y. *et al.* Comparative proteomic analysis reveals cytotoxicity induced by graphene oxide exposure in A549 cells. *Journal of applied toxicology : JAT* 41, 1103–1114 (2021).
graphene oxide, reduced graphene oxide	ecotoxicity	all of the GBNs could enter pepper fruit and induce physiological disorders	Li, X., Sun, S., Guo, S. & Hu, X. Identifying the Phytotoxicity and Defense Mechanisms Associated with Graphene-Based Nanomaterials by Integrating Multiomics and Regular Analysis. *Environmental science & technology* 55, 9938–9948 (2021).
graphene oxide	toxicity	Coexposure to cis-BF and GO deteriorated the lipid homeostasis disruption in tadpoles. The up- or down-regulation of lipogenesis genes expression and enzymes activity were amplified in the coexposure groups. Furthermore, the presence of GO enhanced the deleterious impacts of cis-BF on the hepatic function in tadpoles. This study uniquely shows that GO promotes the lipotoxicity and hepatic function deficit caused by cis-BF exposure in frog.	Li, M., Zhu, J., Wu, Q. & Wang, Q. The combined adverse effects of cis-bifenthrin and graphene oxide on lipid homeostasis in Xenopus laevis. *Journal of hazardous materials* 407, 124876–124876 (2021).
monolayer MoS2	toxicity	Herein, the interfacial interactions (affinity sites and intensity) between monolayer MoS2 and zebrafish embryos mediated by 1 T phase surface atomic arrangement (octahedral coordination) and the 2H phase surface atomic arrangement (triangular prism coordination) MoS2 nanosheets were studied. Compared to 1 T-MoS2, 2H-MoS2 more readily entered embryos, which was facilitated by caveolae-mediated endocytosis, and caused higher developmental toxicity. Furthermore, metabolic pathways related to amino acid and protein biosynthesis and energy metabolism were affected by the nanomaterial surface atomic arrangements.	Li, K. *et al.* Surface atomic arrangement of nanomaterials affects nanotoxicity. *Nanotoxicology* 15, 114–130 (2021).
graphene oxide	toxicity	Herein, the effects of GOs with small (GO-S) and large (GO-L) lateral sizes in three major cell types in the liver, Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and hepatocytes is compared. It is confirmed that the differential cytotoxic responses and toxicity mechanism were similar to GO-S and GO-L, suggesting that it is a universal feature of GO.	Li, J. *et al.* Lateral size of graphene oxide determines differential cellular uptake and cell death pathways in Kupffer cells, LSECs, and hepatocytes. *Nano today* 37, (2021).

2D boron nitride, MoS2	toxicity	Here, the toxicity of BN and MoS2, dispersed in Pluronic F87 (designated BN-PF and MoS2-PF) is compared with aggregated forms of these materials (BN-Agg and MoS2-Agg) in liver cells. MoS2 induces dose-dependent cytotoxicity in KCs, but not other cell types, while the BN derivatives are non-toxic.	Li, J. *et al.* Dissolution of 2D Molybdenum Disulfide Generates Differential Toxicity among Liver Cell Types Compared to Non-Toxic 2D Boron Nitride Effects. *Small (Weinheim an der Bergstrasse, Germany)* 17, e2101084–e2101084 (2021).
graphene	ecotoxicity	In this study, the combined toxic effects of co-exposure to graphene and Triphenyl phosphate (TPP) was investigated in Mytilus galloprovincialis. The present work revealed that TPP could be adsorbed on the surface of graphene. Transcriptomic responses suggested that graphene + TPP could reduce oxidative stress by modulating the glutathione metabolism pathway, and bring down the energy consumption by modulating the glycolysis/gluconeogenesis and TCA cycle pathways.	Li, F., Meng, X., Wang, X., Ji, C. & Wu, H. Graphene-triphenyl phosphate (TPP) co-exposure in the marine environment: Interference with metabolism and immune regulation in mussel Mytilus galloprovincialis. *Ecotoxicology and environmental safety* 227, 112904–112904 (2021).
reduced graphene oxide	toxicity	In this study it is showed that nanoscale carbon materials are highly related to abnormal neural activation and function in both health and neurological disorders. This suggests that chronic exposure to structured carbon nanomaterials can induce neuronal cell death and accelerate the development of degenerative nerve diseases.	Lee, H. *et al.* Effect of carbon nanomaterial dimension on the functional activity and degeneration of neurons. *BIOMATERIALS* 279, (2021).
graphene	toxicity	These results indicate that graphene monolayer scaffold is cytocompatible with connective tissue cells examined and could be beneficial for tissue engineering therapy	Lasocka, I. *et al.* Cytocompatibility of Graphene Monolayer and Its Impact on Focal Cell Adhesion, Mitochondrial Morphology and Activity in BALB/3T3 Fibroblasts. *MATERIALS* 14, (2021).
Pristine graphene	toxicity	This study was conducted to address the knowledge gap related to the risk/safety assessment during the interaction of pristine graphene with the human intestinal barrier using an ex vivo model (a human relevant translation model). authors' results highlighted the pathways affected by graphene upon tissue exposure. It is shown that graphene can stimulate the mRNA expression of genes involved in cell proliferation and growth upon binding/adhering to epithelial tissue.	Lahiani, M. H., Gokulan, K., Williams, K. & Khare, S. Ex Vivo Human Colon Tissue Exposure to Pristine Graphene Activates Genes Involved in the Binding, Adhesion and Proliferation of Epithelial Cells. *International journal of molecular sciences* 22, (2021).
graphene nanoplatelets	toxicity	This research aimed to evaluate the skin sensitization potentials induced by GNPs using two types of alternative to animal testing. It is found that GNPs do not induce skin sensitization. In addition, it was observed that the administration of GNPs did not induce cytotoxicity and skin toxicity.	Kim, S.-H. *et al.* Skin Sensitization Evaluation of Carbon-Based Graphene Nanoplatelets. *Toxics* 9, (2021).

hexagonal boron nitride	toxicity	The results revealed that hBN NPs induce oxidative stress in a dose-dependent manner by modulating thiol/disulfide homeostasis in rats at higher concentrations	Kar, F. *et al.* Hexagonal boron nitride nanoparticles trigger oxidative stress by modulating thiol/disulfide homeostasis. *Human & experimental toxicology* 40, 1572–1583 (2021).
hexagonal boron nitride	toxicity	The aim of authors' study is to investigate the dose-dependent biological system effect of hexagonal boron nitride (hBN) nanoparticles, which is directly produced nanoscale, *in vivo*. The results show that no significant change was observed in the hematological and biochemical parameters when the control group and other low dose groups were compared, except for the 1600 and 3200 µg/kg b.w. dose groups. Histological detection indicated that 1600 and 3200 µg/kg hBN NPs treatment could induce significant damage in the liver, kidney, heart, spleen and pancreas.	Kar, F. *et al. in vivo* Assessment of the Effect of Hexagonal Boron Nitride Nanoparticles on Biochemical, Histopathological, Oxidant and Antioxidant Status. *JOURNAL OF CLUSTER SCIENCE* 32, 517–529 (2021).
carbon blacks, graphene nanoplatelets	toxicity	COMPARATIVE STUDY: DNA damage, oxidative stress, and protein stress were the major mechanisms of action for all the CNMs at sub-cytotoxic concentration levels	Jiang, T. *et al.* Comparative and mechanistic toxicity assessment of structure-dependent toxicity of carbon-based nanomaterials. *Journal of hazardous materials* 418, 126282–126282 (2021).
graphene oxide	toxicity	In this study, GO toxicity, and its dependence on oxidation level, elemental composition, and size, were comprehensively assessed. A newly established quantitative toxicogenomic-based toxicity testing approach, combined with conventional phenotypic bioassays, were employed. the toxicity of graphene oxides (GOs), and its dependence on oxidation level, elemental composition, and size, were comprehensively and systematically evaluated with five GOs, *i.e.*, untreated control GO, UV-treated GO with different elemental compositions, thermally reduced GO with a lower oxidation level, and two sonicated GOs with smaller sizes. The results show that elemental composition and size do indeed exert impacts on GO toxicity, while the oxidation level exhibited no significant effects.	Jiang, T. *et al.* Dependence of Graphene Oxide (GO) Toxicity on Oxidation Level, Elemental Composition, and Size. *International journal of molecular sciences* 22, (2021).

pristine graphene, graphene oxide	toxicity	analysis of the toxicity of pristine graphene (PG) and graphene oxide (GO) using four various biological models: zebrafish (Danio rerio) embryo, duckweed (Lemna minor), human HS-5 cells and bacteria (Staphylococcus aureus). The toxicity of pristine graphene (PG) and graphene oxide (GO) was tested at concentrations of 5, 10, 20, 50 and 100 µg/mL. Higher toxicity was noted after administration of high doses of PG and GO in all tested biological models. Hydrophilic GO shows greater toxicity to biological models living in the entire volume of the culture medium (zebrafish, duckweed, S. aureus). PG showed the highest toxicity to adherent cells growing on the bottom of the culture plates—human HS-5 cells.	Jaworski, S. *et al.* Comparison of the Toxicity of Pristine Graphene and Graphene Oxide, Using Four Biological Models. *Materials (Basel, Switzerland)* 14, (2021).
graphene oxide	toxicity	In this study, it is elucidated the cytotoxic effects of 2D graphene oxide (GO), in relation to differentiation of human induced pluripotent stem cells (hiPSCs). Taken together, authors' results highlight the risk in the uptake and accumulation of GO on the stem cell development by unwanted loss in pluripotency and accelerated initiation of differentiation.	Heo, J. *et al.* 2D graphene oxide particles induce unwanted loss in pluripotency and trigger early differentiation in human pluripotent stem cells. *Journal of hazardous materials* 414, 125472–125472 (2021).
graphene oxide	ecotoxicity	In this study an experiment based on seed germination, seedling morphology, physio-biochemical properties, and antioxidant enzyme activities of five rice genotypes (9311, MH63, R527, K866, and Nipponbare) under six concentrations of GO (0, 5, 10, 50, 100, and 150 mg dm-3) is designed. Graphene oxide treatments significantly enhanced seed germination and root growth and inhibited shoot growth of all genotypes. Furthermore, it is found a significant genotype-dependent response to GO treatments	He, Y. *et al.* Growth response of Oryza sativa seedlings to graphene oxide and its variability among genotypes. *BIOLOGIA PLANTARUM* 65, 39–46 (2021).
graphene oxide nanosheets	toxicity	Sixty serum samples from mice in four different time intervals including 24 and 72 h and 7 and 21 days after injection of 0-, 1-, and 10-mg/kg b.w. were analyzed based on 1HNMR spectra of each sample and multivariate methods. this study shows that after 21 days, the treated groups regardless of their GO nanosheet dose are very close to the control group.	Ghiasvand Mohammadkhani, L. *et al.* Metabolomics approach to study *in vivo* toxicity of graphene oxide nanosheets. JOURNAL OF APPLIED TOXICOLOGY (2021) doi:10.1002/jat.4235.
graphene oxide	toxicity	Authors' research indicated that GO may contribute to the development of intestinal inflammation by inducing IECs autophagy dysfunction.	Gao, Y. *et al.* Graphene oxide aggravated dextran sulfate sodium-induced colitis through intestinal epithelial cells autophagy dysfunction. *The Journal of toxicological sciences* 46, 43–55 (2021).

graphene oxide	toxicity	In this study a dissection of the effects of GO, characterized by a large or small lateral size (GO 1.32 μm and GO 0.13 μm, respectively), and its amino-functionalized counterpart (GONH2 1.32 μm and GONH2 0.13 μm, respectively) on fifteen cell types of human primary peripheral blood mononuclear cells (PBMCs) is performed. It is found that the smallest later size not only evokes pronounced toxicity on the pool of PBMCs compared to larger GOs but also towards the distinct immune cell subpopulations. The amino-functionalization can critically modify graphene impact dampening the immune cell activation.	Fusco, L. *et al.* Lateral dimension and amino-functionalization on the balance to assess the single-cell toxicity of graphene on fifteen immune cell types. *NANOIMPACT* 23, (2021).
graphene oxide	ecotoxicity	Vitellogenin (Vg) has been used as a parameter for evaluating female fertility due to its importance in embryo nutrition. In this study, it is used a promising model organism, Acheta domesticus, which was intoxicated with GO in food for three generations. The aim of the study was to investigate the process of Vg synthesis in crickets depending on the exposure time, GO concentration, and age of the females. The results revealed that chronic GO intoxication had adverse effects on the Vg expression pattern. The 1st generation of insects showing low Vg expression was most affected. The 2nd generation of A. domesticus presented a high Vg expression. The last investigated generation seemed to cope with stress caused by GO, and the Vg expression was balanced. It is pointed out that chronic GO intoxication can disturb the regular formation of the Vg quaternary structure, resulting in consequences for developing an embryo.	Flasz, B., Dziewięcka, M., Kędziorski, A., Tarnawska, M. & Augustyniak, M. Multigenerational graphene oxide intoxication results in reproduction disorders at the molecular level of vitellogenin protein expression in Acheta domesticus. *Chemosphere* 280, 130772–130772 (2021).
graphene oxide, reduced graphene oxide	ecotoxicity	In the present study, the endocrine disruption potential of graphene oxide (GO) and reduced graphene oxide (rGO) was assessed using a T3-induced amphibian metamorphosis assay. The results indicated that GBMs potentiate the effects of exogenous T3 with a more marked effect of GO compared to rGO. This study highlights that the tested GBMs do not disrupt the thyroid pathway in amphibians but indicates that adsorption properties of these nanomaterials may increase the bioavailability and the toxicity of other pollutants.	Evariste, L. *et al.* Graphene oxide and reduced graphene oxide promote the effects of exogenous T3 thyroid hormone in the amphibian Xenopus laevis. *Chemosphere* 281, 130901–130901 (2021).
graphene oxide, reduced graphene oxide	ecotoxicity	this study focuses on the ecotoxicological assessment of GO and rGO toward a biofilm composed of the diatom Nitzschia palea associated to a bacterial consortium. After 48 and 144 h of exposure to these GBMs at 0, 0.1, 1, and 10 mg L−1, their effects on the diatom physiology, the structure, and the metabolism of bacterial communities were measured. authors' study suggests that diatoms benefited from diatom-bacteria interactions and that the biofilm was able to maintain or recover its carbon-related metabolic activities when exposed to GBMs	Evariste, L. *et al.* Graphene-Based Nanomaterials Modulate Internal Biofilm Interactions and Microbial Diversity. *Frontiers in microbiology* 12, 623853–623853 (2021).

graphene oxide	toxicity	This research aims to determine and compare the *in vivo* toxicity potential of GO samples from various manufacturers. Each GO sample is analyzed in two concentrations and applied with food. The physiological reactions of an easy model Acheta domesticus during ten-day lasting exposure were observed. The study proved that the tested GO suspensions have different potential to induce cytotoxicity and the mechanism of interaction with the cell is difficult to standardize and define.	Dziewiecka, M. *et al.* The Structure-Properties-Cytotoxicity Interplay: A Crucial Pathway to Determining Graphene Oxide Biocompatibility. *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 22, (2021).
WS2	toxicity, ecotoxicity	Toxicity analyses on human cells showed that both aqueous 2D WS2 suspensions have not the ability to impact on their viability, and a small capacity to induce oxidative stress. The viability of S. cerevisiae was reduced in the presence of the nanomaterials after long exposure times, although their ability to trigger ROS production in this organism was very low. Additionally, the obtained results indicated that the same concentrations of aqueous suspensions prepared with dry 2D WS2 nanopowders, employing comparable exposure conditions, are able to produce a similar toxicity impact on S. cerevisiae cells	Domi, B. *et al.* Toxicological assessment of commercial monolayer tungsten disulfide nanomaterials aqueous suspensions using human A549 cells and the model fungus Saccharomyces cerevisiae. *Chemosphere* 272, 129603–129603 (2021).
boron nitride	toxicity, ecotoxicity	In the present study, it is assessed two commercial 2D BN samples, namely BN-nanopowder (BN-PW) and BN-nanoplatelet (BN-PL), with the objective to identify whether distinct physico-chemical features may have an influence on the biological responses of exposed cellular models. The impact of the selected nanomaterials in the viability of both unicellular models was very low, with only a slight reduction of S. cerevisiae colony forming units being observed after a long exposure period (24 h) to high concentrations (800 mg/L) of both nanomaterials. Even at the highest concentration and exposure times, no major cytotoxicity indicators were observed in human cells and yeast.	Domi, B. *et al.* Assessment of Physico-Chemical and Toxicological Properties of Commercial 2D Boron Nitride Nanopowder and Nanoplatelets. *International journal of molecular sciences* 22, (2021).
graphene oxide, reduced graphene oxide	toxicity	In this work, by using larval zebrafish in a high-throughput screening system for the analysis of locomotor behavior, it is reported that GO materials with different degrees of thermal reduction affect the sensory-motor nervous system with opposite effects and timing, with GO impairing swimming performance with short latency while reduced materials enhancing it in the long-term period. Electrophysiological evidence obtained in rat neuronal cultures suggests that such effects might depend on the interference of nanomaterials with synaptic communication. It is concluded that the manipulation of a single GRMs chemical property, as the degree of GO reduction, is enough to induce differential effects of nanomaterials on nervous system function.	Di Mauro, G. *et al.* Tuning the Reduction of Graphene Oxide Nanoflakes Differently Affects Neuronal Networks in the Zebrafish. *NANOMATERIALS* 11, (2021).

graphene oxide, reduced graphene oxide	toxicity	In this study, pro-inflammatory responses and genotoxicity were assessed in alveolar epithelial cells (A549) and activated THP-1 macrophages (THP-1a) after exposure to three nanoclays; a pristine (Bentonite) and two surface modified (benzalkonium chloride-coated Nanofil9, and dialkyldimethyl-ammonium-coated NanofilSE3000); graphene oxide (GO) and reduced graphene oxide (r-GO) nanomaterials. GO and r-GO induced a pro-inflammatory response in A549 cells. Strong *in vitro-in vivo* correlation of nanomaterials-induced inflammation. GO caused an increased level of genotoxicity in THP-1a cells.	Di Ianni, E., Møller, P., Vogel, U. B. & Jacobsen, N. R. Pro-inflammatory response and genotoxicity caused by clay and graphene nanomaterials in A549 and THP-1 cells. *Mutation research. Genetic toxicology and environmental mutagenesis* 872, 503405–503405 (2021).
graphene	toxicity	D. melanogaster was used as a model organism in authors' study to identify the potential risks of exposure to graphene (thickness: 2–18 nm) and MWCNTs in different properties (as pure [OD: 10–20 nm short], modified by amide [NH2] [OD: 7–13 nm length: 55 μm], and modified by carboxyl [COOH] [OD: 30–50 nm and length: 0.5–2 μm]) at concentrations ranging from 0.1 to 250 μg/ml. Significant effects were observed at two high doses (100 and 250 μg/ml) of graphene or MWCNTs. This is the first study to report findings of cellular immune response against hematopoiesis and parasitoids, nanogenotoxicity, phenotypic variations, and locomotor behavior in D. melanogaster.	Demir, E. Mechanisms and biological impacts of graphene and multi-walled carbon nanotubes on Drosophila melanogaster: Oxidative stress, genotoxic damage, phenotypic variations, locomotor behavior, parasitoid resistance, and cellular immune response. Journal of applied toxicology : JAT (2021) doi:10.1002/jat.4232.
graphene	toxicity	it was possible to observe that the toxicity of carbon nanomaterials does not vary according to the dimensionality of 0D, 1D, and 2D materials; in other words, for these materials, it is not possible to infer a direct relationship between dimensionality and toxicity.	da Rosa, P. C. C., Leao, M. B., Dalla Corte, C. L. & de Matos, C. F. Evaluation of the Carbon Nanostructures Toxicity as a Function of Their Dimensionality Using Model Organisms: a Review. *WATER AIR AND SOIL POLLUTION* 232, (2021).
graphene oxide	ecotoxicity	In this study, the effects of GO and oxidized multi-walled CNTs were compared in the cyanobacterium Microcystis aeruginosa to determine the similarities or differences in how the two CNMs interact with and induce toxicity to cyanobacteria. The EC50 of the two CNMs were not found to be statistically different. the intrinsic differences in shape, size, and surface properties between CNTs and GO did not result in differences in how they induce toxicity to cyanobacteria.	Cruces, E. *et al.* Similar toxicity mechanisms between graphene oxide and oxidized multi-walled carbon nanotubes in Microcystis aeruginosa. *Chemosphere* 265, 129137–129137 (2021).

graphene	ecotoxicity	In this study it is successfully identified 3946 differentially expressed genes involved in crucial biological pathways (such as biosynthesis of amino acids, isoflavonoid biosynthesis, flavone and flavonol biosynthesis, linoleic acid metabolism, and phenylpropanoid biosynthesis pathways) underlying graphene tolerance in alfalfa.	Chen, Z. *et al.* Integrating transcriptome and physiological analyses to elucidate the essential biological mechanisms of graphene phytotoxicity of alfalfa (Medicago sativa L.). *Ecotoxicology and environmental safety* 220, 112348–112348 (2021).
graphene oxide	ecotoxicity	Results indicated that the co-exposure of Cr6+ (1 mg/L) and GO (0.01 mg/L) inhibited hatching and spontaneous movement of embryos, but no significant changes were found in the single Cr6+ or GO group. Compared with the single GO or Cr6+ exposure, their co-exposure (GO+Cr6+) significantly enhanced the teratogenicity in a concentration-dependent pattern, and the spinal curvature was observed as the main deformity.	Chen, Y., Li, J., Zhou, Q., Liu, Z. & Li, Q. Hexavalent chromium amplifies the developmental toxicity of graphene oxide during zebrafish embryogenesis. *Ecotoxicology and environmental safety* 208, 111487–111487 (2021).
MoS2 nanosheets	ecotoxicity	In this study it is evaluated the biological effects of molybdenum disulfide (MoS2) nanosheets on a N2-fixation cyanobacteria (Nostoc sphaeroides) by monitoring growth and metabolome changes. MoS2 nanosheets did not exert overt toxicity to Nostoc at the tested doses (0.1 and 1 mg/L). On the contrary, the intrinsic enzyme-like activities and semiconducting properties of MoS2 nanosheets promoted the metabolic processes of Nostoc, including enhancing CO2-fixation-related Calvin cycle metabolic pathway	Chen, S. *et al.* MoS2Nanosheets-Cyanobacteria Interaction: Reprogrammed Carbon and Nitrogen Metabolism. *ACS nano* 15, 16344–16356 (2021).
graphene oxide	toxicity	Herein, zebrafish larvae at 3 d post fertilization (dpf) were exposed to BPA, GO, and their mixtures until 7 dpf. GO might relieve the BPA-induced developmental toxicity and endocrine disruption by recovering the genes related to the corresponding pathways.	Chen, P. *et al.* Mechanisms for the impacts of graphene oxide on the developmental toxicity and endocrine disruption induced by bisphenol A on zebrafish larvae. *Journal of hazardous materials* 408, 124867–124867 (2021).
Carboxyl graphene oxide	toxicity	In this study, zebrafish is used to evaluate the toxicity of Carboxyl graphene oxide (GO-COOH). Exposure of zebrafish embryos to 10, 50 and 100 mg/L GO-COOH specifically induced neurodevelopmental abnormalities and altered tendency of locomotor in larval fish. Furthermore, GO-COOH exposure led to increase of AchE and ATPase activities and oxidative stress upregulation, and disrupted the expression of genes involved in neurodevelopment and neurotransmitter pathway.	Cao, Z. *et al.* Carboxyl graphene oxide nanoparticles induce neurodevelopmental defects and locomotor disorders in zebrafish larvae. *Chemosphere* 270, 128611–128611 (2021).

graphdiyne, graphene oxide	toxicity	In this study, the toxicity of Graphdiyne (GDY) and GO with human umbilical vein endothelial cells (HUVECs) are compared. Exposure to up to 100-μg/ml GDY and GO induced cytotoxicity, but there was no statistically significant difference between GDY and GO. GO was more potent to activate endothelial activation probably due to the activation of ER stress and pyroptosis genes.	Cao, Y., Xiao, W., Li, S. & Qiu, D. A comparative study of toxicity of graphdiyne and graphene oxide to human umbilical vein endothelial cells. *Journal of applied toxicology : JAT* 41, 2021–2030 (2021).
graphene oxide	ecotoxicity	This study explored the joint phytotoxicity of GO and arsenic species (arsenite [As (III)], arsenate [As (V)]) to monocot (Triticum aestivum L.) and dicot (Solamun lycopersicum) plant species. Under the environmentally relevant concentrations, GO (1 mg/L) significantly increased the phytotoxicity of As (III) and As (V) (1 mg/L), with effects being both As- and plant species-specific. co-exposure with GO resulted in more severe oxidative stress than single As exposure, which could subsequently induce damage in root plasma membranes and compromise key arsenic detoxification pathways such as complexation with glutathione and efflux. Co-exposure to GO and As also led to more significant reduction in macro- and micronutrient content.	Cao, X. *et al.* New insight into the mechanism of graphene oxide-enhanced phytotoxicity of arsenic species. *Journal of hazardous materials* 410, 124959–124959 (2021).
few layer graphene	toxicity	This investigation seeks to assess the inhalation hazard of industrially relevant FLG engineered with: (i) no surface functional groups (neutral), (ii) amine, and (iii) carboxyl group functionalization. The findings of the present study have demonstrated the capability of neutral-FLG and amine-FLG to induce genotoxicity in 16HBE14o- cells through primary indirect mechanisms, suggesting a possible role for carboxyl groups in scavenging radicals produced via oxidative stress	Burgum, M. J. *et al. In vitro* Primary-Indirect Genotoxicity in Bronchial Epithelial Cells Promoted by Industrially Relevant Few-Layer Graphene. *Small (Weinheim an der Bergstrasse, Germany)* 17, e2002551–e2002551 (2021).
few layer graphene	toxicity	FLG genotoxicity when examined in monocultures, results in primary-indirect DNA damage; whereas co-cultured cells reveal secondary mechanisms of DNA damage	Burgum, M. J. *et al.* Few-layer graphene induces both primary and secondary genotoxicity in epithelial barrier models *in vitro*. *Journal of nanobiotechnology* 19, 24–24 (2021).
graphene oxide	ecotoxicity	the present study aimed to investigate the influence of sediment on the impacts caused by GO exposure. Graphene Oxide (GO) did not induce toxicity under the tested conditions. Higher concentrations of GO caused only some modulations in the biochemical parameters.Different concentrations of GO weren't able to generate oxidative damages.	Britto, R. S. *et al.* Oxidative stress in Ruditapes philippinarum after exposure to different graphene oxide concentrations in the presence and absence of sediment. *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP* 240, 108922–108922 (2021).

graphene oxide	ecotoxicity	In this work, it is evaluated the chemical degradation of graphene oxide by sodium hypochlorite (NaClO, bleach water) and its consequences over toxicity, on the nematode Caenorhabditis elegans. an increase of over 100% in nematode survival was verified for the degraded material when compared to GO at 10 mg L−1.	Bortolozzo, L. S. *et al.* Mitigation of graphene oxide toxicity in C. elegans after chemical degradation with sodium hypochlorite. *Chemosphere* 278, 130421–130421 (2021).
graphene	toxicity	In the current study, it is investigated the long-term pulmonary exposure model of graphene and carbon black and discovered that long-term pulmonary exposure of the materials led to lung cancer metastasis and progression. authors' findings elucidate the how the pulmonary cell deaths induced by graphene reshaped the tumor microenvironment by releasing DAMPs and promoting tumor metastasis	Bi, Z. F. *et al.* Graphene promotes lung cancer metastasis through Wnt signaling activation induced by DAMPs. *NANO TODAY* 39, (2021).
graphene oxide	ecotoxicity	In this study, a modified Hummer's GO (ARGO) was systematically reduced by thermal annealing at 200, 500, or 800 °C and toxicity towards bacteria (Escherichia coli), alga (Scenedesmus obliquus), cyanobacteria (Microcystis aeruginosa), and invertebrates (Daphnia magna) was assessed by measuring the effective concentrations inducing 50% inhibition (EC50). The EC50–carbon/oxygen ratio relationships show similar trends for bacteria and invertebrates, where toxicity increases as the material is reduced. Conversely, cyanobacterial inhibition decreases as GO is reduced.	Barrios, A. C. *et al.* Emerging investigator series: a multispecies analysis of the relationship between oxygen content and toxicity in graphene oxide. *ENVIRONMENTAL SCIENCE-NANO* 8, 1543–1559 (2021).
graphene oxide, graphene nanoplatelets, graphene nanoribbons	toxicity, method	In the present study, an ex vivo exposure method is conducted in order to study the complexity of the secretome given by the interactions between GBN and blood cells. Blood samples from different healthy donors were exposed to three different types of GBN widely used in the biomedical field. In this sense, graphene oxide (GO), graphene nanoplatelets (GNPs), graphene nanoribbons (GNRs) and a panel of 105 proteins representatives of the blood secretome were evaluated. As a conclusion, this study describes an innovative approach to study the potential harmful effects of GBN, providing relevant data to be considered in the biomedical context when GBN are planned to be used in patients.	Ballesteros, S., Domenech, J., Velázquez, A., Marcos, R. & Hernández, A. Ex vivo exposure to different types of graphene-based nanomaterials consistently alters human blood secretome. *Journal of hazardous materials* 414, 125471–125471 (2021).
graphene oxide	toxicity	The research concerned the possibility of improving the muscle structure by using CEME extract from physiologically mature muscles and GO flakes as a carrier of the extract, administered in ovo. Basic *in vitro* studies have documented the biocompatibility of CEME and the GO-CEME complex administered at concentrations 1–5%. No toxicity was observed in in ovo studies.	Balaban, J. *et al.* Effect of Muscle Extract and Graphene Oxide on Muscle Structure of Chicken Embryos. *ANIMALS* 11, (2021).

graphene, graphene oxide	toxicity	The main objective of the current study was to investigate the toxicities of GR and GO in predicted environmental relevant concentrations in adult zebrafish (Danio rerio), particularly on their behaviors. Treated groups displayed behavior abnormalities, especially in predator response. Biomarker alterations were shown, especially in the high concentration of graphene group.	Audira, G. *et al.* Comparison of the chronic toxicities of graphene and graphene oxide toward adult zebrafish by using biochemical and phenomic approaches. *Environmental pollution (Barking, Essex : 1987)* 278, 116907–116907 (2021).
graphene oxide, graphene nanoplatelets	toxicity	In this investigation, a study on 22 GBMs to investigate their potential SARs by performing a complete physicochemical characterization and *in vitro* toxicity assessment (on RAW264.7 cells) is conducted. GBMs of variable lateral size (0.5–38 µm), specific surface area (SSA, 30–880 m^2/g), and surface oxidation (2–17%) were used. It is observed that reduced graphene oxides (RGOs) were more reactive than graphene nanoplatelets (GNPs), potentially highlighting the role of GBM's surface chemistry and surface defects density in their biological impact. It is also observed that for GNPs, a smaller lateral size caused higher cytotoxicity. Lastly, GBMs showing a SSA higher than 200 m^2/g were found to induce a higher ROS production.	Achawi, S., Feneon, B., Pourchez, J. & Forest, V. Structure-Activity Relationship of Graphene-Based Materials: Impact of the Surface Chemistry, Surface Specific Area and Lateral Size on Their *In vitro* Toxicity. *Nanomaterials (Basel, Switzerland)* 11, (2021).
reduced graphene oxide, graphene nanoplatelets	toxicity, method	In this study, it is explored the relevance and feasibility of the FRAS for grouping, working on 22 GBMs and 2 carbon blacks. It is concluded that with few adjustments, the FRAS method appeared perfectly adapted to these materials and allowed a classification as "reactive" or "non-reactive" in agreement with results of ROS production for 84% of authors' GBMs.	Achawi, S., Feneon, B., Pourchez, J. & Forest, V. Assessing biological oxidative damage induced by graphene-based materials: An asset for grouping approaches using the FRAS assay. *Regulatory toxicology and pharmacology : RTP* 127, 105067–105067 (2021).
graphene oxide nanosheets	combined ecotoxicity	GO significantly reduced the mortality and malformation rates of zebrafish induced by tris(1,3–dichloro–2–propyl) phosphate (TDCIPP) maximumly by 28.6% and 41.8%, respectively.	Zou, W., Zhang, X., Ouyang, S., Hu, X. & Zhou, Q. Graphene oxide nanosheets mitigate the developmental toxicity of TDCIPP in zebrafish via activating the mitochondrial respiratory chain and energy metabolism. *The Science of the total environment* 727, 138486–138486 (2020).

graphene oxide	ecotoxicity	In this study, the acute exposures of the multilayer Nano-graphene oxide (MNGO) at different dosages were conducted in order to investigate its integrated effects on the formation of the biofilm, mature biofilm and the microbial activity of the activated sludge. The microbial metabolic activity, viability, and the biological removal of the nutrients were significantly affected with the more than 100 mg/L of the MNGO.	Zhu, C. *et al.* In situ investigation of acute exposure of graphene oxide on activated sludge: Biofilm characteristics, microbial activity and cytotoxicity. *Ecotoxicology and environmental safety* 199, 110639–110639 (2020).
graphene oxide	ecotoxicity	In this study it is investigated the effect of GO exposure on protein-protein interactions by using Caenorhabditis elegans as an animal model. Data highlights the potential of GO exposure in affecting the protein-protein interactions in organisms.	Zhao, Y., Chen, H., Yang, Y., Wu, Q. & Wang, D. Graphene oxide disrupts the protein-protein interaction between Neuroligin/NLG-1 and DLG-1 or MAGI-1 in nematode Caenorhabditis elegans. *The Science of the total environment* 700, 134492–134492 (2020).
graphene oxide	ecotoxicity	the effect of photo-transformation on GO toxicity to freshwater algae (Chlorella pyrenoidosa) was investigated. The toxicological investigation showed that 8-day sunlight irradiation significantly increased growth inhibition of GO (25 mg/L) to algal cells by 11.2%, due to enhanced oxidative stress and stronger membrane damage.	Zhao, J. *et al.* Photo-transformation of graphene oxide in the presence of co-existing metal ions regulated its toxicity to freshwater algae. *Water research* 176, 115735–115735 (2020).
graphene oxide	ecotoxicity	In this study, Paeonia ostii was exposed to GO under drought stress. GO did not have toxic effects on P. ostii and was an effective soil water retention agent; therefore, it could be economically beneficial for the production of plants under drought stress.	Zhao, D., Fang, Z., Tang, Y. & Tao, J. Graphene Oxide as an Effective Soil Water Retention Agent Can Confer Drought Stress Tolerance to Paeonia ostii without Toxicity. *Environmental science & technology* 54, 8269–8279 (2020).
graphene	ecotoxicity	In this study, a matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)-based untargeted metabolomics was used to investigate the metabolic response of juvenile earthworms (Eisenia fetida) to graphene exposure in soil tests for the first time. Results revealed that graphene-exposure significantly disturbs earthworm metabolome, and graphene toxicity on earthworm shows non-concentration-dependent effect.	Zhang, Y. *et al.* Metabolite changes associated with earthworms (Eisenia fetida) graphene exposure revealed by matrix-assisted laser desorption/ionization mass spectrometry imaging. *Ecotoxicology and environmental safety* 205, 111102–111102 (2020).

graphene oxide	toxicity	In this study pleiotropic cytokine IL-6 was chosed as the model parameter to investigate inflammation responses upon exposure to GOs. The results demonstrated that large-sized GOs (L-GO) induced much stronger IL-6 activation. A detailed analysis uncovered that L-GO induced ROS production and TLR-4 activation promoted macrophage polarization and secretion of pro-inflammatory cytokines IL-1β and TNF-α, activated via> the NF-κB signaling pathway, which in turn initiated the expression of IL-6 in hepatocytes.	Zhang, Y. L. *et al.* Large-sized graphene oxide synergistically enhances parenchymal hepatocyte IL-6 expression monitored by dynamic imaging. *NANOSCALE* 12, 8147–8158 (2020).
graphene oxide	combined ecotoxicity	Triphenyl phosphate (TPhP) disperses in water and poses an increasing hazard to the ecosystem and human health. It is critical to study the environmental responses and molecular mechanisms of GO and TPhP together to assess both chemicals; however, this information is lacking. The present work revealed that GO promoted the bioaccumulation of TPhP in zebrafish larvae by 5.0%–24.3%. The TPhP-induced growth inhibition of embryos (malformation, mortality, heartbeat, and spontaneous movement) at environmentally relevant concentrations was significantly amplified by GO,	Zhang, X., Zhou, Q., Li, X., Zou, W. & Hu, X. Integrating omics and traditional analyses to profile the synergistic toxicity of graphene oxide and triphenyl phosphate. *Environmental pollution (Barking, Essex : 1987)* 263, 114473–114473 (2020).
graphene oxide	toxicity, method	In this study, a new label-free method to quantify, track, and in situ image graphene and graphene oxide (GO) in plants based on their inherent metallic impurities as fingerprints is proposed. The present method is more straightforward, accessible, and economical than normally used isotopic or metal-labeling methods. It also avoids the uncertainties or alterations of properties caused by the labeling process and thus has great promise in analysis and risk assessment of carbon nanomaterials.	Zhang, T. Y. *et al.* 3 Metallic Fingerprints of Carbon: Label-Free Tracking and Imaging of Graphene in Plants. *ANALYTICAL CHEMISTRY* 92, 1948–1955 (2020).
graphene oxide	toxicity	In this study, repetitive exposure to 5 mg/L GO for 200 subcultures (400 days), evolved Escherichia coli (E. coli) cells (EGO) differed significantly from their ancestor cells according to transcriptomic and metabolomic analyses. Contact with GO surfaces transformed E. coli by activating the Cpx envelope stress response (ESR), resulting in more than twofold greater extracellular protease release and biofilm formation. It is pointed out that awareness should be raised of the possible occurrence of microbial adaptation to antimicrobial nanomaterials, which may be implicated in cross-adaptation to harsh environments and eventually the prevalence of virulence.	Zhang, Q. R. & Zhang, C. D. Chronic Exposure to Low Concentration of Graphene Oxide Increases Bacterial Pathogenicity via the Envelope Stress Response. *ENVIRONMENTAL SCIENCE & TECHNOLOGY* 54, 12412–12422 (2020).
graphene oxide, reduced graphene oxide	ecotoxicity	The present study compared the effects of graphene oxide (GO) and reduced GO (rGO) on rice seedling growth under hydroponic conditions for 3 weeks. GO induced pH alteration of nutrient solution and subsequent Fe overaccumulation and oxidative damage in plant leaves. The apparently different impact of GO and rGO on plant growth suggests that the phytotoxicity of GBMs is highly related to their surface oxygen content.	Zhang, P. *et al.* Graphene Oxide-Induced pH Alteration, Iron Overload, and Subsequent Oxidative Damage in Rice (Oryza sativa L.): A New Mechanism of Nanomaterial Phytotoxicity. *Environmental science & technology* 54, 3181–3190 (2020).

multi-layer graphenes	ecotoxicity	In this study, the response of earthworms (Eisenia fetida) to three types of multi-layer graphenes (MLGs) (G1, G2 and G3 with variable morphology, thicknesses, and hydrophobicity is investigated. The study highlight the importance of these properties in determining absorption potential of GBNs and their interaction with earthworm membranes, and also suggests a need to consider multiple endpoints in assessing the ecological risks of these materials.	Zhang, H., Vidonish, J., Lv, W., Wang, X. & Alvarez, P. Differential histological, cellular and organism-wide response of earthworms exposed to multi-layer graphenes with different morphologies and hydrophobicity. *Environmental pollution (Barking, Essex : 1987)* 263, 114468–114468 (2020).
graphene oxide	toxicity	In this study, toxicity and mechanism of GO exposure in the rat astroglioma-derived F98 cell line is systematically explored. The exposure of F98 cells to GO can elicit concentration- and time-dependent toxicological effects.	Zhang, C., Feng, X., He, L., Zhang, Y. & Shao, L. The interrupted effect of autophagic flux and lysosomal function induced by graphene oxide in p62-dependent apoptosis of F98 cells. *Journal of nanobiotechnology* 18, 52–52 (2020).
few layer graphene	ecotoxicity	In this study, the effects of few-layer graphene (FLG) and a similarly layered phyllosilicate, muscovite mica (MICA), were tested *in vivo* on the reproductive structures, *i.e.*, pollen and stigma, of Cucurbita pepo L. ssp. pepo 'greyzini' (summer squash, zucchini). it was shown that FLG is as safe as a naturally occurring nanomaterial.	Zanelli, D. *et al.* Effects of Few-Layer Graphene on the Sexual Reproduction of Seed Plants: An *in vivo* Study withCucurbita pepoL. *NANOMATERIALS* 10, (2020).
reduced graphene oxide	toxicity	In this work, a reduced graphene oxide membrane (rGOM) for its use in ocular Regenerative Medicine is developed and characterized. In addition, *in vitro* and *in vivo* biocompatibility and genotoxicity with different types of human ocular cells is studied. It is proved that rGOM allowed the growth of different ocular cells without inducing *in vitro* or *in vivo* cytotoxicity or genotoxicity in the short-term.	Zambrano-Andazol, I. *et al.* Reduced graphene oxide membranes in ocular regenerative medicine. *Materials science & engineering. C, Materials for biological applications* 114, 111075–111075 (2020).
graphene oxide	ecotoxicity	In this study, GO toxicity to green algae (Chlorella vulgaris, Scenedesmus obliquus, Chlamydomonas reinhardtii), cyanobacteria (Microcystis aeruginosa) and diatoms (Cyclotella sp.) is investigated. The results show that GO toxicity to algae varied according to species, with S. obliquus being the most susceptible and C. reinhardtii being the most tolerant of all investigated species. Cells with locomotive organelles and low specific area such as C. reinhardtii were more tolerant to the shading effects because of cell migration. Variations in membrane damage were mainly caused by differences in cell wall composition and interactions with GO, while oxidative stress and membrane damage were found to be involved in the GO mechanism of toxicity in all algal species investigated.	Yin, J. *et al.* The toxicity of graphene oxide affected by algal physiological characteristics: A comparative study in cyanobacterial, green algae, diatom. *Environmental pollution (Barking, Essex : 1987)* 260, 113847–113847 (2020).

graphene oxide	ecotoxicity	the interaction between graphene oxide (GO) and nitrogen-fixing bacterium Azotobacter chroococcum was studied to reveal the potential impact of graphene materials on biological nitrogen fixation. authors' results collectively indicated that GO was bio-reduced and toxic to nitrogen-fixation bacteria.	Yilihamu, A. *et al.* Interaction between graphene oxide and nitrogen-fixing bacterium Azotobacter chroococcum: Transformation, toxicity and nitrogen fixation. *CARBON* 160, 5–13 (2020).
mono layer graphene oxide, multi layer graphene oxide,	toxicity	In this work, DC2.4 cell line to investigate the *in vitro* immunotoxicity of two types of GO, mono-layer GO (mono-GO) and multi-layer GO (multi- GO) were used. It is found that mono-GO had less effect on cell viability than multi-GO, but both mono-GO and multi-GO significantly induced the generation of ROS in DC2.4 cells. These results suggested that both mono-GO and multi-GO are immunotoxic to DC2.4 cells	Yang, Z. *et al.* Cytotoxicity and Immune Dysfunction of Dendritic Cells Caused by Graphene Oxide. *Frontiers in pharmacology* 11, 1206–1206 (2020).
graphene oxide	toxicity	This study aims to investigate the toxic effects and molecular mechanisms of GO exposure in larval and adult zebrafish. Taken together, these informations demonstrated that GO induced hepatic dysfunction mainly through the ROS and PPAR-α mediated innate immune signaling in zebrafish	Xiong, G. *et al.* Graphene oxide nanoparticles induce hepatic dysfunction through the regulation of innate immune signaling in zebrafish (Danio rerio). *Nanotoxicology* 14, 667–682 (2020).
graphene, graphene oxide	toxicity	Our study provided experimental evidences that carbonaceous NPs could be used as surrogate models to study the toxic mechanisms of PM. NPs and PM reduced cell viability, increased ROS production, and decreased ATP content to varying extents.	Wu, Y. *et al.* Comparative Toxic Effects of Manufactured Nanoparticles and Atmospheric Particulate Matter in Human Lung Epithelial Cells. *International journal of environmental research and public health* 18, (2020).
graphene oxide	toxicity	In this study, the differential distribution of GO in lobules in the liver, with a higher amount surrounding portal triad zones than the central vein zones is investigated. Even though no significant exhibition of overall hepatotoxicity could be observed in mice under relatively low-dose GO exposure, tremendous disruption of liver functional zonation patterns was uncovered, associated with detrimental changes in cellular components and signaling transduction, as evidenced by the surface adhesion molecule P450 superfamily members and other cardinal molecules involved in liver metabolism	Wu, Y., Feng, W., Liu, R., Xia, T. & Liu, S. Graphene Oxide Causes Disordered Zonation Due to Differential Intralobular Localizatio n in the Liver. *ACS nano* 14, 877–890 (2020).

MXene	toxicity	In this study, the cytotoxicity of Ti3C2 (the most studied MXenes) using primary neural stem cells (NSCs) and NSCs-derived differentiated cells was studied. It is found that Ti3C2 induced dose-dependent cytotoxicity to NSCs and NSCs-derived differentiated cells and 12.5 μg/mL Ti3C2 had no observable adverse effect. When the concentration reached 25 μg/mL, Ti3C2 nanosheets induced significant apoptosis and disturbed the cell membrane.	Wu, W. *et al.* Evaluating the Cytotoxicity of Ti3C2 MXene to Neural Stem Cells. *CHEMICAL RESEARCH IN TOXICOLOGY* 33, 2953–2962 (2020).
Few-layered black phosphorus	toxicity	In this study, the potential toxicity of BP on the unicellular organism, Tetrahymena thermophila was studied. After the exposure for 8 h at 10 μg/mL, the reproduction of T. thermophila significantly decreased by 46.3%. Severe cell membrane and cilium damage were observed. authors' study also revealed that BP induced the increase of intracellular reactive oxidant species and formed oxidative stress-dependent toxicity to T. thermophila.	Wu, Q. *et al.* Cellular Uptake of Few-Layered Black Phosphorus and the Toxicity to an Aquatic Unicellular Organism. *ENVIRONMENTAL SCIENCE & TECHNOLOGY* 54, 1583–1592 (2020).
graphene oxide	ecotoxicity	In this study, it is concluded that GO exposure decreased the root uptake area and root activity, and decreased the expression of NRTs, which may have consequently suppressed the NO3− uptake rate, leading to adverse nitrate accumulation in stressed plants.	Weng, Y. N. *et al.* Graphene oxide exposure suppresses nitrate uptake by roots of wheat seedlings. *ENVIRONMENTAL POLLUTION* 262, (2020).
graphene oxide	ecotoxicity	In the present study, the responses of nutrient removal performance and microbial community to long-term GO exposure were investigated. The results showed that reduction in performance of COD, ammonia and phosphate removals was observed during the whole experiment.	Wang, J. H., Zhang, L. Q., Zhang, S. Q., Li, S. G. & Yao, H. N. Responses of Performance and Microbial Community to Long-Term Graphene Oxide Exposure in a Sequencing Batch Reactor. *POLISH JOURNAL OF ENVIRONMENTAL STUDIES* 29, 4371–4384 (2020).
graphene, graphene oxide	toxicity	This study aimed to investigate whether mast cells influence the response of the epithelium upon irritant exposure. To conclude, it is found that the studied environmental irritants do not directly or indirectly activate HMC-1 MAST cells.	Van Den Broucke, S., Vanoirbeek, J., Alfaro-Moreno, E. & Hoet, P. Contribution of mast cells in irritant-induced airway epithelial barrier impairment*in vitro*. *TOXICOLOGY AND INDUSTRIAL HEALTH* 36, 823–834 (2020).

graphene oxide	ecotoxicity	this work aimed to assess the potential harm of GO to the environment by investigating the toxicity of GO to the M. sextelata, and the effects of M. sextelata to GO. Low GO concentrations stimulated the growth of M. sextelata meanwhile GO still tightly wrapped on the surface of the mycelia. However, a high GO concentration could inhibit the growth of M. sextelata and change the general morphology and microstructure of M. sextelata. Excessive emission of GO and the release of GO into M. sextelata lead to severe ecological consequence as the biomass and SOD activity of M. sextelata was partly lost at high GO concentrations	Tan, R. H. *et al.* Interaction between Graphene Oxide and the Mycelia of Morchella sextelata. *NANO* 15, (2020).
Pristine graphene	toxicity	In this work, multi-walled carbon nanotubes (MWCNT) and two different types of pristine GP in their potential to activate inflammasome NLRP3 (The nod-like receptor family pyrin domain containing 3) *in vitro* were compared. This study demonstrates a possible proinflammatory potential of GP and MWCNT acting through NLRP3 activation.	Svadlakova, T. *et al.* Proinflammatory Effect of Carbon-Based Nanomaterials: *In vitro* Study on Stimulation of Inflammasome NLRP3 via Destabilisation of Lysosomes. *NANOMATERIALS* 10, (2020).
graphene nanoplatelets	ecotoxicity	Here, an assessment to understand the toxicity of commercial polycarboxylate functionalized graphene nanoplatelets (GN) on the unicellular fungal model Saccharomyces cerevisiae was performed. While cell proliferation was not negatively affected even in the presence of 800 mg L−1 of the nanomaterial for 24 hours, oxidative stress was induced at a lower concentration (160 mg L−1), after short exposure periods (2 and 4 hours).	Suarez-Diez, M., Porras, S., Laguna-Teno, F., Schaap, P. J. & Tamayo-Ramos, J. A. Toxicological response of the model fungus Saccharomyces cerevisiae to different concentrations of commercial graphene nanoplatelets. *Scientific reports* 10, 3232–3232 (2020).
graphene oxide	ecotoxicity	Here, GO with positive and negative charges were synthesized. The negatively charged GO with the limited uptake has been found to cause enhancement in the chloroplast activity without any significant stress both in ex vivo and *in vivo*. Whereas, amine modified GO have been found to damage photosynthetic machinery by disturbing the photosystem structure.	Sharma, S. *et al.* Effect of galvanotaxic graphene oxide on chloroplast activity: Interaction quantified with Biolayer-Interferometry coupled confocal microscopy. *CARBON* 162, 147–156 (2020).
graphene oxide	toxicity	In the present study, two different sizes of native GO samples, GO and NanoGO, as well as PF-functionalized GO, GO-PF and NanoGO-PF, were prepared. Toxicological assessment of all GO samples (0– 100 µg/mL) on zebrafish embryonic developmental stages (survival, hatching and heart rates, and morphological changes) was recorded daily for up to 96 hours post-fertilization (hpf). The toxicity effects of each GO sample were observed to be higher at increasing concentrations and upon prolonged exposure. NanoGO demonstrated lower toxicity effects compared to GO.	Shamsi, S., Alagan, A. A., Sarchio, S. N. E. & Md Yasin, F. Synthesis, Characterization, and Toxicity Assessment of Pluronic F127-Functionalized Graphene Oxide on the Embryonic Development of Zebrafish (Danio rerio). *International journal of nanomedicine* 15, 8311–8329 (2020).

MoS2	toxicity	The aim of this investigation was to assess the effects of different MoS2 concentrations (5×10^{-1}, 5×10^{-2} and 5×10^{-3} mg/ml) on the embryonated eggs of Gallus gallus domesticus, according to Beck method. The investigation showed the toxicity of both MoS2 powders with deaths and growth defects.	Scalisi, E. M. *et al.* Toxicity assessment of two-dimensional nanomaterials molybdenum disulfide in Gallus gallus domesticus. *Ecotoxicology and environmental safety* 200, 110772–110772 (2020).
graphene	toxicity	The objective of authors' study was to explore the possible graphene impact on microorganism growth as well as on laboratory animal overall condition. authors' data confirm the applicability of graphene both in scientific and practical biomedical purposes.	Savicheva, A. *et al.* Secure application of graphene in medicine. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology* 36, 48–52 (2020).
graphene oxide, reduced graphene oxide	toxicity	Various *in vivo* studies have been conducted along with the in vitro studies to assess the genotoxic potential of graphene and its derivatives . Although the inflammatory effects of graphene and its derivatives were observed in different studies, little evidence is available confirming their genotoxic effect	Samadian, H. *et al.* Genotoxicity assessment of carbon-based nanomaterials; Have their unique physicochemical properties made them double-edged swords? *MUTATION RESEARCH-REVIEWS IN MUTATION RESEARCH* 783, (2020).
graphene oxide	toxicity	In this study, the effects of lateral dimensions of GO sheets in acute and chronic pulmonary responses after single intranasal instillation in mice were compared. Micrometer-sized GO induces stronger pulmonary inflammation than nanometer-sized GO, despite reduced translocation to the lungs.	Rodrigues, A. F. *et al.* Size-dependent pulmonary impact of graphene oxide: towards safe-by-design. *Advanced Science 7*, 1903200 (2020)
graphene oxide	ecotoxicity	In this study, the responses of wheat seedlings to graphene oxide (GO) were investigated at a wide concentration range of 0–1000 mg L^{-1}. GO exposure caused significant concentration-dependent membrane depolarization in roots, and significantly inhibited H+ efflux and extracellular Ca2+ influx in root cap.	Ramesh, A. M., Gangadhar, A., Chikkamadaiah, M., Krishnegowda, J. & Shivanna, S. Synthesis of graphene nanosheets by emitted black carbon and its sustainable applications. *JOURNAL OF ENVIRONMENTAL CHEMICAL ENGINEERING* 8, (2020).
few layer graphene	toxicity	GNS is highly stable and shows less toxicity against the Human embryonic kidney (HEK-293) cell lines and safer to the environment.	

graphene oxide nanosheets	toxicity	The cell viability significantly decreased when A549 cells only exposed to GO (called GO group); whilst more cells survived after exposure to GO regulated by Pb^{2+} (called GO + Pb group).	Qiang, S. R. *et al.* Effects of morphology regulated by Pb2+ on graphene oxide cytotoxicity: Spectroscopic and *in vitro* investigations. *MATERIALS CHEMISTRY AND PHYSICS* 239, (2020).
graphene oxide	toxicity	The objectives of the study were to determine the combinatorial actions of GO and antibiotics against Gram-positive and Gram-negative bacteria and the toxicological effects of GO towards human epidermal keratinocytes (HaCaT). Cytotoxicity of GO was found to be dose-dependent towards HaCaT cell line, it is found to impose negligible toxic effects against the skin cells at concentration below 100 μg/mL.	Pulingam, T. *et al.* Synergistic antibacterial actions of graphene oxide and antibiotics towards bacteria and the toxicological effects of graphene oxide on human epidermal keratinocytes. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 142, 105087–105087 (2020).
reduced graphene oxide	toxicity	The results show no genome-wide or global DNA methylation changes associated with either condition. authors' observations thus suggest that medium-term rGO exposure does not have significant effects on the DNA methylation patterns of human lung epithelial cells.	Pérez, R. F. *et al.* No genome-wide DNA methylation changes found associated with medium-term reduced graphene oxide exposure in human lung epithelial cells. *Epigenetics* 15, 283–293 (2020).
few layer graphene, graphene oxide	toxicity	this study investigates if HaCaT skin keratinocytes exposed to high concentrations of few-layer graphene (FLG) or partially dehydrated graphene oxide (d-GO) for a short time can recover from the cytotoxic insult. This study demonstrates a partial reversibility of GBM-induced cytotoxicity in skin HaCaT keratinocytes. In particular, recovery experiments suggest that the cytotoxicity induced by 24 h exposure to GBMs is only partially reduced by the material's removal followed by culturing of cells for additional 48 h.	Pelin, M. *et al.* Partial Reversibility of the Cytotoxic Effect Induced by Graphene-Based Materials in Skin Keratinocytes. *Nanomaterials (Basel, Switzerland)* 10, (2020).
graphene oxide nanosheets	toxicity	A single dose of sheets (100 μg) was intravenously administered to healthy rats (average weight – 220 g) and changes in hematological and biochemical parameters were recorded until 4 weeks. Cells (erythrocytes and SKBR3) incubated with bare sheets exhibited marked changes at the membrane surface. Histological examination of liver, spleen, lung and kidney showed the symptoms of injury which coincided with biochemical analyses.	Patil, R. *et al.* Biosafety assessment of P103 stabilized graphene oxide nanosheets. *MATERIALS TODAY COMMUNICATIONS* 25, (2020).

graphene oxide	toxicity	In this study, the potential consequences of accumulation and the fate of the spleen-residing GO over a period of nine months were studied. It is shown that thoroughly characterized GO materials are not associated with any detectable pathological consequences in the spleen.	Newman, L. *et al.* Splenic Capture and *in vivo* Intracellular Biodegradation of Biological-Grade Graphene Oxide Sheets. *ACS NANO* 14, 10168–10186 (2020).
graphene oxide	toxicity	Here, RNA sequencing is utilized to unearth responses of human lung cells to GO exposure to GO for 48 h results in mitochondrial dysfunction. In contrast, exposure to GO for 28 days is characterized by engagement of apoptosis pathways. These studies reveal the sensitivity of RNA-sequencing approaches and show that acute exposure to GO is not a good predictor of the long-term effects of GO.	Mukherjee, S. P. *et al.* Next-Generation Sequencing Reveals Differential Responses to Acute versus Long-Term Exposures to Graphene Oxide in Human Lung Cells. *Small (Weinheim an der Bergstrasse, Germany)* 16, e1907686–e1907686 (2020).
graphene oxide	toxicity	Purified and starting samples were tested toward human-derived fibroblastic cell culture. Strong correlation between cytotoxicity and purity of GO samples were revealed. Biocompatibility of GO can be improved by throughout removing of impurities introduced in oxidation step within GO synthesis.	Mrózek, O. *et al.* Salt-washed graphene oxide and its cytotoxicity. *Journal of hazardous materials* 398, 123114–123114 (2020).
graphene oxide nanoparticles	toxicity	this study estimated the possible genotoxicity and mutagenicity of GO nanoparticles as well as possible oxidative stress induction in the mice liver and brain tissues. Acute and subacute oral administration of GO nanoparticles induced genomic instability and mutagenicity by induction of oxidative stress in the mice liver and brain tissues.	Mohamed, H. R. H., Welson, M., Yaseen, A. E. & El-Ghor, A. A. Estimation of genomic instability and mutation induction by graphene oxide nanoparticles in mice liver and brain tissues. *Environmental science and pollution research international* 27, 264–278 (2020).
graphene	combined ecotoxicity	This study aimed to assess the joint effects of graphene and TPP on Mytilus galloprovincialis hemocytes. This study demonstrated that graphene and TPP could induce intracellular oxidative stress and cytotoxicity in mussel hemocytes.	Meng, X. *et al.* Toxicological effects of graphene on mussel Mytilus galloprovincialis hemocytes after individual and combined exposure with triphenyl phosphate. *Marine pollution bulletin* 151, 110838–110838 (2020).
graphene oxide	ecotoxicity	Herein, authors address the crucial quality criteria when evaluating the ecotoxicology of GO using an alga (Raphidocelis subcapitata) and a shrimp (Paratya australiensis). It was observed that under the same exposure conditions the behavior of GO and the estimated outcomes (IC50 values) in modified algae media differed in comparison to the referent media.Shrimps which were exposed to GO-algae aggregates via the food intake did not indicate stress or accumulation of GO.	Markovic, M. *et al.* Addressing challenges in providing a reliable ecotoxicology data for graphene-oxide (GO) using an algae (Raphidocelis subcapitata), and the trophic transfer consequence of GO-algae aggregates. *CHEMOSPHERE* 245, (2020).

graphene oxide	ecotoxicity	In this study the interaction of three differently oxidized GO systems with planktonic and benthic crustaceans are studied.These experiments proved that GO is not a hazardous material in complex aquatic environments because its acute toxicity can be successfully mitigated through the interaction with algae even at very high concentrations (25 mg/L).	Malina, T., Maršálková, E., Holá, K., Zbořil, R. & Maršálek, B. The environmental fate of graphene oxide in aquatic environment. Complete mitigation of its acute toxicity to planktonic and benthic crustaceans by algae. *Journal of hazardous materials* 399, 123027–123027 (2020).
boron nitride	toxicity	In this study, silkworm (Bombyx mori) was used as a model to investigate the toxicity of BN NSs, by continuously feeding silkworm larvae with BN NSs at various mass concentrations (1%, 2%, 3%, 4%). The results show that the exposure to BN NSs causes no obvious adverse effects on the growth, silk properties or tissues of silkworm, but the expressions of genes in midgut concerned with some specific functions and pathways are significantly changed, indicating that BN NSs may have potential danger to lead to dysfunction.	Ma, L. *et al. in vivo* toxicity evaluation of boron nitride nanosheets in Bombyx mori silkworm model. *Chemosphere* 247, 125877–125877 (2020).
graphene oxide	toxicity	In this study, the interactions between THP-1 macrophages and GO of different sizes (GO of size 500–5000 nm, denoted as GO-L; GO of size < 500 nm, denoted as GO-S) was investigated. In conclusion, the result suggested that GO size-dependently altered lipid profiles in THP-1 macrophages that might be related with PPAR signaling pathway.	Luo, Y., Peng, J., Huang, C. & Cao, Y. Graphene oxide size-dependently altered lipid profiles in THP-1 macrophages. *Ecotoxicology and environmental safety* 199, 110714–110714 (2020).
graphene oxide nanoplatelets, graphene oxide nanoribbons	toxicity	In this work, the GODs with a wide range of shapes (sheets, helical/longitudinal ribbons, caps, dots), sizes (10 nm to 20 μm), and chemistry (partially to fully oxidized) are synthesized, and their cytotoxicity in normal cells (NIH3T3) and colon cancer cells (HCT116) are evaluated. the extent of GOD-induced cytotoxicity (reduction of cell viability) to the two cell lines is similar	Lu, P. *et al.* Mechanistic Insights into the Cytotoxicity of Graphene Oxide Derivatives in Mammalian Cells. *Chemical research in toxicology* 33, 2247–2260 (2020).
graphene oxide	toxicity	In this study, Caenorhabditis elegans was used as an *in vivo* assay model to investigate the effect of GO exposure on intestinal Wnt signaling. Results imply that the association of dysregulation in physiological and functional states of intestinal barrier with toxicity induction of GO in organisms.	Liu, P., Shao, H., Kong, Y. & Wang, D. Effect of graphene oxide exposure on intestinal Wnt signaling in nematode Caenorhabditis elegans. *Journal of environmental sciences (China)* 88, 200–208 (2020).
few layer graphene	toxicity	The effects of the GNSs on plasma membrane, lysosomal and mitochondrial membranes were investigated using rat basophilic leukemia (RBLsingle bond2H3) cells. Specially, the exposure to 25 mg/L GNSs caused severest cell mortality, plasma membrane damage, ROS generation, MMP depolarization and apoptosis.	Liu, L., Zhang, M. M., Zhang, Q. & Jiang, W. Graphene nanosheets damage the lysosomal and mitochondrial membranes and induce the apoptosis of RBL-2H3 cells. *SCIENCE OF THE TOTAL ENVIRONMENT* 734, (2020).

graphene oxide	toxicity	In this study it is shown that blood exposure to GO under the maximum safe starting dose may cause accidental death of mammals, including non-human primates (1 in 5 Macaca fascicularis and 7 in 121 mice), while remains general amenable in others.	Lin, Y. F. *et al.* Blood exposure to graphene oxide may cause anaphylactic death in non-human primates. *NANO TODAY* 35, (2020).
graphene oxide	ecotoxicity	In this study, the effect of reduced graphene oxide synthesized from eucalyptus leaf extracts (EL-rGO) on Zn^{2+} and Cu^{2+} bioleaching from sludge was investigated. These new findings provide evidence that green synthesized rGO is toxic to microorganisms and that toxicity increased with rGO dose.	Lin, J., Xue, C., Guo, S., Owens, G. & Chen, Z. Impact of green reduced graphene oxide on sewage sludge bioleaching with Acidithiobacillus ferrooxidanse. *Environmental pollution (Barking, Essex : 1987)* 267, 115455–115455 (2020).
few layer graphene, MoS2	toxicity	In this study, M1 and M2 macrophage viability and activation are mainly found to be unaffected by few-layer graphene (FLG) and MoS2 at doses up to 50 μg mL−1. Overall, FLG and MoS2 are of little toxicity in human macrophages even though they are found to trigger cell stress and inflammatory responses.	Lin, H. *et al.* Comparative Effects of Graphene and Molybdenum Disulfide on Human Macrophage Toxicity. *Small (Weinheim an der Bergstrasse, Germany)* 16, e2002194–e2002194 (2020).
graphene, graphene oxide	ecotoxicity	In this study, the interaction of graphene oxide (GO) or graphene (G) at different concentrations with microbial communities in sequential batch reactors was investigated. Further, it was exhibited that the composition and dynamic of microbial communities changed under GFNs exposure by highthroughput sequencing. Molecular ecological networks showed that there were more complex and negative interaction in the presence of GFNs	Lian, S. *et al.* Interaction of graphene-family nanomaterials with microbial communities in sequential batch reactors revealed by high-throughput sequencing. *Environmental research* 184, 109392–109392 (2020).
black phosphorus nanosheets	ecotoxicity	In this study, a series of toxicity tests and discussed the underlying toxic mechanisms of BPNSs on Chlorella vulgaris (C. vulgaris) were carried out. After a 120-h exposure, BPNSs at 1 mg/L promoted the growth of C. vulgaris, while BPNSs at higher concentrations (5 and 10 mg/L) inhibited its growth.	Li, P. *et al.* Perturbation of Normal Algal Growth by Black Phosphorus Nanosheets: The Role of Degradation. *ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS* 7, 35–41 (2020).
graphene oxide	combined ecotoxicity	Xenopus laevis (X. laevis) tadpoles were exposed to various concentrations of typical pyrethroid (cis-bifenthrin; cis-BF), either alone or in combination with graphene oxide (GO), for 21 days. These results indicate that GO enhance the bioconcentration of cis-BF and promote the conversion of its 1R-enantiomer to the 1S form, which lead to disruption of neurotransmitter systems as well as interference in metamorphic development.	Li, M. *et al.* Coexposure to environmental concentrations of cis-bifenthrin and graphene oxide: Adverse effects on the nervous system during metamorphic development of Xenopus laevis. *Journal of hazardous materials* 381, 120995–120995 (2020).

graphene oxide nanosheets	combined ecotoxicity	In this study, the mutual effects of cadmium (Cd) at 1 mg/L and different concentrated GO nanosheets (0, 1 and 10 mg/L) on the rice seed germination, further seedling growth, Cd uptake and accumulation in rice roots and shoots in a hydroponic system was investigated. GO nanosheets could uptake and transport water due to their hydrophilic nature, thus promoting the rice seed germination and further seedling growth. The inhibitive effects of Cd on the seed germination was alleviated by the co-accurring GO nanosheets. Besides, the presence of GO nanosheets at 10 mg/L improved Cd uptake	Li, J. *et al.* The mutual effects of graphene oxide nanosheets and cadmium on the growth, cadmium uptake and accumulation in rice. *Plant physiology and biochemistry : PPB* 147, 289–294 (2020).
carbon blacks, graphene nanoplatelets	toxicity, method	In this study, it is developed an improved lung burden assay for CNMs with an accuracy > 94% and a recovery rate > 90% using PK digestion and UV-Vis spectrophotometry. This method can be applied to any nanomaterial with sufficient absorbance in the near-infrared band and can differentiate nanomaterials from elements in the body, as well as the soluble fraction of the nanomaterial. Furthermore, a combination of PK digestion and other instrumental analysis specific to the nanomaterial can be applied to organ burden analysis.	Lee, D.-K., Jeon, S., Jeong, J., Song, K. S. & Cho, W.-S. Carbon nanomaterial-derived lung burden analysis using UV-Vis spectrophotometry and proteinase K digestion. *Particle and fibre toxicology* 17, 43–43 (2020).
graphene oxide	ecotoxicity	Here, an assessment to understand the toxicity of different commercial graphene oxide nanomaterials on the unicellular fungal model organism Saccharomyces cerevisiae was performed. In addition, an assessment to understand the toxicity of different commercial graphene oxide nanomaterials on the unicellular fungal model organism Saccharomyces cerevisiae was performed. Significant common and specific changes in genes linked to homeostasis and ribosomal indicate major changes in the physiological state of yeast cells in the presence of these nanomaterials.	Laguna-Teno, F., Suarez-Diez, M. & Tamayo-Ramos, J. A. Commonalities and Differences in the Transcriptional Response of the Model Fungus Saccharomyces cerevisiae to Different Commercial Graphene Oxide Materials. *Frontiers in microbiology* 11, 1943–1943 (2020).
carbon black	toxicity	Here, macrophage-like THP-1 cells, exposed to ten different CNM for 48 h in low-cytotoxic concentration of 10 μg mL^{-1}, are characterized by secretion of different cytokines and global transcriptional changes. Taken together, authors' results suggest that complex regulatory events, resulting from both ENM exposure and secondary microenvironment changes, trigger macrophages to move toward M1 and M2 activation.	Kinaret, P. A. S., Scala, G., Federico, A., Sund, J. & Greco, D. Carbon Nanomaterials Promote M1/M2 Macrophage Activation. *SMALL* 16, (2020).
graphene oxide	toxicity	In this study, neurotoxic potential of GO and its underlying molecular and cellular mechanism were investigated using the nematode, Caenorhabditis elegans. The present study suggested the GO possesses neurotoxic potential, especially on neurotransmitters and AFD neuron in C. elegans	Kim, M., Eom, H.-J., Choi, I., Hong, J. & Choi, J. Graphene oxide-induced neurotoxicity on neurotransmitters, AFD neurons and locomotive behavior in Caenorhabditis elegans. *Neurotoxicology* 77, 30–39 (2020).

graphene oxide	combined ecotoxicity	To examine this relationship, the marine shrimp Litopenaeus vannamei was exposed for 48 h to As, graphene oxide (GO; two different concentrations) or a combination of both. The main finding was that GO modulated the As toxic effect	Josende, M. E. *et al.* Graphene oxide and GST-omega enzyme: An interaction that affects arsenic metabolism in the shrimp Litopenaeus vannamei. *The Science of the total environment* 716, 136893–136893 (2020).
graphene oxide	ecotoxicity	The effects of different concentrations of graphene oxide (GO) on intracellular metabolism in Chlorella vulgaris (C. vulgaris) and removal of nitrogen and phosphorus nutrients by C. vulgaris from synthetic wastewater were studied. High concentrations of 10 mg/L GO affected the ultrastructure, causing severe plasmolysis. Furthermore, severe GO stress dramatically reduced the generation of protein-like EPS. The biological responses to GO included increase in ROS level, inhibition of saccharide metabolism, and degradation of amino acids. Furthermore, high concentrations of GO weakened the carbon fixation process in algal cells.	Ji, X. Y., Li, X., Wu, S. C., Hou, M. F. & Zhao, Y. J. Effects of graphene oxide on algal cellular stress response: Evaluating metabolic characters of carbon fixation and nutrient removal. *CHEMOSPHERE* 252, (2020).
graphene oxide	toxicity	The effect of two concentrations (100 and 200 µg/mL) of nano- and microsized graphene oxide (nGO and mGO) on apoptosis, cell cycle, and ROS generation was studied. The effect of both sizes on viability and genotoxicity of the embryonic fibroblast cell cycle was evaluated. In conclusion, graphene oxide at both nano- and micro-scale damages cell physiology and increases cell population in the S phase of the cell cycle.	Hashemi, E. *et al.* Graphene Oxide Negatively Regulates Cell Cycle in Embryonic Fibroblast Cells. *INTERNATIONAL JOURNAL OF NANOMEDICINE* 15, 6201–6209 (2020).
reduced graphene oxide	ecotoxicity	Rice seedlings were exposed to different types of carbon-based nanomaterials (CNMs), including reduced graphene oxide (rGO), multi-walled carbon nanotubes (MWCNTs), and fullerene (C60), at 10–250 mg L^{-1} under hydroponic conditions for 20 days to investigate the impact of CMN exposure on the composition of the rice endophyte community. The results suggest that fungal endophytes in rice were sensitive to CNM exposure even at 10 mg L^{-1} CNM exposures.	Hao, Y. *et al.* Carbon-based nanomaterials alter the composition of the fungal endophyte community in rice (Oryza sativa L.). *ENVIRONMENTAL SCIENCE-NANO* 7, 2047–2060 (2020).
reduced graphene oxide, carboxylated graphene nanosheets, hydroxylated graphene nanosheets, aminated graphene nanosheets	toxicity	Here, the effects of reduced graphene oxide (RGO), and carboxylated (G-COOH), hydroxylated (G-OH) and aminated (G-NH_2) graphene nanosheets on human neuroblastoma cells (SK-N-SH) were compared. All GFNs inhibited cellular growth at concentrations of 0.1–10 mg L^{-1} after 24 h exposure. The toxicity was attenuated over longer exposure times, with the exception of G-NH2. This study provides significant insights into the neurological effects of GFNs, and suggests that G-NH2 is not as safe as reported in many previous studies.	Guo, Z. *et al.* Elucidating the mechanism of the surface functionalization dependent neurotoxicity of graphene family nanomaterials. *Nanoscale* 12, 18600–18605 (2020).

graphene oxide	toxicity	In this study, Caenorhabditis elegans as an *in vivo* model to investigate the biological function and molecular basis of mir-235 in the regulation of GO toxicity was used. The results indicate that mir-235 mediates a protective mechanism against GO toxicity by suppressing the function of DAF-12-DAF-16 and DAF-12-PMK-1 signaling cascade in the intestine of nematodes	Guo, T. *et al.* The C. elegans miR-235 regulates the toxicity of graphene oxide via targeting the nuclear hormone receptor DAF-12 in the intestine. *Scientific reports* 10, 16933–16933 (2020).
nanographene oxide	toxicity	The purpose of this study was to evaluate the biocompatibility potential of graphene oxide (nGO), chitosan functionalized graphene oxide (nGO-CS), and carboxylated graphene (nGO-COOH) when exposed to human dental pulp stem cells (hDPSCs). These results demonstrated the lower toxicity and higher cytocompatibility of nGO-CS and nGO-COOH compared to nGO. nGO-COOH not only has any adverse effect on the cell membrane and mitochondrial activity but also shows slight antioxidant activity at some concentrations.	Gholami, A. *et al.* Functionalization of Graphene Oxide Nanosheets Can Reduce Their Cytotoxicity to Dental Pulp Stem Cells. *JOURNAL OF NANOMATERIALS* 2020, (2020).
Carbon nanohorns, carbon nanoplatelets, graphene oxide, reduced graphene oxide	toxicity	In this study, *in vitro* toxicity of various carbon nanomaterials in human epithelial colorectal adenocarcinoma (Caco-2) cells and human breast adenocarcinoma (MCF-7) cells are investigated. Carbon nanohorns (CNH), carbon nanotubes (CNT), carbon nanoplatelets (CNP), graphene oxide (GO), reduced graphene oxide (GO) and nanodiamonds (ND) were systematically compared, using Pluronic F-127 dispersant. Cell viability after carbon nanomaterial treatment followed the order CNP < CNH < RGO < CNT < GO < ND, being the effect more pronounced on the more rapidly dividing Caco-2 cells.	Garriga, R. *et al.* Toxicity of Carbon Nanomaterials and Their Potential Application as Drug Delivery Systems: *In vitro* Studies in Caco-2 and MCF-7 Cell Lines. *Nanomaterials (Basel, Switzerland)* 10, (2020).
few layer graphene, graphene oxide, reduced graphene oxide	toxicity, method	Skin irritation was assessed using the SkinEthic™ Reconstructed human Epidermis (RhE), following the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 439. Even though not validated for nanomaterials, the OCED TG 439 turned out to be applicable also for GBM testing, since no interference with the methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction, used as a final readout, was found. On the whole, these results demonstrate that GBMs prepared with non-irritant exfoliation agents do not induce skin irritation after a single acute exposure.	Fusco, L. *et al.* Skin irritation potential of graphene-based materials using a non-animal test. *Nanoscale* 12, 610–622 (2020).
few layer graphene	toxicity, method	In the work described here, the effect of sub-lethal doses of sFLG on the biology of human HaCaT keratinocytes was examined. A powerful methodology is described to detect changes that are not evident with other classical techniques. It was observed that sFLG generates a whole series of changes in human skin cells, even on applying sub-lethal doses. In a multi-experimental approach, a remodeling of the cellular energetic metabolism was observed along with alterations in the calcium and redox homeostasis. This metabolic reshaping shares some characteristics with classic tumor cell metabolism.	Frontiñan-Rubio, J., Gomez, M. V., González, V. J., Durán-Prado, M. & Vázquez, E. Sublethal exposure of small few-layer graphene promotes metabolic alterations in human skin cells. *Scientific reports* 10, 18407–18407 (2020).

graphene oxide	ecotoxicity	Two strains of Acheta domesticus, which are selected for longevity, were tested. The main aim was to investigate how GO, when administrated in food, affects: the condition of cells, DNA stability, ROS generation and the reproduction potential (the Vitellogenin (Vg) protein expression). The "recovery effect" – after removing GO from the diet for 15 days – was also measured. he results indicated a GO concentration-dependent outcome. In both strains, removing the GO from the food led to a high Vg expression.	Flasz, B., Dziewiecka, M., Kedziorski, A., Tarnawska, M. & Augustyniak, M. Vitellogenin expression, DNA damage, health status of cells and catalase activity in Acheta domesticus selected according to their longevity after graphene oxide treatment. *SCIENCE OF THE TOTAL ENVIRONMENT* 737, (2020).
graphene oxide	ecotoxicity	This study is aimed to comprehensively assess the acute toxicity of a well-characterized GO suspension to Daphnia magna. Conventional ecotoxicological endpoints (lethality, immobilization) and more sensitive, sublethal endpoints (heartbeat rate, feeding activity, and reactive oxygen species (ROS)) production were used. Alterations of normal physiology (heart rate) and feeding activity may be associated with increased risk of predation and reproductive decline, highlighting that GO may have impacts on population and food web dynamics in aquatic ecosystems.	Fekete-Kertesz, I. *et al.* Ecotoxicity Assessment of Graphene Oxide by Daphnia magna through a Multimarker Approach from the Molecular to the Physiological Level including Behavioral Changes. *NANOMATERIALS* 10, (2020).
graphene, graphene oxide	toxicity, method	The present study indicated some toxic effects of graphene-based materials through systems toxicology assessment. Integrating gene expression and PPI network may help explaining biological responses of graphene. By using the topological and functional investigations, authors identified several key genes involved in graphene–cell interactions, including CDK1, CCNB1, PLK1, TOP2A, and CCNA2. Toxicity can occur by the disruption of activity of cell cycle regulators	Farahani, M., Rezaei-Tavirani, M., Zali, H., Hatamie, S. & Ghasemi, N. Systems toxicology assessment revealed the impact of graphene-based materials on cell cycle regulators. *Journal of biomedical materials research. Part A* 108, 1520–1533 (2020).
graphene oxide	ecotoxicity	In this work, experiments were achieved using microcosm systems to expose a reconstituted food chain to GO at environmentally-relevant concentrations (0.05 and $0.1\,mg\,L^{-1}$). Monitoring of multiple ecotoxicological and ecological endpoints allowed to observe changes in bacterial communities while no toxic effects were noticed in chironomids. However, chironomids feeding behaviour changed as a consequence of GO contamination, leading to an increase in leaf litter consumption. Genotoxic effects were noticed in Pleurodeles larvae.	Evariste, L. *et al.* Assessment of graphene oxide ecotoxicity at several trophic levels using aquatic microcosms. *CARBON* 156, 261–271 (2020).

graphene oxide	toxicity	In this study authors investigate the multigenerational effects of graphene oxide (GO) which was given to Acheta domesticus in low doses (0.2, 2 and 20 $\mu g \cdot g^{-1}$ of food) for three subsequent generations. Low concentrations of GO cause differences in the life cycle of A. domesticus DNA damage was increased in the third generation of insects. During three generations of insects decrease of fertility was observed	Dziewięcka, M. *et al.* Graphene oxide as a new anthropogenic stress factor - multigenerational study at the molecular, cellular, individual and population level of Acheta domesticus. *JOURNAL OF HAZARDOUS MATERIALS* 396, 122775–122775 (2020).
graphene oxide	ecotoxicity	In the present study, the rice seedlings were exposed to GO (5 mg/L) under hydroponic condition for fifteen days with periodic stir. The cellular structures damage, GO deposition and oxidative stress were found in rice root after GO exposure. The relative abundance of many endophytic bacterial communities in rice roots decreased due to GO exposure. The relative abundance of Gram-negative, stress-tolerant and biofilm-forming phenotypes were predicted to increase by BugBase.	Du, J. *et al.* Graphene oxide enters the rice roots and disturbs the endophytic bacterial communities. *Ecotoxicology and environmental safety* 192, 110304–110304 (2020).
graphene oxide	toxicity	The resulting cytotoxicity tests confirm that the materials had no toxic effects against dental cells after 24 h. Following graphene dental materials implantation, the animals did not present any symptoms of acute toxicity or local inflammation. No alterations were detected in relative organ weights and in correlation with hepatic and renal histological findings. The materials' lack of systemic organ toxicity was confirmed.	Dreanca, A. *et al.* Systemic and Local Biocompatibility Assessment of Graphene Composite Dental Materials in Experimental Mandibular Bone Defect. *Materials (Basel, Switzerland)* 13, (2020).
graphene oxide	toxicity	The obtained results using distinct unicellular models and biomolecules display significant changes in the toxicological potential of GO and GOC: the former had a higher ability to induce oxidative stress in human alveolar carcinoma epithelial cells A549, and the yeast Saccharomyces cerevisiae, while provoking a higher luminescence inhibition capacity on the bacteria Vibrio fischeri too.	Domi, B. *et al.* Interaction Analysis of Commercial Graphene Oxide Nanoparticles with Unicellular Systems and Biomolecules. *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 21, (2020).
graphene oxide	toxicity	In this work, authors studied the impact of repeated exposure of GO on a three-dimensional (3D) reconstruct of human bronchial tissue, using a nebulizer system focusing on short-term effects. Experimental results did not show strong toxic effects of GO in terms of viability and integrity of the lung tissue. However, since 2 weeks of treatment, repeated GO exposure elicited a proinflammatory response, moderate barrier impairment, and autophagosome accumulation. These findings indicate that prolonged exposure to GO produces a time window (during the 30 days of treatment set for this study) for which GO-mediated autophagy inhibition along with inflammation may potentially increase the susceptibility of exposed humans to pulmonary infections and/or lung diseases.	Di Cristo, L. *et al.* Repeated exposure to aerosolized graphene oxide mediates autophagy inhibition and inflammation in a three-dimensional human airway model. *Materials today. Bio* 6, 100050–100050 (2020).

graphene oxide	combined ecotoxicity	The metabolic effects of such nanomaterials and their combination with two common pollutants, zinc and cadmium, on the freshwater fish Geophagus iporangensis are analyzed. this metabolic rate alone reveals that combined exposure potentiates effects of trace elements on fish metabolism.	de Medeiros, A. M. Z., Coa, F., Alves, O. L., Martinez, D. S. T. & Barbieri, E. Metabolic effects in the freshwater fish Geophagus iporangensis in response to single and combined exposure to graphene oxide and trace elements. *CHEMOSPHERE* 243, (2020).
graphene oxide	toxicity	Present study was designed to evaluate GO as an endocrine disrupting chemical (EDC) using the model Japanese medaka (Oryzias latipes). LD50 was found to be sex-dependent. Fecundity tended to reduce in a dose-dependent manner during early post-injection days; however, the overall evaluation showed no significant difference. The hatchability of embryos was reduced significantly in the 200 µg/g group; edema (yolk and cardiovascular) and embryo-mortality remained unaltered. Histopathological assessment identified black particles, probably agglomerated GO, in the gonads of GO-treated fish. However, folliculogenesis in stromal compartments of ovary and the composition of germinal elements in testis remained almost unaltered. Moreover, granulosa and Leydig cells morphology did not indicate any significant EDC-related effects.	Dasmahapatra, A. K., Powe, D. K., Dasari, T. P. S. & Tchounwou, P. B. Assessment of reproductive and developmental effects of graphene oxide on Japanese medaka (Oryzias latipes). *Chemosphere* 259, 127221–127221 (2020).
graphene like layer	toxicity	This study represents an important first report of a complete *in vivo* toxicity assessment of GL layers on embryonic zebrafish (Danio rerio). authors' results show that GL layers do not lead to any perturbations in the different biological parameters evaluated, indicating their good biocompatibility on a vertebrate model.	d'Amora, M., Alfe, M., Gargiulo, V. & Giordani, S. Graphene-Like Layers from Carbon Black: *in vivo* Toxicity Assessment. *Nanomaterials (Basel, Switzerland)* 10, (2020).
graphene oxide	toxicity	Interactions and cellular responses were assessed *in vitro* using both classic cell lines and patient derived GSCs. no significant toxicity was detected in brain astrocytes and endothelial cells.	Costa, P. M. *et al.* Selective toxicity of functionalised graphene oxide to patients-derived glioblastoma stem cells and minimal toxicity to non-cancerous brain tissue cells. *2D MATERIALS* 7, (2020).
graphene oxide	toxicity	In this work, authors exploited an integrated approach combining systematic analysis of cytotoxicity, angiogenic potential, and metabolomics to shed light on the effects of graphene oxide (GO) on primary human endothelial Huvec cells. Significant toxicity was found in Huvec cells at high GO concentrations (25 and 50 µg/mL). the combination of the physical hindrance of internalized GO aggregates, induction of oxidative stress, and alteration of some metabolic pathways leads to a significant antiangiogenic effect in primary human endothelial cells.	Cibecchini, G. *et al.* Antiangiogenic Effect of Graphene Oxide in Primary Human Endothelial Cells. *ACS applied materials & interfaces* 12, 22507–22518 (2020).

graphene oxide	toxicity	In the present study, authors systematically investigated the dose-dependent effects of three different-sized GO particles (50–200 nm, <500 nm, and >500 nm) on zebrafish during the very early developmental stages (4–124 h post-fertilization). Developmental toxicity and their effects on larval behaviors were observed. The occurrence of oxidative stress and the induction of apoptosis are suggested. The impact was dependent on exposure concentration and associated particle size.	Chen, Z. *et al.* Toxic effects of different-sized graphene oxide particles on zebrafish embryonic developmen t. *Ecotoxicology and environmental safety* 197, 110608–110608 (2020).
graphene oxide, reduced graphene oxide	toxicity, method	In this study, autors reported on the use of label-free mass spectrometry and mass spectrometry imaging (MSI) for graphene oxide (GO) and reduced graphene oxide (rGO) analyses in rodent tissues.our study confirms that LDI-MSI is a relevant approach for biodistribution studies of carbon-based nanoparticles, as quantification can be achieved	Cazier, H. *et al.* Development of a Mass Spectrometry Imaging Method for Detecting and Mapping Graphene Oxide Nanoparticles in Rodent Tissues. *JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY* 31, 1025–1036 (2020).
graphene oxide	ecotoxicity	this study was carried out to assess the toxic effects of GO on Artemia franciscana, a well-established model organism for marine ecotoxicological studies. A. franciscana stage I nauplii or adults were exposed to GO (1–100 μg mL^{-1}) up to 72 h, which induced a significant mortality only in adults exposed to the highest concentration for 72 h.	Cavion, F. *et al.* Ecotoxicological impact of graphene oxide: toxic effects on the model organism Artemia franciscana. *ENVIRONMENTAL SCIENCE-NANO* 7, 3605–3615 (2020).
few-layer graphene, graphene oxide, reduced graphene oxide	ecotoxicity	Here, Corylus avellana L. (common Hazel) pollen was germinated in-vitro with and without 1−100 μg mL^{-1} few-layer graphene (FLG), GO and reduced GO (rGO) to identify GRMs effects. Few Layer Graphene and graphene oxide affect sexual reproduction of a seed plant. GO affects pollen tube growth by lowering extracellular pH and Ca^{2+} bioavailability. Both FLG and GO alter the tip-focused ROS distribution along the pollen tube. Reduced GO affects neither pollen germination nor pollen tube elongation.	Candotto Carniel, F. *et al.* Beyond graphene oxide acidity: Novel insights into graphene related materials effects on the sexual reproduction of seed plants. *JOURNAL OF HAZARDOUS MATERIALS* 393, 122380–122380 (2020).
graphene oxide	combined ecotoxicity	the main goal of this study was to evaluate the effect of exposure of clam Ruditapes philippinarum to GO when acting alone and in the combination with copper (Cu), under two pH levels (control 7.8 and 7.3). it can be stated that the effects induced by carbon nanomaterials (GO) and/or metals (Cu) in clams were strongly modulated by abiotic factors such as pH, since it was observed that clams living in control pH (7.8) were slightly affected by isolated or under co-exposure of GO and Cu, whereas animals at low pH showed increased defenses (ETS and GSTs activities and GSH levels) although without induction of cellular damage (LPO).	Britto, R. S. *et al.* The effects of co-exposure of graphene oxide and copper under different pH conditions in Manila clam Ruditapes philippinarum. *Environmental science and pollution research international* 27, 30945–30956 (2020).

graphene oxide	toxicity	Toxicological assessment of the GO small intestinal digesta over 24 h does not point to acute cytotoxicity, and examination of the intestinal epithelium under electron microscopy does not reveal histological alterations.	Bitounis, D. *et al.* Synthesis and Physicochemical Transformations of Size-Sorted Graphene Oxide during Simulated Digestion and Its Toxicological Assessment against an *In vitro* Model of the Human Intestinal Epithelium. *Small (Weinheim an der Bergstrasse, Germany)* 16, e1907640–e1907640 (2020).
graphene oxide, few-layer graphene, multi-layer graphene	toxicity	On human endothelial cells, the graphenes showed antioxidant effects and were less toxic than on colon cancer cells, especially the one with low content of nitrogen (NGr-1).	Baldea, I. *et al.* Cytotoxicity mechanisms of nitrogen-doped graphene obtained by electrochemical exfoliation of graphite rods, on human endothelial and colon cancer cells. *CARBON* 158, 267–281 (2020).
MXene	toxicity	Herein, authors aim to scrutinize the potential toxicity of MXene nanosheets on the early stage of the embryo as well as angiogenesis. authors' study clearly suggests that MXene at studied concentration might induce a toxic effect on the early stage of embryogenesis	Alhussain, H. *et al.* MXene Nanosheets May Induce Toxic Effect on the Early Stage of Embryogenesis. *Journal of biomedical nanotechnology* 16, 364–372 (2020).
reduced graphene oxide	toxicity	Nanomaterials of rGO and TiO2 (anatase and rutile) exhibited toxicity impact towards both B. subtilis and E. coli. In general, anatase TiO2 is more toxic compared to rGO and rutile TiO2. NMs caused reduction in growth rate for B. subtilis with significant reduction in growth rate constant. In contrast, observation on the toxicity impact was slightly different in E. coli, where upon NMs exposure, the early onset of death phase was observed.	Ahmad, N. S., Abdullah, N. & Yasin, F. M. Toxicity assessment of reduced graphene oxide and titanium dioxide nanomaterials on Gram-positive and Gram-negative bacteria under normal laboratory lighting condition. *Toxicology reports* 7, 693–699 (2020).
reduced graphene oxide	Combined toxicity	Combined effects of RGO and Cd was investigated in HepG2 cells. RGO alone were not toxic whereas Cd alone induced toxicity in HepG2 cells. In co-exposure group, RGO effectively mitigates Cd-induced cytotoxicity & apoptosis. In co-exposure group, RGO significantly attenuates Cd-induced oxidative stress. Toxicity of Cd was alleviated due to its strong adsorption on RGO surface.	Ahamed, M., Akhtar, M. J., Khan, M. A. M. & Alhadlaq, H. A. Reduced graphene oxide mitigates cadmium-induced cytotoxicity and oxidative stress in HepG2 cells. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 143, 111515–111515 (2020).

graphene oxide	ecotoxicity	In this study, the toxic effects of GO at concentration of 10 and 100 mg/L in 4 h and 10 days were evaluated with sealed reactors operated in sequencing batch mode. Short-term exposure of GO had no negative effect on nitrification and N2O emission. Long-term exposure of GO weakened nitrifying capability and promoted N2O generation. GO enhanced ROS generation and impaired the antioxidant enzymes SOD and CAT. The inhibition of GO to NOB and CAOB led to higher production of NO- 2-N and N2O.	Zhou, N. *et al.* The effects of graphene oxide on nitrification and N2O emission: Dose and exposure time dependent. *Environmental pollution (Barking, Essex : 1987)* 252, 960–966 (2019).
graphene, graphene oxide, reduced graphene oxide	toxicity	In this study, zebrafish were fed diets containing three GFNs, namely, monolayer graphene powder (GR), graphene oxide nanosheet (GO) and reduced graphene oxide powder (rGO), or appropriate control for 21 days. Only GR markedly reduced diversity of gut microbiota. GR, GO and rGO caused critical taxa alternation. GFNs exposure lead to more damage in intestines by generating more vacuolation and goblet cells. GFNs pose potential health risks to aquatic organisms through alteration of gut microbiota.	Zheng, M. *et al.* Dysbiosis of gut microbiota by dietary exposure of three graphene-family materials in zebrafish (Danio rerio). *Environmental pollution (Barking, Essex : 1987)* 254, 112969–112969 (2019).
graphene, graphene oxide, reduced graphene oxide	ecotoxicity	This work systematically investigated the effect of natural organic matter on the toxicity of GFNs to algae (Chlorella pyrenoidosa). Toxicity antagonism was observed between humic acid (HA) and all the three types of GFNs, and the degree of antagonism in the presence of HA followed the order reduced graphene oxide (rGO) > graphene oxide (GO) > graphene (G).	Zhao, J. *et al.* Humic acid mitigated toxicity of graphene-family materials to algae through reducing oxidative stress and heteroaggregation. *ENVIRONMENTAL SCIENCE-NANO* 6, 1909–1920 (2019).
graphene oxide	ecotoxicity	In this study, authors investigated the photochemical reactivity of four types of GO with different oxidation degrees in aqueous environment, and their related toxicity to two bacterial models Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) was further compared. The quantity of ROS increased with increasing GO oxidation degrees. Toxicity of GO to E. coli and S. aureus were enhanced after photo-transformation. Better charge transfer and smaller size contributed to stronger toxicity of RGO. This study revealed that the oxidation degrees play important roles in photochemical transformation and the resulting toxicity of GO	Zhao, F.-F. *et al.* Effects of oxidation degree on photo-transformation and the resulting toxicity of graphene oxide in aqueous environment. *Environmental pollution (Barking, Essex : 1987)* 249, 1106–1114 (2019).
graphene oxide	ecotoxicity	In this study, authors conducted several toxicity tests (*i.e.*, acute toxicity and oxidative damage) with Scenedesmus obliquus (S. obliquus) and Daphnia magna (D. magna), as well as a chronic toxicity test with D. magna, to investigate the toxicity of GO with or without the presence of humic acid (HA). authors' results showed that GO induced significant toxicity to S. obliquus and D. magna, and the median lethal concentrations (72 h-LC50 and 48 h-LC50) for acute toxicity were 20.6 and 84.2 mg L^{-1}, respectively, while the 21 d-LC50 for chronic toxicity to D. magna was 3.3 mg L^{-1}. authors' study provides useful and basic biotoxicity data of GO with a consideration of its interaction with NOM which could aid in preventing an overestimation of the risks of GO to the natural aquatic environment.	Zhang, Y. *et al.* The effects of humic acid on the toxicity of graphene oxide to Scenedesmus obliquus and Daphnia magna. *The Science of the total environment* 649, 163–171 (2019).

carbon nitride nanosheets	toxicity	In this study, authors systematically investigate the potential cytotoxicity of C2N nanosheets, a newly emerging 2D nitrogenized graphene with uniform holes in the basal plane. authors' *in vitro* experiments show that C2N is toxic to human umbilical vein/vascular endothelium cells. Compared with graphene oxide, C2N exerts a relatively milder cytotoxicity, and importantly, this novel material shows negligible physical destruction effects on cell membranes, suggesting that C2N might be a potential alternative to graphene and its derivatives in biomedical research.	Zhang, S. *et al.* Cytotoxicity of C2N Originating from Oxidative Stress Instead of Membrane Stress. *ACS applied materials & interfaces* 11, 34575–34585 (2019).
boron nitride	toxicity	In this paper, authors show that BN nanosheets can cause degradation of bacterial cell membranes via experimental and simulation-based approaches. authors' current findings may aid in the understanding of how BNNSs affect the structure and properties of bacterial membranes	Zhan, Y. H. *et al.* Nanotoxicity of Boron Nitride Nanosheet to Bacterial Membranes. *LANGMUIR* 35, 6179–6187 (2019).
graphene oxide	ecotoxicity	In this study, the toxic effect of GO to E. coli was studied before and after its aggregation equilibrium in the synthetic surface waters (the soft water, moderately hard water, and hard water) to reveal the effects of GO aggregation and solution hardness. GO exhibited much lower toxicity to bacteria after homoaggregation. The GO-bacterial heteroaggregation increased with increasing water hardness. The GO-bacterial heteroaggregation regulated the toxicity of GO to the bacteria. The bactericidal effect of GO increased with increasing water hardness.	Zeng, Z., Yang, K. & Lin, D. The effect of water hardness on the toxicity of graphene oxide to bacteria in synthetic surface waters. *Aquatic toxicology (Amsterdam, Netherlands)* 216, 105323–105323 (2019).
graphene oxide	ecotoxicity	Typical synthetic surface water (soft water) with representative multisalts was used to study the aggregation and sedimentation of GO. The self-assembly of carbonate nanoparticles on GO sheets occurred in the soft water. The formed carbonate nanoparticles facilitated GO aggregation through bridging action. High pH favored the formation of carbonate nanoparticles and the induced GO aggregation. These findings show a new mechanism of GO aggregation in environmentally relevant waters and help us to better evaluate the environmental fate of GO.	Zeng, Z. Y., Wang, Y. L., Zhou, Q. B., Yang, K. & Lin, D. H. New insight into the aggregation of graphene oxide in synthetic surface water: Carbonate nanoparticle formation on graphene oxide. *ENVIRONMENTAL POLLUTION* 250, 366–374 (2019).
graphene oxide	toxicity	In this study, authors used proteomics based on stable isotope labeling with amino acids in cell culture (SILAC) to quantify the proteomic changes in macrophage RAW 264.7 cells following GO treatment. Authors confirmed a GO concentration-dependent increase in membrane rafts and the production of phagosomes. GO exposure also induced necrotic cell death and an inflammation response in RAW 264.7 cells.	Yang, X. *et al.* Proteomic profiling of RAW264.7 macrophage cells exposed to graphene oxide: insights into acute cellular responses. *Nanotoxicology* 13, 35–49 (2019).

graphene oxide	toxicity	his study was focused on the zebrafish and RAW264.7 cell line as *in vivo* and *in vitro* models to assess the potential developmental neurotoxicity and immunotoxicity of GO. No obvious acute developmental toxicity was observed upon treatments with 0.01, 0.1, and 1 μg/mL GO for five consecutive days. However, decreased hatching rate, increased malformation rate, heart beat rate and hypoactivity of locomotor behavior were detected when exposed to 10 μg/mL GO. Taken together, authors' results demonstrated that immunotoxicity is a sensitive indicator for assessment of bio-compatibility of GO.	Yang, X. *et al.* Developmental neurotoxicity and immunotoxicity induced by graphene oxide in zebrafish embryos. *Environmental toxicology* 34, 415–423 (2019).
reduced graphene oxide	ecotoxicity	Herein, authors incubated reduced graphene oxide (RGO) in the white rot fungus Phanerochaete chrysosporium culture system for 4 weeks and investigated the transformation of RGO by multiple techniques. Authors pointed out that the fungal transformation of graphene materials has significant environmental importance in evaluating their environmental risks and safety.	Yang, H. *et al.* Fungal transformation of graphene by white rot fungus Phanerochaete chrysosporium. *Chemosphere* 216, 9–18 (2019).
graphene oxide	Combined toxicity	In this study, authors analyzed the interaction and combined toxicity of graphene oxide (GO) and zinc oxide nanoparticles (nano-ZnO) in the human lung carcinoma epithelial A549 cell line. Furthermore, in this study, authors found that GO increased the toxicity of Zn^{2+}, which differed from the effects of GO on nano-ZnO. This difference might be due to different modes of action, such that GO decreased the uptake of nano-ZnO, but inhibited the efflux of Zn^{2+} in cells	Wu, B. *et al.* Combined effects of graphene oxide and zinc oxide nanoparticle on human A549 cells: bioavailability, toxicity and mechanisms. *ENVIRONMENTAL SCIENCE-NANO* 6, 635–645 (2019).
MoS2	toxicity	In this study, C57BL/6 mice were exposed to nano-MoS2 and MoS2 micromaterials (micro-MoS2) via food premixed with 15 and 150 mg MoS2 per kg food for 90 days. The results showed that nano-MoS2 and micro-MoS2 exposure induced Mo accumulation in mouse organs, especially in the small intestine (SI) and large intestine (LI). Additionally, both types of MoS2 exposure obviously changed the intestinal microbiota, especially in the LI.	Wu, B. *et al.* Differential influence of molybdenum disulfide at the nanometer and micron scales in the intestinal metabolome and microbiome of mice. *ENVIRONMENTAL SCIENCE-NANO* 6, 1594–1606 (2019).
graphene oxide	ecotoxicity	The current study evaluated the impact of GO sheets at different concentrations (500, 1000 and 2000 mg/L) on physiological, biochemical and genetic levels to determine the possible toxic action. No significant effects were observed at a morphological level. GO treatments increased mitotic indices and number of aberrations of wheat cells. Chlorophyll a levels were decreased by 2000 mg/L GO treatment. TEM images revealed accumulation of GO particles in root cells of wheat seedlings.	Vochita, G. *et al.* Graphene oxide effects in early ontogenetic stages of Triticum aestivum L. seedlings. *Ecotoxicology and environmental safety* 181, 345–352 (2019).
Single-layer molybdenum disulfide nanoholes	ecotoxicity	This work proposes that nanoholes cause obvious effects on the environmental fate and ecotoxicity of SLMoS2	Tong, Y., Feng, A., Hou, X., Zhou, Q. & Hu, X. Nanoholes Regulate the Phytotoxicity of Single-Layer Molybdenum Disulfide. *Environmental science & technology* 53, 13938–13948 (2019).

graphene oxide	toxicity, method	To determine the actual toxicological mechanisms of zebrafish brains induced by graphene oxide (GO, a popular carbon-based nanomaterial applied in various fields) at nonlethal concentrations, multi-omics and regular analyses were combined. Graphene oxide induced adult zebrafish brain toxicity at nonlethal concentrations. Graphene oxide induced mitochondria damage and cytoskeletal collapse.	Sun, J., Zhou, Q. & Hu, X. Integrating multi-omics and regular analyses identifies the molecular responses of zebrafish brains to graphene oxide: Perspectives in environmental criteria. *Ecotoxicology and environmental safety* 180, 269–279 (2019).
graphene nanoplatelets	toxicity	An experimental probabilistic approach for health risk assessment was applied for graphene nanoplatelets (GNPs). The hazard assessment indicated a low level of toxicity for the GNPs.	Spinazze, A. *et al.* Probabilistic approach for the risk assessment of nanomaterials: A case study for graphene nanoplatelets. *INTERNATIONAL JOURNAL OF HYGIENE AND ENVIRONMENTAL HEALTH* 222, 76–83 (2019).
graphene oxide	toxicity	In this study, authors evaluated the effects of GO on the antioxidant metabolism of zebrafish after 48 h of sub-lethal exposure, and the fish recovery after 168 h in nanoparticle-free water. authors' study suggests that GO exposure disrupts the antioxidant metabolism of adult zebrafish. Even after 168 h of recovery in clean water, homeostasis was not completely restored, although organisms developed mechanisms of recovery, and toxic effects were more subtle.	Souza, J. P., Mansano, A. S., Venturini, F. P., Santos, F. & Zucolotto, V. Antioxidant metabolism of zebrafish after sub-lethal exposure to graphene oxide and recovery. *Fish physiology and biochemistry* 45, 1289–1297 (2019).
graphene oxide	toxicity	Based on Illumina HiSeq2500 sequencing, authors here identified 43 dysregulated circRNAs in graphene oxide (GO) (1 mg L^{-1}) exposed nematodes. Five of these candidate circRNAs could be further dysregulated by GO exposure in the range of μg L^{-1}.	Shi, L. F. *et al.* A circular RNA circ_0000115 in response to graphene oxide in nematodes. *RSC ADVANCES* 9, 13722–13735 (2019).
graphene oxide nanoparticles	ecotoxicity	Five rice varieties, Huanghuazhan (HHZ), Jinghuazhan (JHZ), Shenyou9521 (SY9521), Jingyouhuazhan (JYHZ), and Jingchuxiang (JCX), were treated with different concentrations of GO (0, 5, 15, 25, and 50 mg/L), and their physiological response was measured. The results demonstrate that GO had significant effects on the development of HHZ, JCX, JHZ, JYHZ, and SY9521 roots, but the effect varied by GO concentration and rice variety. The correlation analysis indicates that GO could affect the IAA content to modulate rice root growth.	Shen, S. S. *et al.* Graphene Oxide Regulates Root Development and Influences IAA Concentration in Rice. *JOURNAL OF PLANT GROWTH REGULATION* 38, 241–248 (2019).

graphene oxide platelets	toxicity	The objective of this study was to evaluate the influence of three carbon nanostructures on the activity and expression at the mRNA and protein levels of CYP2C9 isoenzyme from the CYP2C subfamily: Diamond nanoparticles, graphite nanoparticles, and graphene oxide platelets. The experiments have shown that all examined nanostructures inhibit the enzymatic activity of the studied isoenzymes. Moreover, a decrease in the expression at the mRNA and protein levels was also observed. This indicates that despite low toxicity, the nanostructures can alter the enzymatic function of CYP450 enzymes, and the molecular pathways involved in their expression.	Sekretarska, J. *et al.* Influence of Selected Carbon Nanostructures on the CYP2C9 Enzyme of the P450 Cytochrome. *Materials (Basel, Switzerland)* 12, (2019).
Pristine graphene	toxicity	Here authors analyzed the effects of PG on BMVECs in an *in vitro* model of the BBB. BMVECs were treated with PG at 0, 10, 50 and 100 μg/mL for 24 hours and viability and functional analyses of BBB integrity were performed. PG increased lactate dehydrogenase release at 50 and 100 μg/mL, suggesting the induction of necrosis. n conclusion, these results suggest that PG negatively affects the viability and function of the BBB endothelial cells *in vitro*.	Rosas-Hernandez, H. *et al.* Cytotoxicity profile of pristine graphene on brain microvascular endothelial cells. *Journal of applied toxicology : JAT* 39, 966–973 (2019).
graphene oxide	toxicity	This study describes adverse effects of GO and amino-functionalized GO (GONH2) during Caenorhabditis elegans development and ageing upon acute or chronic exposure. Chronic GO treatment throughout the C. elegans development causes decreased fecundity and a reduction of animal size, while acute treatment does not lead to any measurable physiological decline.	Rive, C. *et al.* Improved Biocompatibility of Amino-Functionalized Graphene Oxide in Caenorhabditis elegans . *Small (Weinheim an der Bergstrasse, Germany)* 15, e1902699–e1902699 (2019).
carbon black	toxicity	Authors employed two cell lines of osteoblasts, MC3T3-E1 and MG-63, upon exposure to 4 different CB samples with differential physicochemical properties in research of mechanistic insights. Carbon black particles are toxic to osteoblasts in a dose-dependent manner.	Ren, Q. *et al.* Carbon black-induced detrimental effect on osteoblasts at low concentrations: Remar kably compromised differentiation without significant cytotoxicity. *Ecotoxicology and environmental safety* 178, 211–220 (2019).
graphene oxide	toxicity	Various concentrations of GO were mixed with the fly food and flies were transferred to the vial. Various behavioral and morphological as well as genetic defects were checked on the different developmental stages of the offspring. the current study finds oral administration of GO which acts as a mutagen and causes various behavioral and developmental defects in the offspring.	Priyadarsini, S. *et al.* Oral administration of graphene oxide nano-sheets induces oxidative stress, genotoxicity, and behavioral teratogenicity in Drosophila melanogaster. *Environmental science and pollution research international* 26, 19560–19574 (2019).

graphene oxide	toxicity, method	Here, authors proposed a structured approach for cytotoxicity assessment complemented with cells' mechanical responses represented as the variations of elastic Young's modulus in conjunction with conventional biochemical tests. Monitoring the mechanical properties responses at various times allowed understanding the effects of NMs to the filamentous actin cytoskeleton. Authors confirmed the production of radical oxygen species (ROS) on cells exposed to CBNs, which is related to the disruption of the cytoskeleton. Altogether, the changes in mechanical properties and the length of F-actin fibers confirmed that disruption of the F-actin cytoskeleton is a major consequence of cellular toxicity	Pastrana, H. F., Cartagena-Rivera, A. X., Raman, A. & Ávila, A. Evaluation of the elastic Young's modulus and cytotoxicity variations in fibroblasts exposed to carbon-based nanomaterials. *Journal of nanobiotechnology* 17, 32–32 (2019).
graphene oxide	toxicity	Authors have investigated the toxic impacts of sub lethal doses of GO on selected oxidative stress physiology markers, protein and nucleic acid content along with haematological parameters in A. testudineus. GO administration causes tissue specific oxidative stress in fish Anabas Testudineus. Activity of redox responsive and bio-transformation enzymes are elevated. Reduction in RBC and haemoglobin level with elevated WBC count was noticed. Reduced glutathione level was noticed indicating its important role. Results of the present study indicate that GO induces oxidative stress in cell and mitochondria in fish.	Paital, B. *et al.* Ecotoxic impact assessment of graphene oxide on lipid peroxidation at mitochondrial level and redox modulation in fresh water fish Anabas testudineus. *Chemosphere* 224, 796–804 (2019).
graphene, graphene oxide	ecotoxicity	The model fungi investigated are Aspergillus niger and Aspergillus flavus. Their exposure to graphene (G) and graphene oxide (GO) presents 62% reduction in biomass and abnormal hyphae aspect. The stress response of these microorganisms in the presence of these nanomaterials is further determined by overall cell changes through apoptosis analyses and production of reactive oxygen species (ROS) by the fungal hyphae. Overall, A. niger was more sensitive to GO and A. flavus was more sensitive to G.	Nguyen, H. N., Chaves-Lopez, C., Oliveira, R. C., Paparella, A. & Rodrigues, D. E. Cellular and metabolic approaches to investigate the effects of graphene and graphene oxide in the fungi Aspergillus flavus and Aspergillus niger. *CARBON* 143, 419–429 (2019).
few layer graphene	toxicity	In this study authors were particularly interested in understanding the impact of few layer graphene (FLG) on primary murine lymphocytes. Authors' results point out that FLG neither impacts the viability and activation of T and B cells nor their autophagic activity.	Murera, D. *et al.* Few layer graphene does not affect the function and the autophagic activity of primary lymphocytes. *NANOSCALE* 11, 10493–10503 (2019).
graphene	combined ecotoxicity	the toxicities of graphene and its combinations with contaminants remain largely unexplored. In this work, authors investigated the toxicological effects of graphene and TPP to mussel Mytilus galloprovincialis. Results indicated that graphene could damage the digestive gland tissues, but no significant changes were found in the graphene + TPP co-exposure group.	Meng, X. *et al.* Combinatorial immune and stress response, cytoskeleton and signal transduction effects of graphene and triphenyl phosphate (TPP) in mussel Mytilus galloprovincialis. *Journal of hazardous materials* 378, 120778–120778 (2019).

graphene oxide, reduced graphene oxide	ecotoxicity	Therefore, this study aims to assess the toxicity of two commonly used derivatives of GBNs, graphene oxide (GO) and reduced graphene oxide (rGO), in the soil invertebrate Enchytraeus crypticus using a reduced full life cycle test. At higher exposure concentrations, GO induced high mortality and severe impairment in the reproduction rate, while rGO showed little adverse effect up to 1000 mg/kg.	Mendonca, M. C. P. *et al.* Graphene-Based Nanomaterials in Soil: Ecotoxicity Assessment Using Enchytraeus crypticus Reduced Full Life Cycle. *NANOMATERIALS* 9, (2019).
graphene oxide	ecotoxicity	Authors developed an integrative experimental design to investigate the long-term effects of two important classes of carbon nanomaterials with different dimensionalities (*i.e.*, 1D oxidized multiwalled carbon nanotube, ox-MWCNT, and 2D graphene oxide, GO) on the development of the generalist insect Spodoptera frugiperda (Lepidoptera: Noctuidae). The results showed that the type and concentration of CNMs in the diet negatively affected the reproductive parameters and the digestive and metabolic efficiency of S. frugiperda. quantitative differences in digestive enzyme activities were not observed between all treatments.	Martins, C. H. Z., de Sousa, M., Fonseca, L. C., Martinez, D. S. T. & Alves, O. L. Biological effects of oxidized carbon nanomaterials (1D versus 2D) on Spodoptera frugiperda: Material dimensionality influences on the insect development, performance and nutritional physiology. *CHEMOSPHERE* 215, 766–774 (2019).
graphene, graphene oxide	toxicity	In the current study, the oxidation potential measured by dithiothreitol (DTT) decay rate and the cytotoxicity to murine macrophage cells of different functionalized carbon nanomaterials were investigated to understand the role of functionalization in their toxicities. This implies that functionalization of carbon nanomaterials might not pose an enhanced cytotoxicity risk to macrophages when compared with the corresponding control materials although the oxidized carbon nanomaterials were still toxic as far as metabolic activity was considered.	Liu, Y. C. *et al.* Influence of functional groups on toxicity of carbon nanomaterials. *ATMOSPHERIC CHEMISTRY AND PHYSICS* 19, 8175–8187 (2019).
graphene oxide	toxicity	Our data provide the important molecular basis for neuronal Gαo signaling in response to GO. Additionally, authors' results imply that certain neuronal GPCRs may sense the GO exposure, and the affected neuronal GPCRs may further regulate the functions of goa-1/Gαo-mediated signaling cascade to regulate the GO toxicity.	Liu, P. *et al.* Dysregulation of Neuronal Gαo Signaling by Graphene Oxide in Nematode Caenorhabditis elegans. *Scientific reports* 9, 6026–6026 (2019).
graphene oxide	combined ecotoxicity	This study explored the DBP formation when only GO was present. The DBP formation were multiplied increased when simulated sunlight were also introduced into the reaction system. The possible reason is that GO was decomposed and more reactive site were formed under irradiation	Liu, M. *et al.* Disinfection byproduct formation and toxicity of graphene oxide in water treatment system. *Chemosphere* 217, 68–75 (2019).
graphene oxide	toxicity	The present study chose the Xenopus laevis tadpole as a model to assess the thyroid endocrine disruption as well as the lipid metabolic disturbance of GO. Taken together, the results revealed for the first time that GO could induce thyroid endocrine disruption and produce obvious disturbance effect on lipid synthesis and metabolism.	Li, M. *et al.* Exposure to graphene oxide at environmental concentrations induces thyroid endocrine disruption and lipid metabolic disturbance in Xenopus laevis. *Chemosphere* 236, 124834–124834 (2019).

graphene oxide, reduced graphene oxide	toxicity	The present study gave a systematic description of the *in vitro* and *in vivo* toxicities of GDs. Six kinds of GDs (GO, rGO, GQD, GQD-NH2, GQD-COOH, and GOQD) were used. Consistent with the *in vitro* results, GO and rGO rather than GQD, GQD-NH2, GQD-COOH, and GOQD, caused significant toxicity *in vivo*.	Li, J. *et al.* Systematic Assessment of the Toxicity and Potential Mechanism of Graphene Derivatives *In vitro* and *In Vivo*. *Toxicological sciences : an official journal of the Society of Toxicology* 167, 269–281 (2019).
graphene oxide	ecotoxicity	The performance, microbial community and toxic mechanism of anammox-based unplanted subsurface-flow constructed wetlands (USFCWs) were investigated under the long-term exposure of different graphene oxides (GOs) and Ag NP concentrations. GO did not cause significant damage to the cell integrity though there was an increase in ROS concentrations.	Li, H., Chi, Z. & Yan, B. Long-term impacts of graphene oxide and Ag nanoparticles on anammox process: Performance, microbial community and toxic mechanism. *Journal of environmental sciences (China)* 79, 239–247 (2019).
graphene	toxicity	In this study, authors aimed to provide the recommended occupational exposure limits (OELs) for multi-walled carbon nanotubes (MWCNTs) and graphene nanomaterials based on data from a subchronic inhalation toxicity study using a lung dosimetry model. In summary, authors proposed the recommended OELs for MWCNTs (6 μg m−3) and graphene (18 μg m−3) from subchronic inhalation study data in rats by using the MPPD model, which is a simple and reliable tool in estimating the lung deposition of poorly soluble particles.	Lee, Y.-S. *et al.* Derivation of occupational exposure limits for multi-walled carbon nanotubes and graphene using subchronic inhalation toxicity data and a multi-path particle dosimetry model. *Toxicology research* 8, 580–586 (2019).
few layer graphene	toxicity	Herein, four types of carbon nanomaterials, include long and short carbon nanotubes and graphene nanosheets, at low and high concentrations, were functionalized and dispersed in the biocompatible buffer for assessment. In summary, authors' data demonstrated that these nanomaterials could regulate cell cycle and lead to apoptosis at high concentrations,	Lee, K.-C., Lo, P.-Y., Lee, G.-Y., Zheng, J.-H. & Cho, E.-C. Carboxylated carbon nanomaterials in cell cycle and apoptotic cell death regulation. *Journal of biotechnology* 296, 14–21 (2019).
Pristine graphene	toxicity	During the first phase of study, three representative commensal bacterial species (L. acidophilus, B. longum, and E. coli) were exposed to different concentrations (1, 10, and 100 μg/mL) of pristine graphene for 3, 6, and 24 h in the Bioreactor Rotary Cell Culture System (BRCCS) which allowed a continuous interaction of intestinal microbiota with the pristine graphene without precipitation of test material. The results showed that pristine graphene had dose-dependent effects on the growth of selective bacteria. Our study further demonstrates that the toxicity of pristine graphene to the intestinal microbiome are time and dose dependent.	Lahiani, M. H., Gokulan, K., Williams, K. & Khare, S. Impact of Pristine Graphene on Intestinal Microbiota Assessed Using a Bioreactor-Rotary Cell Culture System. *ACS applied materials & interfaces* 11, 25708–25719 (2019).

graphene nanoplatelets, graphene oxide, reduced graphene oxide	toxicity	Based upon the results of these investigations, exposure to graphene nanoparticles produced physiological and alterations in ROS and gene expression that may lead to cardiovascular dysfunction. Evidence indicates that the effects of these particles may be dependent upon dose and graphene form to which an individual may be exposed to	Krajnak, K. *et al.* Exposure to graphene nanoparticles induces changes in measures of vascular/renal function in a load and form-dependent manner in mice. *Journal of toxicology and environmental health. Part A* 82, 711–726 (2019).
graphene oxide	ecotoxicity	Unfiltered natural surface water samples were exposed to GO and Ag-GO at a final concentration of 10 to 100 mg L−1 for 48 h. Authors' results indicate that the activities of microorganisms inhabiting natural surface waters may have been inhibited by oxidative stress and cell membrane damage induced by GO and Ag-GO.	Ko, K., Kim, M.-J., Lee, J.-Y., Kim, W. & Chung, H. Effects of graphene oxides and silver-graphene oxides on aquatic microbial activity. *The Science of the total environment* 651, 1087–1095 (2019).
graphene oxide	ecotoxicity	The aim of this study was to evaluate the effects of GO exposures in a marine filter-feeding bivalve (Crassostrea virginica) using sublethal biomarker approaches that can contribute to the development of an AOP. Graphene oxide (GO) exposures are associated with elevated lipid peroxidation in Eastern oysters. 14-d GO exposures led to elevated activity of glutathione-s-transferase (GST) enzyme. Oxidative damage and GST-associated signaling are putative key events in the proposed adverse outcome pathway. Oyster gills and digestive glands are susceptible to GO-induced adverse effects.	Khan, B., Adeleye, A. S., Burgess, R. M., Russo, S. M. & Ho, K. T. Effects of graphene oxide nanomaterial exposures on the marine bivalve, Crassostrea virginica. *Aquatic toxicology (Amsterdam, Netherlands)* 216, 105297–105297 (2019).
graphene oxide	ecotoxicity	Authors present results from a 72-h static renewal oyster study using 1 and 10 mg/L graphene oxide, which, to authors' knowledge, is the first report on *in vivo* effects of graphene oxide exposures in marine bivalves. The results indicate that short-term graphene oxide exposures can induce oxidative stress and epithelial inflammation and adversely affect overall oyster health.	Khan, B. *et al.* A 72-h exposure study with eastern oysters (Crassostrea virginica) and the nanomaterial graphene oxide. *Environmental toxicology and chemistry* 38, 820–830 (2019).
graphene oxide	toxicity	In both cell lines, CNFs appeared to have higher toxicity than GO and the highest degree of graphitization in fibers was associated with lower toxicity.	Kalman, J., Merino, C., Fernández-Cruz, M. L. & Navas, J. M. Usefulness of fish cell lines for the initial characterization of toxicity and cellular fate of graphene-related materials (carbon nanofibers and graphene oxide). *Chemosphere* 218, 347–358 (2019).

graphene, graphene oxide	toxicity	*In vitro* assays using HEK 293T cells revealed that the small and large sizes of G and GO significantly reduced the cell viability and increased DNA damage, accompanying with activated reactive oxygen species (ROS) generation and induced various expressions of associated critical genetic markers. Moreover, the bacterial assays highlighted that G and GO caused strong acute toxicity on Tox2 bacteria. *in vivo* assays revealed that exposure to G and GO caused the developmental toxicity, induced ROS generation, and activated related pathways (specifically GO) in zebrafish. Taken together, G showed stronger ability to decrease the survival rate and induce the acute toxicity, while GO showed obvious toxicity in terms of DNA damages, ROS generation, and abnormal gene expressions.	Jia, P.-P. *et al.* Nanotoxicity of different sizes of graphene (G) and graphene oxide (GO) *in vitro* and *in vivo*. *Environmental pollution (Barking, Essex : 1987)* 247, 595–606 (2019).
graphene oxide	toxicity	In this study, authors investigated the effects of GO chronic exposure (25 days) on the composition of the intestinal microbiota and immune response in female & male zebrafish at different concentrations (0.05, 0.5, and 5 mg L^{-1}). Taken all together, chronic exposure to GO disturbed the diversity and richness of intestinal microbes, increased the pathogenic bacterial community in zebrafish, induced damage to the gut tissues, and activated the inflammation response.	Jia, P. P. *et al.* Chronic exposure to graphene oxide (GO) induced inflammation and differentially disturbed the intestinal microbiota in zebrafish. *ENVIRONMENTAL SCIENCE-NANO* 6, 2452–2469 (2019).
graphene oxide	toxicity	In the present study authors have evaluated genotoxicity of pristine and ammonia-modified graphene oxide (GO-NH_2) nanoparticles (NPs) in a human lung epithelial cell line, A549, exposed for 24 h to different concentrations of NPs (0.1, 1, 10, 20 and 50 μg/ml). The presented results suggest that ammonia-modified GO NPs applied at concentrations higher than 20 μg/ml induced stronger toxicity effect in A549 cells compared to pristine GO and that the use of low concentrations of GO and GO-NH_2 NPs is important to avoide adverse biological effects.	Hristova-Panusheva, K. *et al.* Dose-dependent genotoxicity of ammonia-modified graphene oxide particles in lung cancer cells. in *20TH INTERNATIONAL SCHOOL ON CONDENSED MATTER PHYSICS* vol. 1186 (2019).
graphene oxide	ecotoxicity	In this work, authors reveal the GO-promoted cadmium (Cd) uptake by rice in a Cd-contaminated soil system. it can be concluded that GO exhibited indirect toxicity to plants in heavy metal-enriched soil.	He, Y. J. *et al.* Graphene Oxide Promoted Cadmium Uptake by Rice in Soil. *ACS SUSTAINABLE CHEMISTRY & ENGINEERING* 7, 10283–10292 (2019).
graphene oxide	ecotoxicity	The current study aimed to investigate the impacts of different concentrations of GO/PANI nanocomposites (25, 50 and 100 mg L^{-1}), in comparison with GO and PANI, on seed germination behaviors, morpho-physiological and biochemical traits in intact (mucilaginous) and demucilaged seeds, and young seedlings of the medicinal plant Salvia mirzayanii. Upon exposure to GO, seed germination was delayed and reduced, and growth attributes (root and shoot length, shoot fresh weight, and total chlorophyll content) declined, all of which could be attributed to the reductions in water uptake and oxidative stress particularly in demucilaged seeds.	Hatami, M., Hosseini, S. M., Ghorbanpour, M. & Kariman, K. Physiological and antioxidative responses to GO/PANI nanocomposite in intact and demucilaged seeds and young seedlings of Salvia mirzayanii. *Chemosphere* 233, 920–935 (2019).

graphene oxide, reduced graphene oxide	ecotoxicity	In this study, the recently updated Water Quality Analysis Simulation Program (WASP8) is used to simulate time-dependent environmental exposure concentrations of GO and its major phototransformation product, rGO. Simulation of natural recovery after removal of the GO source suggests that free GO and rGO are the immediate contaminants of concern in the studied surface water system, while rGO heteroaggregated with suspended solids can have a long-term ecological impact on both the water column and sediments.	Han, Y. *et al.* Simulating graphene oxide nanomaterial phototransformation and transport in surface water. *Environmental science. Nano* 6, 180–194 (2019).
graphene oxide	toxicity	To investigate, for the first time, the cyto- and geno- toxic effects of different sizes of GO in two different cell types, Leydig (TM3) and Sertoli (TM4) cells, authors synthesized different sized GO nanosheets with an average size of 100 and 20 nm. Authors' results showed that GO-20 has more potent toxic effects than GO-100, and that the loss of MMP and apoptosis are the main toxicity responses to GO-100 and GO-20 treatments, which likely occur due to EGFR/AKT pathway regulation. Collectively, authors' results suggest that both GO-100 and GO-20 exhibit size-dependent germ cell toxicity in male somatic cells, particularly TM3 cells, which seem to be more sensitive compared to TM4	Gurunathan, S., Kang, M.-H., Jeyaraj, M. & Kim, J.-H. Differential Cytotoxicity of Different Sizes of Graphene Oxide Nanoparticles in Leydig (TM3) and Sertoli (TM4) Cells. *Nanomaterials (Basel, Switzerland)* 9, (2019).
graphene oxide	toxicity	Authors exposed HEK293 cells to different concentrations of graphene oxide for 24 h and performed several cellular assays. authors' analysis provides mechanistic insights into how exposure to graphene oxide induces changes in cellular responses and massive cell death in HEK293 cells.	Gurunathan, S. *et al.* Evaluation of Graphene Oxide Induced Cellular Toxicity and Transcriptome Analysis in Human Embryonic Kidney Cells. *Nanomaterials (Basel, Switzerland)* 9, (2019).
graphene oxide	toxicity	In-house prepared graphene oxide (GO) was processed via base washing, sonication, cleaning and combinations of these processing techniques to evaluate the impact on the flake morphology, composition and cytotoxicity of the material. These results indicate that flake morphology, followed by flake size has the greatest impact on the cytotoxicity of the material.	Gies, V. *et al.* The impact of processing on the cytotoxicity of graphene oxide. *NANOSCALE ADVANCES* 1, 817–826 (2019).
reduced graphene oxide	ecotoxicity	The rGO could be used as safe nematocide because it is safe, cheep, could be produced at large scale, and it is a good additive for the soil.	Gareeb, R. Y. *et al.* New Trend for Using the Reduced Graphene Oxide as Effective and Eco-friendly Nematicide. *MATERIALE PLASTICE* 56, 59–64 (2019).
graphene oxide	ecotoxicity	This study clearly shows how the environmental fate and risk of GO are modified by UV and VL irradiations.	Gao, Y., Ren, X., Zhang, X. & Chen, C. Environmental fate and risk of ultraviolet- and visible-light-transformed graphene oxide: A comparative study. *Environmental pollution (Barking, Essex : 1987)* 251, 821–829 (2019).

reduced graphene oxide	ecotoxicity	Authors investigated the effects of CNTs, rGO and aGO, at three time points (7, 14 and 30 days), and over a range of concentrations (1 ng, 1 µg and 1 mg kg dry soil^{-1}), on soil bacterial diversity using 16S rRNA amplicon sequencing. authors' study highlights that carbon nanomaterials can induce changes in soil bacterial diversity, even at doses that are environmentally realistic.	Forstner, C., Orton, T. G., Wang, P., Kopittke, P. M. & Dennisa, P. G. Effects of carbon nanotubes and derivatives of graphene oxide on soil bacterial diversity. *SCIENCE OF THE TOTAL ENVIRONMENT* 682, 356–363 (2019).
graphene oxide	ecotoxicity	Here, authors characterized the effects of GO and graphite, over time (7, 14 and 30 days) and at three concentrations (1 ng, 1 µg and 1 mg kg dry soil^{-1}), on soil bacterial and fungal diversity using 16S rRNA and ITS2 gene amplicon sequencing. The composition of bacterial and fungal communities, however, was significantly influenced by both materials at all doses. With the exception of the lowest GO dose on day 14, these effects were apparent for all treatments over the course of the experiment.	Forstner, C. *et al.* Effects of graphene oxide and graphite on soil bacterial and fungal diversity. *SCIENCE OF THE TOTAL ENVIRONMENT* 671, 140–148 (2019).
graphene oxide	toxicity	Herein, authors explored the possibility to develop hybrid graphene oxide/amorphous carbon (GO/a-C) coatings with different oxidation degree and analysed the influence of surface oxygen composition on biocompatibility. Human osteoblasts cell toxicity increased considerably with increasing content of carbonyl and carboxyl groups on the surface, suggesting that these functional groups had an important role in inducing oxidative stress in hFOB cells.	Fedel, M. *et al.* Hybrid graphene oxide/amorphous carbon coatings and their effect on the viability and toxicity of different cell types. *SURFACE & COATINGS TECHNOLOGY* 374, 95–102 (2019).
graphene oxide	Combined toxicity	In this work, the ecotoxicological effects of GO, cadmium, zinc and the interactions between GO and these trace elements (co-exposure) were evaluated through acute toxicity tests and routine metabolism (*i.e.*, oxygen consumption and ammonia excretion) in Palaemon pandaliformis (shrimp). the GO potentiated the ecotoxicological effects of Cd and Zn in the shrimp model.	de Melo, C. B., Coa, F., Alves, O. L., Martinez, D. S. T. & Barbieri, E. Co-exposure of graphene oxide with trace elements: Effects on acute ecotoxicity and routine metabolism in Palaemon pandaliformis (shrimp). *CHEMOSPHERE* 223, 157–164 (2019).
graphene oxide	toxicity	In this work, authors found that 7-day exposure of 2.5 mg/kg/day CNMs, including C60, single-walled carbon nanotubes, and graphene oxides significantly elevated the level of HMGB1 in blood and lung lavage fluids in C57BL/6 mice.	Cui, X. *et al.* Carbon Nanomaterials Stimulate HMGB1 Release From Macrophages and Induce Cell Migration and Invasion. *Toxicological sciences : an official journal of the Society of Toxicology* 172, 398–410 (2019).

graphene oxide	toxicity	In this study, zebrafish embryos were analyzed after 5 and 7 days of exposure to GO (100 mg L^{-1}) and HA (20 mg L^{-1}) alone or together. The results indicated that, regardless of the presence of HA, larvae exposed to GO for 5 days showed an increase in locomotor activity, reduction in the yolk sac size, and total length and inhibition of AChE activity, but there was no difference in enzyme expression. The results indicated that HA is associated with the toxicity risk modulation by GO and that some compensatory homeostasis mechanisms may be involved in the developmental effects observed in zebrafish.	Clemente, Z. *et al.* Exploring the mechanisms of graphene oxide behavioral and morphological changes in zebrafish. *Environmental science and pollution research international* 26, 30508–30523 (2019).
reduced graphene oxide	ecotoxicity	After the chemical reduction of graphene oxide (GO), the reduced GO (RGO) was translocated from roots into leaves and directly inhibited the activity of photosystem II (PS II) by damaging the oxygen-evolving-complex on the donor side, which was attributed to oxidative stress. These findings highlight the critical role of chemical reduction in the translocation of graphene materials in plants, and the potential risks of such materials to disrupt important metabolic processes in carbon cycle.	Chen, L. Y. *et al.* Chemical reduction of graphene enhances *in vivo* translocation and photosynthetic inhibition in pea plants. *ENVIRONMENTAL SCIENCE-NANO* 6, 1077–1088 (2019).
graphene oxide	ecotoxicity	In this study assessed the effect of graphene oxide on rice plants at low concentration. All these results indicate graphene oxide has some concentration-dependent phytotoxicity on seeds germination and seedling development, but also decreases damage by improving activities of antioxidant enzymes.	Chen, J. Z., Mu, Q. L. & Tian, X. H. Phytotoxicity of graphene oxide on rice plants is concentration-dependent. *MATERIALS EXPRESS* 9, 635–640 (2019).
graphene oxide	ecotoxicity	Results show no difference in colony-forming units, indicating that inhibition of cell growth is a result of the adsorption of bacterial cells on the GO material. By comparing different GO samples at their EC50, this study reveals that reduction of GO alters both the mechanisms of cellular interaction and the degree of toxicity to bacteria.	Barrios, A. C., Wang, Y., Gilbertson, L. M. & Perreault, F. Structure-Property-Toxicity Relationships of Graphene Oxide: Role of Surface Chemistry on the Mechanisms of Interaction with Bacteria. *Environmental science & technology* 53, 14679–14687 (2019).
graphene oxide	toxicity	In the present study, the risk-related information of GO was evaluated to examine the potential ecological and health risks of developmental toxicity. Therefore, this study was conducted to further evaluate the toxicity of GO on embryonic development and cardiovascular defects in zebrafish embryos used as an in-vivo animal model. As a result, the presence of GO at a small concentration (0.1–0.3 mg/mL) does not affect the embryonic development. However, GO at higher concentrations (0.4–1 mg/mL) induces significant embryonic mortality, increase heartbeat, delayed hatching, cardiotoxicity, cardiovascular defects, retardation of cardiac looping, increased apoptosis and decreased hemoglobinization.	Bangeppagari, M., Park, S. H., Kundapur, R. R. & Lee, S. J. Graphene oxide induces cardiovascular defects in developing zebrafish (Danio rerio) embryo model: In-vivo toxicity assessment. *The Science of the total environment* 673, 810–820 (2019).

few layer graphene, graphene oxide	ecotoxicity	The effects of two graphene-based materials (GBMs), few-layers graphene (FLG) and graphene oxide (GO), were studied in the aeroterrestrial green microalga Trebouxia gelatinosa. Algae were subjected to short- and long-term exposure to GBMs at 0.01, 1 and 50 µg mL^{-1}. Results suggest that interactions between FLG and plasma membrane were harmless, activating a down-regulation of the HSP70-1 gene similar to that induced by H2O2.	Banchi, E. *et al.* Graphene-based materials do not impair physiology, gene expression and growth dynamics of the aeroterrestrial microalga Trebouxia gelatinosa. *Nanotoxicology* 13, 492–509 (2019).
Nanographene oxide	toxicity	The aim of the present study was to evaluate the toxicity of nano-graphene oxide (nano-GO) in the rat cardiomyoblast cell line H9c2 and the involvement of oxidative processes. In conclusion, the nano-GO caused cardiotoxicity in authors' *in vitro* model, with mitochondrial disturbances, generation of reactive species and interactions with DNA	Arbo, M. D. *et al. In vitro* cardiotoxicity evaluation of graphene oxide. *Mutation research. Genetic toxicology and environmental mutagenesis* 841, 8–13 (2019).
graphene oxide	toxicity	Authors present a study to evaluate, how highly soluble 2-dimensional GO constituted of large or small flakes affects human monocyte derived macrophages (hMDM). Authors' data revealed that the GO sheet size had a significant impact on different cellular parameters (*i.e.* cellular viability, ROS generation, and cellular activation). Indeed, the more the lateral dimensions of GO were reduced, the higher were the cellular internalization and the effects on cellular functionality.	Russier, J. *et al.* Evidencing the mask effect of graphene oxide: a comparative study on primary human and murine phagocytic cells. *Nanoscale* 5, 11234–11247 (2013).
graphene oxide	toxicity	In this study, authors focused on the role of lateral size by preparing a panel of GO samples with differential lateral sizes using the same starting material. Authors found that, in comparison to its smaller counterpart, larger GO showed a stronger adsorption onto the plasma membrane with less phagocytosis, which elicited more robust interaction with toll-like receptors and more potent activation of NF-κB pathways. Together, authors' study delineated the size dependent M1 induction of macrophages and pro-inflammatory responses of GO *in vitro* and *in vivo*.	Ma, J. *et al.* Crucial Role of Lateral Size for Graphene Oxide in Activating Macrophages and Stimulating Pro-inflammatory Responses in Cells and Animals. *ACS nano* 9, 10498–10515 (2015).
graphene oxide	toxicity	GO sheets produced under sterile conditions by a modified Hummers' method are found to be taken up by macrophages without signs of cytotoxicity. these studies show that inflammasome activation is independent of the lateral dimensions of the GO sheets. These studies provide novel insights regarding the immunomodulatory properties of endotoxin-free GO.	Mukherjee, S. P., Kostarelos, K. & Fadeel, B. Cytokine Profiling of Primary Human Macrophages Exposed to Endotoxin-Free Graphene Oxide: Size-Independent NLRP3 Inflammasome Activation. *Advanced Healthcare Materials* 7, 1700815 (2018).
graphene oxide	toxicity	This study examined how macrophage, the primary immune cell type engaging microbes, responded to GO treatment. Authors uncovered that incubation of macrophage cell RAW264.7 with GO elicited autophagy in a concentration-dependent manner. Altogether, authors demonstrated that GO treatment of cells simultaneously triggers autophagy and TLR4/TLR9-regulated inflammatory responses, and the autophagy was at least partly regulated by the TLRs pathway.	Chen, G.-Y. *et al.* Simultaneous induction of autophagy and toll-like receptor signaling pathways by graphene oxide. *Biomaterials* 33, 6559–6569 (2012).

few layer graphene	toxicity	FLG can induce cytotoxicity in RAW 264.7 macrophages by decreasing mitochondrial membrane potential (MMP), causing the accumulation of intracellular ROS, and triggering apoptosis through activation of the mitochondrial pathway. The mitogen-associated protein kinases (MAPKs) and TGF-b-related signaling pathways may also be involved	Li, Y. *et al.* The triggering of apoptosis in macrophages by pristine graphene through the MAPK and TGF-beta signaling pathways. *Biomaterials* 33, 402–411 (2012).
pristine graphene	toxicity	Pristine graphene nanosheets produce holes in the membranes of RAW 264.7 macrophages, reducing cell viability. This was due to strong interactions between pristine graphene and membrane phospholipid tails	Duan, G. *et al.* Graphene-Induced Pore Formation on Cell Membranes. *Scientific reports* 7, 42767–42767 (2017).
MoS2	toxicity	A comprehensive analysis of the pulmonary hazard potential of three aqueous suspended forms of MoS2 —aggregated MoS2 (Agg-MoS2), MoS2 exfoliated by lithiation (Lit-MoS2), and MoS2 dispersed by Pluronic F87 (PF87-MoS2)— is presented. No cytotoxicity is detected in THP-1 and BEAS-2B cell lines. However, Agg-MoS2 induces strong proinflammatory and profibrogenic responses *in vitro*.	Wang, X. *et al.* Differences in the Toxicological Potential of 2D versus Aggregated Molybdenum Disulfide in the Lung. *Small (Weinheim an der Bergstrasse, Germany)* 11, 5079–5087 (2015).
MoS2 nanosheets	toxicity	Here, authors report about the *in vitro* toxicity of MoS2 nanosheets. Specifically, the more exfoliated the MoS2 nanosheets, the stronger its cytotoxic influence, which may be due to an increase in surface area and active edge sites.	Chng, E. L. K., Sofer, Z. & Pumera, M. MoS2 exhibits stronger toxicity with increased exfoliation. *NANOSCALE* 6, 14412–14418 (2014).
boron nitride	toxicity	Boron has shown to be nontoxic in general, pos- sibly due to its suppression of macrophage phagocytosis, although it has been shown to induce inflammatory responses which could be indirectly linked to its inhibitory effects on cellular uptake	Lin, H., Song, Z. & Bianco, A. How macrophages respond to two-dimensional materials: a critical overview focusing on toxicity. *Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes* 56, 333–356 (2021).
phosphorus chalcogenides NiPS3, FePS3, CoPS3	toxicity	Here, authors investigated the cytotoxicity of NiPS3, FePS3 and CoPS3 on human lung carcinoma cells (A549) and normal human bronchial cells (BEAS-2B). Authors found that CoPS3 was most toxic followed by FePS3 with intermediate toxicity while NiPS3 showed the lowest toxicity among the three materials tested for both cell lines.	Latiff, N. M. *et al.* Cytotoxicity of layered metal phosphorus chalcogenides (MPXY) nanoflakes; FePS3, CoPS3, NiPS3. *FLATCHEM* 12, 1–9 (2018).
black phosphorus	toxicity	The cytotoxicity of as-exfoliated layered BP is evaluated by a label-free real-time cell analysis technique, displaying a concentration-, size-, and cell type-dependent response. Given the results from authors' present study, the mechanisms of BP's cytotoxicity are strikingly complicated and have significant implications for the risk evaluation and safe biomedical applications of BP	Zhang, X. J. *et al.* Size Effect on the Cytotoxicity of Layered Black Phosphorus and Underlying Mechanisms. *SMALL* 13, (2017).

black phosphorus	toxicity	The toxicological effects were found to be dose-dependent, with BP reducing cell viabilities to 48 % (WST-8) and 34 % (MTT) at 50 μg mL^{-1} exposure. This toxicity was observed to be generally intermediate between that of graphene oxides and exfoliated transition-metal dichalcogenides (MoS2, WS2, WSe2). The relatively low toxicity paves the way to utilization of black phosphorus.	Latiff, N. M., Teo, W. Z., Sofer, Z., Fisher, A. C. & Pumera, M. The Cytotoxicity of Layered Black Phosphorus. *Chemistry (Weinheim an der Bergstrasse, Germany)* 21, 13991–13995 (2015).
graphene oxide	toxicity	Authors performed *in vitro* and *in vivo* studies to evaluate the toxicity of graphene oxide (GO) exposure to the eye. Primary human corneal epithelium cells (hCorECs) and human conjunctiva epithelium cells (hConECs) were exposed to GO (12.5–100 μg/mL). Acute GO exposure (2 h) did not induce cytotoxicity to hCorECs. However, short-term GO exposure (24 h) exerted significant cytotoxicity to hCorECs and hConECs with increased intracellular reactive oxygen species (ROS).	Wu, W. *et al.* Evaluation of the toxicity of graphene oxide exposure to the eye. *Nanotoxicology* 10, 1329–1340 (2016).
graphene oxide	toxicity	Authors carried out detailed studies on genotoxicity and *in vivo* biocompatibility of G-OH in this work. he ocular fundus photography results showed that G-OH could be diffused in the vitreous body gradually without any damage caused. Injection of G-OH had caused few damages on eyesight related functions such as intraocular pressure, electroretinogram and histological structures of the retina.	Lin, M. M. *et al.* Ocular biocompatibility evaluation of hydroxyl-functionalized graphene. *MATERIALS SCIENCE & ENGINEERING C-MATERIALS FOR BIOLOGICAL APPLICATIONS* 50, 300–308 (2015).
graphene oxide	toxicity	Here, authors report authors' recent studies on intraocular biocompatibility and cytotoxicity of graphene oxide (GO) both *in vitro* and *in vivo*. GO intravitreally injected eyes showed few changes in eyeball appearance, intraocular pressure (IOP), eyesight, and histological photos. authors' results suggested that GO did not cause any significant toxicity to the cell growth and proliferation. Intravitreal injection of GO into rabbits' eyes did not lead to much change in the eyeball appearance, IOP, electroretinogram, and histological examination.	Yan, L. *et al.* Can graphene oxide cause damage to eyesight? *Chemical research in toxicology* 25, 1265–1270 (2012).
graphite nanoplates	toxicity	The goal of this study was to comparatively assess pulmonary and systemic toxicity of graphite nanoplates, a member of the graphene-based nanomaterial family, with respect to nanoplate size. Pulmonary and systemic toxicity of graphite nanoplates may be dependent on lateral size and/or surface reactivity, with the graphite nanoplates > 5 μm laterally inducing greater toxicity which peaked at the early time points post-exposure relative to the 1–2 μm graphite nanoplate.	Roberts, J. R. *et al.* Evaluation of pulmonary and systemic toxicity following lung exposure to graphite nanoplates: a member of the graphene-based nanomaterial family. *Particle and fibre toxicology* 13, 34–34 (2016).

graphene nanoplatelets	toxicity	This study highlights the importance of nanoplatelet form as a driver for *in vivo* and *in vitro* inflammogenicity by virtue of their respirable aerodynamic diameter, despite a considerable 2-dimensional size which leads to frustrated phagocytosis when they deposit in the distal lungs and macrophages attempt to phagocytose them. authors' data suggest that nanoplatelets pose a novel nanohazard and structure-toxicity relationship in nanoparticle toxicology.	Schinwald, A., Murphy, F. A., Jones, A., MacNee, W. & Donaldson, K. Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. *ACS nano* 6, 736–746 (2012).
graphene nanoplatelets	toxicity	In this study authors focussed on medium-term effects of graphene in lung tissue by investigating the pulmonary inflammation 6 weeks after pharyngeal aspiration of unoxidised multilayered graphene platelets. authors' results show non-inflammogenicity at medium time pointsfollowing inhalation exposure. Additionally, the biopersistence of pristine GP *in vivo* is not associated inflammation.	Schinwald, A. *et al.* Minimal oxidation and inflammogenicity of pristine graphene with residence in the lung. *Nanotoxicology* 8, 824–832 (2014).
few layer graphene	toxicity	Carbon-14 labeled FLG was used to quantify the *in vivo* distribution of FLG in mice after oral gavage or intratracheal instillation for up to 3 or 28 days after exposure, respectively. Graphene persistence in the lung only caused transient pulmonary effects.	Mao, L., Hu, M., Pan, B., Xie, Y. & Petersen, E. J. Biodistribution and toxicity of radio-labeled few layer graphene in mice after intratracheal instillation. *Particle and fibre toxicology* 13, 7–7 (2016).
graphene nanoplatelets	toxicity	In this study, authors explored toxic response of commercially available graphene nanoplatelets (GNPs) *in vivo* and *in vitro*. Based on these results, authors suggest that GNPs provoked a subchronic inflammatory response in mice and that GNPs induced autophagy accompanying apoptosis via mitochondria damage *in vitro*.	Park, E.-J. *et al.* Toxic response of graphene nanoplatelets *in vivo* and *in vitro*. *Archives of toxicology* 89, 1557–1568 (2015).
graphene nanoplatelets	toxicity	In this study, authors sought to evaluate the local and systemic health effect after a long pulmonary persistence of GNP. As expected, GNP remained in the lung on day 90 after a single intratracheal instillation (1.25, 2.5 and 5 mg kg^{-1}). In conclusion, authors suggest that the long persistence of GNP in the lung may cause adverse health effects by disturbing immunological- and physiological-homeostasis of authors' body.	Park, E.-J. *et al.* Pulmonary persistence of graphene nanoplatelets may disturb physiological and immunological homeostasis. *Journal of applied toxicology : JAT* 37, 296–309 (2017).
graphene nanoplatelets	toxicity	Thus, a 5-day repeated inhalation toxicity study of graphene was conducted using a nose-only inhalation system for male Sprague-Dawley rats. A total of three groups (20 rats per group) were compared: (1) control (ambient air), (2) low concentration (0.68 ± 0.14 mg/m^3 graphene) and (3) high concentration (3.86 ± 0.94 mg/m^3 graphene). The rats were exposed to graphene for 6 h/day for 5 days, followed by recovery for 1, 3, 7 or 28 days. these results suggest that the 5-day repeated exposure to graphene only had a minimal toxic effect at the concentrations and time points used in this study.	Shin, J. H. *et al.* 5-Day repeated inhalation and 28-day post-exposure study of graphene. *Nanotoxicology* 9, 1023–1031 (2015).

graphene nanoplatelets	toxicity	An inhalation toxicology study of graphene was conducted using a nose-only inhalation system for 28 days (6 h/day and 5 days/week) with male Sprague-Dawley rats that were then allowed to recover for 1-, 28-, and 90-day post-exposure period. No dose-dependent effects were recorded for the body weights, organ weights, bronchoalveolar lavage fluid inflammatory markers, and blood biochemical parameters at 1-day post-exposure and 28-day post-exposure. The inhaled graphenes were mostly ingested by macrophages. No distinct lung pathology was observed at the 1-, 28- and 90-day post-exposure. The inhaled graphene was translocated to lung lymph nodes. The results of this 28-day graphene inhalation study suggest low toxicity and a NOAEL of no less than 1.88 mg/m3.	Kim, J. K. *et al.* 28-Day inhalation toxicity of graphene nanoplatelets in Sprague-Dawley rats. *Nanotoxicology* 10, 891–901 (2016).
graphene nanoplatelets, graphene oxide nanosheets	toxicity	The pulmonary effects of GO nanosheets dispersed in water (0.5−2 nm/ 1 nm) were compared to those of graphene nanoplatelets dispersed in water (1.2−5 nm/1−5 nm) or in 2% pluronic F108 in water (1.2− 5 nm/1−5 nm). GO-treated animals exhibited severe pulmonary inflammation but no signs of fibrosis. In contrast, GNPs were less inflammogenic, and this was further minimized when the GNPs were well-dispersed using the block copolymer pluronic. The authors suggested that oxidation of graphene is a major contributor to its pulmonary toxicity	Duch, M. C. *et al.* Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. *Nano letters* 11, 5201–5207 (2011).
graphene oxide	toxicity	Herein, the fabrication of highly pure, colloidally stable, and evenly dispersed GO in physiologically relevant aqueous buffers in comparison to conventional GO is investigated. The purified GO prepared and characterized here does not induce significant cytotoxic responses *in vitro*, or inflammation and granuloma formation *in vivo* following intraperitoneal injection.	Ali-Boucetta, H. *et al.* Purified graphene oxide dispersions lack *in vitro* cytotoxicity and *in vivo* pathogenicity. *Advanced healthcare materials* 2, 433–441 (2013).
graphene oxide, reduced graphene oxide	toxicity	Authors investigated toxicity of 2–3 layered >1 μm sized graphene oxide (GO) and reduced graphene oxide (rGO) in mice following single intratracheal exposure with respect to pulmonary inflammation, acute phase response (biomarker for risk of cardiovascular disease) and genotoxicity. In conclusion, pulmonary exposure to GO and rGO induced inflammation, acute phase response and genotoxicity but no fibrosis.	Bengtson, S. *et al.* Differences in inflammation and acute phase response but similar genotoxicity in mice following pulmonary exposure to graphene oxide and reduced graphene oxide. *PloS one* 12, e0178355–e0178355 (2017).
graphene nanoplatelets, graphene oxide nanosheets	toxicity, method	Wang *et al.* compared large (1676 nm) versus small (179 nm) GO nanosheets and BSA-dispersed (640 nm) versus pluronic F108 dispersed (45 nm) graphene nanoplatelets and reported that all materials, with the exception of the GNPs dispersed in pluronic F108, induced collagen deposition/fibrosis 21 days after pharyngeal aspiration.106 Overall, F108-dispersed GNPs were less inflammogenic and not fibrogenic compared to BSA- dispersed GNPs, which were both inflammogenic and fibrogenic, whereas both small and large GO sheets were inflammogenic and fibrogenic, and large GO sheets induced more pronounced effects than the small GO sheets.	Wang, X. *et al.* Use of a pro-fibrogenic mechanism-based predictive toxicological approach for tiered testing and decision analysis of carbonaceous nanomaterials. *ACS nano* 9, 3032–3043 (2015).

graphene oxide	toxicity	Endotoxin-free materials with distinct lateral dimensions, s-GO (50–200 nm) and l-GO (5–15 μm), were produced and thoroughly characterized. In conclusion, the lateral dimension of GO played a more important role than serum protein coating in determining biological responses to the material. It was also demonstrated that time-lapse imaging of live cells interacting with label-free GO sheets can be used as a tool to assess GO-induced cytotoxicity.	Vranic, S. *et al.* Live Imaging of Label-Free Graphene Oxide Reveals Critical Factors Causing Oxidative-Stress-Mediated Cellular Responses. *ACS nano* 12, 1373–1389 (2018).
graphene oxide, graphene nanoplatelets	toxicity	A 3D human lung model was combined with a commercial aerosolization system to study potential side effects of graphene oxide and graphene nanoplatelets. Single exposure to all tested GRM at the two different exposure concentrations (~300 and 1000 ng/cm2) did not initiate an observable adverse effect to the 3D lung model under acute exposure scenarios.	Drasler, B. *et al.* Single exposure to aerosolized graphene oxide and graphene nanoplatelets did not initiate an acute biological response in a 3D human lung model. *CARBON* 137, 125–135 (2018).
graphene oxide, reduced graphene oxide	toxicity	A qualitative analysis of the viability, cellular uptake, and internalization of particles was carried out using GO (~ 54% content of oxygen) and LRGO (~ 37% content of oxygen) and graphite. GO and LRGO reduce the viability of cardiac cells at IC50 of 652.1 ± 1.2 and 129.4 ± 1.2 μg/mL, respectively. This shows that LRGO particles produce a five-fold increase in cytotoxicity when compared to GO	Contreras-Torres, F. F. *et al.* Differential cytotoxicity and internalization of graphene family nanomaterials in myocardial cells. *Materials science & engineering. C, Materials for biological applications* 73, 633–642 (2017).
graphene oxide, reduced graphene oxide	toxicity	Here authors report for the first time that atomically thin GO sheets elicited strong aggregatory response in platelets through activation of Src kinases and release of calcium from intracellular stores. Compounding this, intravenous administration of GO was found to induce extensive pulmonary thromboembolism in mice. Prothrombotic character of GO was dependent on surface charge distribution as reduced GO (rGO) was significantly less effective in aggregating platelets.	Singh, S. K. *et al.* Thrombus Inducing Property of Atomically Thin Graphene Oxide Sheets. *ACS NANO* 5, 4987–4996 (2011).
graphene nanoplatelets	toxicity	We, therefore, evaluated the effect of amine modification of graphene on platelet reactivity. Remarkably, authors' results revealed for the first time that amine-modified graphene (G-NH_2) had absolutely no stimulatory effect on human platelets nor did it induce pulmonary thromboembolism in mice following intravenous administration. Further, it did not evoke lysis of erythrocytes, another major cellular component in blood. These findings contrasted strikingly the observations with GO and reduced GO (rGO). Authors conclude that G-NH_2 is not endowed with thrombotoxic property unlike other commonly investigated graphene derivatives and is thus potentially safe for *in vivo* biomedical applications.	Singh, S. K. *et al.* Amine-modified graphene: thrombo-protective safer alternative to graphene oxide for biomedical applications. *ACS nano* 6, 2731–2740 (2012).

graphene nanoplatelets	toxicity	Herein, both pristine and functionalized graphene are extensively characterized for their interactions with murine macrophage RAW 264.7 cells and human primary blood components. both types of graphene show excellent compatibility with red blood cells, platelets, and plasma coagulation pathways, and minimal alteration in the cytokine expression by human peripheral blood mononuclear cells. Further, both samples do not cause any premature immune cell activation or suppression up to a relatively high concentration of 75 μg mL^{-1} after 72 h of incubation under *in vitro* conditions.	Sasidharan, A. *et al.* Hemocompatibility and macrophage response of pristine and functionalized graphene. *Small (Weinheim an der Bergstrasse, Germany)* 8, 1251–1263 (2012).
graphene oxide	toxicity	Here, the biological effects of GO suspended in phosphate buffered saline (PBS) with or without 1% nonionic surfactant Tween 80 were investigated. After intravenous administration, GO suspension with or without 1% Tween 80 was quickly eliminated by the mononuclear phagocyte system. Nevertheless, GO suspension without Tween 80 showed greater accumulation in lungs than that containing 1% Tween 80. In contrast, less GO was found in livers for GO suspension compared to Tween 80 assisted GO suspension. Organs including hearts, livers, lungs, spleens, kidneys, brains, and testes did not reveal histological alterations.	Qu, G. *et al.* The ex vivo and *in vivo* biological performances of graphene oxide and the impact of surfactant on graphene oxide's biocompatibility. *Journal of environmental sciences (China)* 25, 873–881 (2013).
graphene oxide	toxicity	In this study, authors investigated the antibacterial properties of GO against human intestinal bacteria. The cytotoxicity of GO was also studied *in vitro* using the Caco-2 cell line derived from a colon carcinoma. The results show that weak adsorption of medium nutrients may contribute to GO's low toxicity.	Nguyen, T. H. D., Lin, M. & Mustapha, A. Toxicity of graphene oxide on intestinal bacteria and Caco-2 cells. *Journal of food protection* 78, 996–1002 (2015).
graphene oxide	toxicity	The aim of authors' study was to investigate the interaction of label-free graphene oxide (GO) with the intestinal cell line Caco-2 *in vitro* and to shed light on the influence of the cell phenotype given by the differentiation status on cellular uptake behaviour. Authors' results show that the internalisation of GO is highly dependent on the cell differentiation status of human intestinal cells. During differentiation Caco-2 cells undergo intense phenotypic changes which lead to a dramatic decrease in GRM internalisation.	Kucki, M. *et al.* Uptake of label-free graphene oxide by Caco-2 cells is dependent on the cell differentiation status. *Journal of Nanobiotechnology* 15, 46 (2017).
graphene oxide, graphene nanoplatelets	toxicity	In this study authors have focused on the interaction of GRM, especially graphene oxide (GO), and Caco-2 cells *in vitro*. Authors mimiked stomach transition by acid-treatment of two representative GRM followed by analysis of their physicochemical properties. No significant changes in the material properties or cell viability of exposed Caco-2 cells in respect to untreated GRM could be detected. Graphene nanoplatelet aggregates led to low acute toxicity at high concentrations, indicating that aggregation, the number of layers or the C/O ratio have a more pronounced effect on the cell viability than the lateral size alone.	Kucki, M. *et al.* Interaction of graphene-related materials with human intestinal cells: an *in vitro* approach. *Nanoscale* 8, 8749–8760 (2016).

graphene oxide, few-layer graphene	toxicity	The biotransformation and biological impact of few layer graphene (FLG) and graphene oxide (GO) are studied, following ingestion as exposure route. Chronic exposure to digested graphene does not affect intestinal barrier integrity and is not associated with inflammation and cytotoxicity, though possible long-term adverse effects cannot be ruled out.	Guarnieri, D. *et al.* Biotransformation and Biological Interaction of Graphene and Graphene Oxide during Simulated Oral Ingestion. *SMALL* 14, (2018).
graphene oxide, reduced graphene oxide	toxicity	To evaluate the impact of nanoparticles on the eyes, authors investigated the ocular toxicity of reduced graphene oxide (RGO) and graphene oxide (GO) using morphological and molecular biological methods *in vivo* and *in vitro* in the present work. The findings show that short-term repeated GO exposure can cause obvious intraocular inflammation, an incrassated corneal stromal layer, cell apoptosis in the cornea, iris neovascularization and significant cytotoxicity of rat corneal epithelial cells (rCECs), while RGO causes no significant ocular toxicity in mice.	An, W. *et al.* Ocular toxicity of reduced graphene oxide or graphene oxide exposure in mouse eyes. *Experimental eye research* 174, 59–69 (2018).
graphene oxide	toxicity	This study revealed that GO adhered to and enveloped the chorion of zebrafish embryos mainly via hydroxyl group interactions, blocked the pore canals of the chorionic membrane, and caused marked hypoxia and hatching delay. Furthermore, GO spontaneously penetrated the chorion, entered the embryo via endocytosis, damaged the mitochondria and primarily translocated to the eye, heart and yolk sac regions, which are involved in the circulatory system of zebrafish. In these organs, GO induced excessive generation of reactive oxygen species and increased oxidative stress, DNA damage and apoptosis. Graphene oxide also induced developmental malformation of the eye, cardiac/yolk sac edema, tail flexure and heart rate reduction. In contrast to the common dose-effect relationships of nanoparticles, the adverse effects of GO on heart rate and tail/spinal cord flexure increased and then decreased as the GO concentration increased.	Chen, Y., Hu, X., Sun, J. & Zhou, Q. Specific nanotoxicity of graphene oxide during zebrafish embryogenesis. *Nanotoxicology* 10, 42–52 (2016).
MXene	toxicity	The present study, for the first time, shows some aspects of the *in vitro* toxicity of 2D sheets of Ti3C2 MXene. The biological activity of the MXene was determined on two normal (MRC-5 and HaCaT) and two cancerous (A549 and A375) cell lines. The cytotoxicity results indicated that the observed toxic effects were higher against cancerous cells compared to normal ones.	Jastrzębska, A. M. *et al. In vitro* studies on cytotoxicity of delaminated Ti3C2 MXene. *Journal of hazardous materials* 339, 1–8 (2017).

MXene	toxicity	Authors prepared a selection of multi-, few-, and single-layered Ti3C2Tx, as well as TiC, Ti2AlC, and Ti3AlC2 and authors investigated and compared several biological effects (cytotoxicity, membrane permeability, reactive oxygen stress, and mechanical stress) induced by MXenes, TiC, and parental MAX phases on the human fibroblasts (MSU1.1) and cervical cancer cells (HeLa), as model cells differing by their tumorigenicity. The analyses revealed that exposure to higher concentrations (≥400 μg/mL) of TiC, Ti2AlC, and Ti3AlC2 particles with the sizes <44 μm could be harmful, inducing a significant cytotoxic effect via oxidative and mechanical stress generation. All of the Ti3C2Tx forms remained safe to MSU1.1 cells with only slight cytotoxic behavior in the highest concentration regime. The cytotoxic behavior was also cell-type dependent, with higher cytotoxicities observed for cells of cancer origin.	Scheibe, B. *et al.* Cytotoxicity Assessment of Ti–Al–C Based MAX Phases and Ti3C2Tx MXenes on Human Fibroblasts and Cervical Cancer Cells. *ACS Biomater. Sci. Eng.* 5, 6557–6569 (2019).
MXene	toxicity	In this study authors focused on the oxidation-state-related *in vitro* cytotoxicity of delaminated V2CTz onto immortalized keratinocytes (HaCaT) and malignant melanoma (A375) human cell lines. Authors found that the oxidation of V2CTz highly increases their cytotoxicity toward human cells, which is also time and dose dependent. The identified mode of action relates to the cell cycle as well as cellular membrane disintegration through direct physicochemical interactions.	Jastrzębska, A. M. *et al.* On the rapid in situ oxidation of two-dimensional V2CTz MXene in culture cell media and their cytotoxicity. *Materials Science and Engineering: C* 119, 111431 (2021).
Mxene	toxicity	The biocompatibility of Ti2NTx MXene was evaluated *in vitro* towards human skin malignant melanoma cells, human immortalized keratinocytes, human breast cancer cells, and normal human mammary epithelial cells. The multilayered 2D sheets of Ti2NTx show higher toxicity towards cancerous cell lines (MCF-7and A365) in comparison to normal ones. The decrease in the cells' viability is dose-dependent, 2D Ti2NTx is not toxic towards non-malignant cells (MCF-10A and HaCaT). The identified mechanisms of toxicity are the generation of reactive oxygen species as well as the 2D sheets' internalization.	Szuplewska, A. *et al.* Multilayered stable 2D nano-sheets of Ti2NTx MXene: synthesis, characterization, and anticancer activity. *Journal of nanobiotechnology* 17, 114–114 (2019).

MXene	ecotoxicity	Herein, authors have studied the biocompatibility of Ti3C2Tx by analyzing its potential toxicity *in vivo* using a zebrafish embryo model and the aggregation patterns of Ti3C2Tx suspensions in seawater were investigated. he acute toxicity of attached/absorbed Ti3C2Tx was tested at concentrations of 25, 50, 100 and 200 μg mL^{-1}. According to the 96-hour sigmoidal mortality curve, the LC50 of Ti3C2Tx was calculated to be 257.46 μg mL^{-1} and the highest NOEC (<20% mortality) was 50 μg mL^{-1}. The LOEC (≥20% mortality) of Ti3C2Tx was detected to be 100 μg mL^{-1}, as this concentration showed a slight increase in mortality (21%). However, no significant teratogenic effects were observed on zebrafish embryos at 100 μg mL^{-1}. This nontoxicity was confirmed by locomotion and neurotoxicity assays, as 50 μg mL^{-1} of Ti3C2Tx showed no harmful effects on neuromuscular activities. In conclusion, because the LC50 of Ti3C2Tx was greater than 100 μg mL^{-1}, it can be classified as within the "practically nontoxic" group according to the Acute Toxicity Rating Scale by the Fish and Wildlife Service	Nasrallah, G. K., Al-Asmakh, M., Rasool, K. & Mahmoud, K. A. Ecotoxicological assessment of Ti3C2Tx (MXene) using a zebrafish embryo model. *ENVIRONMENTAL SCIENCE-NANO* 5, 1002–1011 (2018).
graphene oxide	ecotoxicity	The toxicity of nano-graphene oxide (NGO) on development and angiogenesis was evaluated using zebrafish embryos as *in vivo* model system. Microinjection of NGO resulted in gross morphological defects in a dose-dependent manner partly due to the induction of apoptosis.	Jeong, J. *et al. in vivo* toxicity assessment of angiogenesis and the live distribution of nano-graphene oxide and its PEGylated derivatives using the developing zebrafish embryo. *CARBON* 93, 431–440 (2015).
graphene	ecotoxicity	In this study, authors compared the effect of corannulene (non-planar PAH) and graphene (planar PAH) on embryonic development and sleep/wake behaviors of larval zebrafish. no significant locomotion alterations were induced by graphene graphene did not obviously disturb the sleep behavior and gene expression patterns	Li, X. *et al.* Comparative analysis of biological effect of corannulene and graphene on developmental and sleep/wake profile of zebrafish larvae. Acta biomaterialia 55, 271–282 (2017).
graphene	ecotoxicity	The toxicity of pG was experimentally evaluated using developing zebrafish embryos as *in vivo* model system. To determine this, 4-hpf embryos were exposed to different concentrations of pG (1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μg/L) and different early life-stage parameters were observed at 24, 48, 72, and 96 hpf. Through embryogenesis, pG was observed to induce significant embryonic mortality, delayed hatching, heartbeat, several morphological defects, pericardial toxicity, and bradycardia. Yolk sac edema and pericardial edema were induced by pG in developing embryos. These outcomes would provide new insights into the adverse effects of pG on the developing embryonic cardiac defects in vertebrates and highlight the probable natural environment and health hazards of pG flakes.	Manjunatha, B., Park, S. H., Kim, K., Kundapur, R. R. & Lee, S. J. *in vivo* toxicity evaluation of pristine graphene in developing zebrafish (Danio rerio) embryos. Environmental science and pollution research international 25, 12821–12829 (2018).

graphene oxide	toxicity	Authors compare the toxicity of oxidized carbon nano-onions (oxi-CNOs), oxidized carbon nano-horns (oxi-CNHs) and graphene oxide (GO) in zebrafish (Danio rerio). Authors' results clearly demonstrated the biosafety of oxi-CNOs and oxi-CNHs at the concentrations evaluated and the toxicity of GO s in a dose-dependent manner on zebrafish with regard to hatching and development. Above 50 μg·mL−1, embryos and larvae treated with GO presented a survival rate under 85%, hatching rate/time disturbance, a developmental delay with different malformations and a decrease of spontaneous movements. Moreover, comparing the different values related to the toxicological endpoints of CNOs and CNHs, authors can conclude that CNOs presented a higher biocompatibility than CNHs, with a difference of 5–10 in percentage for the different parameters, showing the most promising features for biological applications.	d'Amora, M. *et al.* Toxicity Assessment of Carbon Nanomaterials in Zebrafish during Development. NANOMATERIALS 7, (2017).
graphene oxide	toxicity	Here, authors show that at low concentrations (that did not directly result in significant cytotoxicity), GO could greatly enhance metal toxicity in macrophages by altering their cellular priming state. Specifically, GO caused impairments to the cellular morphology and membrane integrity of macrophages, and remarkably enhanced the cellular uptake of Cd and other non-essential metal ions (such as Hg and Gd).	Zhu, J. Q. *et al.* Low-dose exposure to graphene oxide significantly increases the metal toxicity to macrophages by altering their cellular priming state. NANO RESEARCH 11, 4111–4122 (2018).
graphene, graphene oxide	ecotoxicity	Authors conduct a study on the toxicity of four GFNs, *i.e.* graphene (G), graphene oxide (GO), carboxyl-modified graphene (G-COOH) and amine-modified graphene (G-NH_2), with or without HA, using Scenedesmus obliquus (S. obliquus) as model organism. results showed that the four GFNs induced significant inhibition on cell growth and Chlorophyll-a (Chl-a) synthesis, loss of cell viability and membrane integrity as well as mitochondrial membrane potential (MMP), where G exhibited the highest toxicity with median effect concentration (EC50) of 8.2 mg L^{-1}, and G-NH_2 exhibited the lowest toxicity with EC50 of 84.0 mg L^{-1}. Meanwhile, HA mitigated the toxicity of GFNs in the order of G-NH_2 > G-COOH > GO > G for the most of endpoints.	Zhang, Y. *et al.* Humic acid alleviates the ecotoxicity of graphene-family materials on the freshwater microalgae Scenedesmus obliquus. *Chemosphere* 197, 749–758 (2018).
graphene oxide	ecotoxicity	Here, Pseudomonas aeruginosa PAO1 was employed to evaluate the effects of non-lethal levels of GO on these bacterial social behaviors. After short-term exposure, GO levels below 40 mg L^{-1} did not affect population propagation but did interfere with biofilm formation. Authors conclude that the short-term effects of GO exposure are not sustained because of bacterial adaptation to GO adsorption. In this regard, there is no adverse ecological impact from GO treatment under non-lethal levels.	Zhang, Y. Y. *et al.* Interference of non-lethal levels of graphene oxide in biofilm formation and adaptive response of quorum sensing in bacteria. ENVIRONMENTAL SCIENCE-NANO 5, 2809–2818 (2018).

graphene oxide	combined ecotoxicity	Here, authors demonstrated how exposure to simulated sunlight or reduction with ferrous iron (Fe^{2+}, an environmentally abundant and mild reductant) significantly impacts the cytotoxicity of GO. The most interesting observation in this study was that Fe^{2+}-reduced GO was less cytotoxic due to reduced cellular uptake and its ability to act as an antioxidant, whereas light-transformed GO was more toxic due to generation of graphene-based radicals and increased cellular uptake.	Zhang, Q. R., Liu, X. L., Meng, H. Y., Liu, S. J. & Zhang, C. D. Reduction pathway-dependent cytotoxicity of reduced graphene oxide. ENVIRONMENTAL SCIENCE-NANO 5, 1361–1371 (2018).
WS2	ecotoxicity	In this study, chemically exfoliated WS2 nanosheets (Ce-WS2, mainly the 1T phase) and annealed exfoliated WS2 nanosheets (Ae-WS2, 2H phase) were fabricated to serve as representative TMDC nanomaterials. Ce-WS2 showed higher levels of cellular uptake, oxidative stress, lipid peroxidation, membrane damage, and inhibition of photosynthesis than Ae-WS2 in Chlorella vulgaris. Metabolomic analysis revealed that Ce-WS2 induced more obvious alterations in metabolites (*e.g.*, amino acids and fatty acids) and metabolic pathways (*e.g.*, starch and sucrose metabolism) than Ae-WS2. These alterations correlated with cell membrane damage, oxidative stress and photosynthesis inhibition.	Yuan, P., Zhou, Q. & Hu, X. The Phases of WS(2) Nanosheets Influence Uptake, Oxidative Stress, Lipid Peroxidation, Membrane Damage, and Metabolism in Algae. Environmental science & technology 52, 13543–13552 (2018).
MoS2	ecotoxicity	Herein, authors used different methods, including metabolomics technology, to investigate the influence of bulk MoS2 (BMS) on yeast cells. The results indicated that high concentrations (1 mg/L and more) of BMS could destroy cell membrane and induce ROS accumulation. When exposed to a low concentration of BMS (0.1 mg/L), the intracellular concentrations of many metabolites (*e.g.*, fumaric acid, lysine) increased. However, most of their concentrations descended significantly as the yeast cells were treated with BMS of high concentrations (1 mg/L and more). Metabolomics analysis further revealed that exposure to high concentrations of BMS could significantly affect some metabolic pathways such as amino acid and citrate cycle related metabolism.	Yu, Y. D. *et al.* Effect of Bulk MoS2 on the Metabolic Profile of Yeast. JOURNAL OF NANOSCIENCE AND NANOTECHNOLOGY 18, 3901–3907 (2018).
graphene oxide	Ecotoxicity	Effects of GO and/or Cd^{2+} on seed germination, seedling growth, and plant uptake to Cd^{2+} were studied in solution culture. GO could rapidly adsorb Cd^{2+} in solution and thus this may change Cd^{2+} effects on organisms. GO and/or Cd2+ had inhibitive effects on rice seed germination, bud growth, and seminal root growth. GO and/or Cd^{2+} also inhibited maize seedling growth. When GO and Cd^{2+} coexisted, GO reduced the inhibitive effects of Cd^{2+} on rice bud growth and seminal root growth as well as maize seedling growth.	Yin, L. Y., Wang, Z., Wang, S. G., Xu, W. Y. & Bao, H. F. Effects of Graphene Oxide and/or Cd2+ on Seed Germination, Seedling Growth, and Uptake to Cd2+ in Solution Culture. WATER AIR AND SOIL POLLUTION 229, (2018).

graphene oxide nanoplatelets	ecotoxicity	In the present study, authors quantitatively evaluated the relative contribution of NPs in their particulate form (NP(particle)) and of dissolved ions released from NPs (NP(ion)) to the combined toxicity of binary mixtures of ZnO NPs and graphene oxide nanoplatelets (GO NPs) to three aquatic organisms of different trophic levels, including an alga species (Scenedesmus obliquus), a cladoceran species (Daphnia magna), and a freshwater fish larva (Danio rerio). authors' results revealed that the effects of ZnO NPs and GO NPs were additive to S. obliquus and D. magna but antagonistic to D. rerio.	Ye, N., Wang, Z., Wang, S. & Peijnenburg, W. J. G. M. Toxicity of mixtures of zinc oxide and graphene oxide nanoparticles to aquatic organisms of different trophic level: particles outperform dissolved ions. Nanotoxicology 12, 423–438 (2018).
graphene oxide	toxicity, method	As natural pulmonary surfactant (PS) film represents the initial barrier of nano–bio interactions in the lungs, a novel *in vitro* experimental method, called constrained drop surfactometry (CDS), is developed to quantitatively evaluate PS inhibition caused by ENMs. The results show that at a very low concentration, four representative ENMs, including carbon nanotubes, graphene oxide, zinc oxide, and silver nanoparticles, all increase *in vitro* minimum surface tension of a modified natural PS, Infasurf. These *in vitro* results are related to the extensive alveolar collapse and inflammation observed *in vivo* in mice exposed to these ENMs in an intratracheal instillation model. Thus, there may be a direct correlation between *in vitro* surface tension increase due to PS inhibition by ENMs and *in vivo* lung toxicity revealed by alveolar collapse and inflammation. Compared to commonly used animal models, CDS holds great promise for the development of an animal-free, easy-to-use, and low-cost precautionary assay for the prediction of acute lung toxicity of inhaled ENMs.	Yang, Y. *et al.* Biophysical Assessment of Pulmonary Surfactant Predicts the Lung Toxicity of Nanomaterials. SMALL METHODS 2, (2018).
Reduced graphene oxide	ecotoxicity	Authors evaluated the influence of reduced graphene oxide (RGO) on the growth, structure and decomposition activity of white-rot fungus, whose decomposition function is vital for carbon cycle. RGO slightly stimulated the fresh weight and dry weight gains of Phanerochaete chrysosporium. RGO had low toxicity to white-rot fungus and was relatively safe for the carbon cycle.	Yang, H. *et al.* Influence of reduced graphene oxide on the growth, structure and decomposition activity of white-rot fungus Phanerochaete chrysosporium. RSC ADVANCES 8, 5026–5033 (2018).
black phosphorus	ecotoxicity	In this study, the bacterial toxicity of exfoliated BP nanosheets was for the first time evaluated against two model bacteria strains, Gram-negative Escherichia coli (E. coli) and Gram-positive Bacillus subtilis (B. subtilis). Time- and concentration-dependent bacterial toxicity profile was observed. Bacterial toxicity to E. coli was decreased over time due to membrane self-healing. ROS generation and membrane damage were the main bactericidal mechanisms. This study indicates the potential environmental risk of BP nanosheets	Xiong, Z. Q. *et al.* Bacterial toxicity of exfoliated black phosphorus nanosheets. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 161, 507–514 (2018).

graphene oxide	ecotoxicity	In the present work, the effects of GO on the migration and transformation of heavy metals and soil bacterial communities in Cd-contaminant soil were systematically evaluated. Soil samples were exposed to different doses of GO (0, 1, and 2 g kg^{-1}) over 60 days. It was shown that Cd was immobilized by GO throughout the entire exposure period. The structure of the bacterial community changed, which was possibly attributed to the reduced toxicity of Cd in the presence of GO. However, GO exerted an adverse influence on the relative abundance of some phyla (*e.g.*, WD272 and TM6). The diversity of bacterial communities was slightly restricted. The functional bacteria related to carbon and the nitrogen cycling were also affected, which, consequently, may influence the nutrient cycling in soil.	Xiong, T. *et al.* Implication of graphene oxide in Cd-contaminated soil: A case study of bacterial communities. Journal of environmental management 205, 99–106 (2018).
graphene oxide	ecotoxicity	This study evaluated the impacts of GO on algal growth and algal organic matter (AOM) of Microcystis aeruginosa. GO exhibited moderate effects on algal growth and the photosynthetic system. There was no evident influence on cell density and chlorophyll-a content at GO concentrations below 10 mg/L. The characteristics of intracellular organic matter on algae's exposure to GO showed no significant difference compared with the control group. GO has a strong adsorption capacity for removing extracellular organic matter, whereas it did not inflict damage to the algal cells, so that little change could be found in intracellular organic matter.	Xin, H. J. *et al.* Impact of Graphene Oxide on Algal Organic Matter of Microcystis aeruginosa. ACS OMEGA 3, 16969–16975 (2018).
graphene oxide	ecotoxicity	In this study, authors employed *in vivo* assay system of Caenorhabditis elegans to investigate the aversive behavior of nematodes to graphene oxide (GO) and the underlying neuronal basis. Using nlg^{-1} mutant as a genetic tool, authors identified the AIY and AIB interneurons required for the regulation of aversive behavior to GO. authors' results provide an important neuronal basis for the aversive response of animals to environmental nanomaterial	Xiao, G., Chen, H., Krasteva, N., Liu, Q. & Wang, D. Identification of interneurons required for the aversive response of Caenorhabditis elegans to graphene oxide. Journal of nanobiotechnology 16, 45–45 (2018).
graphene oxide, reduced graphene oxide	toxicity	In this study, authors compared the biocompatibility aspects (*e.g.* cytotoxicity, pro-inflammatory effects and impairment of cellular morphology) between parental and reduced GOs towards macrophages using primary bone marrow-derived macrophages (BMDMs) and J774A.1 cell line. Two RGOs (RGO1 and RGO2) with differential reduction levels relative to the parental GO were prepared. Cytotoxicity assessment unveiled that the RGOs were more toxic than pristine GO to both types of cells. It was surprising to find for the first time (to authors' knowledge) that GO and RGOs elicited different effects on the morphological changes of BMDMs, as reflected by elongated protrusions from GO treatment and shortened protrusions from the RGOs. Furthermore, RGOs induced greater pro-inflammatory responses than GO, especially in BMDMs.	Wu, Y. *et al.* Reduction of graphene oxide alters its cyto-compatibility towards primary and immortalized macrophages. Nanoscale 10, 14637–14650 (2018).

graphene	ecotoxicity	The mechanism of enhanced accumulation of organic contaminants in crops with engineered nanomaterials (ENMs) were investigated by co-exposure of crops (Ipomoea aquatica Forsk (Swamp morning-glory), Cucumis sativus L. (cucumber), Zea mays L. (corn), Spinacia oleracea L. (spinach) and Cucurbita moschata (pumpkin))to a range of chemicals (polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polybrominated diphenyl ether (PBDE)) and ENMs (TiO2, Ag, Al2O3, graphene, carbon nanotubes (CNTs)) in soil. Engineered nanomaterials may increase the concentrations of contaminants in crops. ENMs competitively adsorb bound fraction of contaminants causing the increments.	Wu, X., Wang, W. & Zhu, L. Z. Enhanced organic contaminants accumulation in crops: Mechanisms, interactions with engineered nanomaterials in soil. ENVIRONMENTAL POLLUTION 240, 51–59 (2018).
graphene oxide	toxicity	The *in vitro* biocompatibility of Graphene Oxide (GO) nanosheets, which were obtained by the electrochemical exfoliation of graphite electrodes in an electrolytic bath containing salts, was compared with the pristine Single Wall Carbon Nanotubes (p-SWCNTs) under the same experimental conditions in different human cell lines. The cells were treated with different concentrations of GO and SWCNTs for up to 48 h. GO did not induce any significant morphological or functional modifications (demonstrating a high biocompatibility), while SWNCTs were toxic at any concentration used after a few hours of treatment.	Valentini, F. *et al.* Metal Free Graphene Oxide (GO) Nanosheets and Pristine-Single Wall Carbon Nanotubes (p-SWCNTs) Biocompatibility Investigation: A Comparative Study in Different Human Cell Lines. International journal of molecular sciences 19, (2018).
graphene oxide	toxicity	In this study, the role of GO-triggered chromatin interactions in the activation of cox2, a hallmark of inflammation, was investigated in normal human cells. authors' results mechanistically link GO-mediated chromatin interactions with the regulation of cox2 and suggest that GO derivatives may minimize toxicity in practical applications.	Sun, Y. *et al.* Graphene oxide regulates cox2 in human embryonic kidney 293T cells via epigenetic mechanisms: dynamic chromosomal interactions. Nanotoxicology 12, 117–137 (2018).
graphene oxide, graphite nanoparticles	toxicity	The objective of this study was to assess if diamond nanoparticles (DN), graphene oxide (GO) or graphite nanoparticles (GN) affect three isoforms of cytochrome P450 (CYP) enzymes, namely, CYP1A2, CYP2D6 and CYP3A4, expressed in the liver. authors' findings revealed that DN, GO and GN might interfere with xenobiotic and drug metabolism in the liver by interactions with CYP isoenzymes responsible for the process.	Strojny, B. *et al.* Nanostructures of diamond, graphene oxide and graphite inhibit CYP1A2, CYP2D6 and CYP3A4 enzymes and downregulate their genes in liver cells. International journal of nanomedicine 13, 8561–8575 (2018).
graphene oxide	ecotoxicity	The potential toxicity of GO to aquatic organism particularly bluegill sun fish cells (BF-2) is unexplored or remains poorly understood. It was found that GO induced dose- and time-dependent cytotoxicity on BF-2 cells. BF-2 cells exposed to lower concentration of GO (40 μg ml^{-1}) for 24 induced morphological changes when compared to their respective controls. authors' findings demonstrate that GO when exposed to BF-2 fish cells cause oxidative stress.	Srikanth, K., Sundar, L. S., Pereira, E. & Duarte, A. C. Graphene oxide induces cytotoxicity and oxidative stress in bluegill sunfish cells. Journal of applied toxicology : JAT 38, 504–513 (2018).

graphene oxide	ecotoxicity	Authors evaluated graphene oxide (GO) effects on the freshwater cladoceran Ceriodaphnia dubia through acute and chronic toxicity, feeding rates, and reactive oxygen species (ROS) generation. The mean effective concentration (EC50) estimated during acute exposure was 1.25 mg L^{-1} of GO. Graphene oxide concentrations cause acute and chronic effects to C. dubia. Feeding and reproduction of C. dubia were affected in the presence of graphene oxide. Graphene oxide can be accumulated in gut tract of C. dubia. Graphene oxide can induce negative damages at individual and at population levels.	Souza, J. P., Venturini, F. P., Santos, F. & Zucolotto, V. Chronic toxicity in Ceriodaphnia dubia induced by graphene oxide. Chemosphere 190, 218–224 (2018).
graphene oxide	toxicity	Effects on sperm quality after pulmonary inflammation induced by carbonaceous nanomaterials were investigated by intratracheally instilling sexually mature male NMRI mice with four different carbonaceous nanomaterials dispersed in nanopure water: graphene oxide (18 µg/mouse/i.t.), Flammruss 101, Printex 90 and SRM1650b (0.1 mg/mouse/i.t. each) weekly for seven consecutive weeks. Despite the sustained pulmonary inflammatory response, an eight week exposure to graphene oxide, Flammruss 101, Printex 90 and the diesel particle SRM1650b in the present study did not appear to affect semen parameters, daily sperm production or testosterone concentration in male NMRI mice.	Skovmand, A. *et al.* Pulmonary exposure to carbonaceous nanomaterials and sperm quality. Particle and fibre toxicology 15, 10–10 (2018).
graphene, graphene oxide	toxicity, method	Our results indicate that hepcidin peptide undergo severe structural deformations when superimposed on the graphene sheet in comparison to graphene oxide sheet. These observations suggest that graphene is more toxic than a graphene oxide nanosheet of similar area. Overall, this study indicates that computational methods based on structural deformation, using molecular dynamics (MD) simulations, can be used for the early evaluation of toxicity potential of novel nanomaterials.	Singh, K. P., Baweja, L., Wolkenhauer, O., Rahman, Q. & Gupta, S. K. Impact of graphene-based nanomaterials (GBNMs) on the structural and functional conformations of hepcidin peptide. Journal of computer-aided molecular design 32, 487–496 (2018).
graphene oxide	toxicity	Authors compared the effects of large GO sheets (l-GO, 1–20 µm) with those of small GO sheets (s-GO, < 1 µm) in terms of mesothelial damage and peritoneal inflammation, after intraperitoneal (i.p.) injection in mice. Authors' aim was to assess whether lateral dimensions can be a predictor of inflammogenicity for GO sheets in a similar fashion as length is for MWCNTs. GO sheets dispersed under similar conditions did not cause any response, regardless of their lateral dimensions. On the other hand, surface reactivity and the ability of some smaller GO sheets to interact more readily with immune cells seem to be key parameters that can be tuned to improve the safety profile of GO.	Rodrigues, A. F. *et al.* Immunological impact of graphene oxide sheets in the abdominal cavity is governed by surface reactivity. Archives of toxicology 92, 3359–3379 (2018).

graphene nanoplatelets	ecotoxicity	Authors used 2D graphene nanoplatelets and isometric carbon black nanoparticles to evaluate the influence of particle morphology and surface properties on adsorption and bioavailability of benzo(a)pyrene, a model aromatic hydrocarbon, to brine shrimp (Artemia franciscana) and a fish liver cell line (PLHC-1). Acellular adsorption studies show that while high surface area carbon black (P90) was most effective at a given concentration, 2D graphene nanoplatelets (G550) adsorbed more benzo(a)pyrene than carbon black with comparable surface area (M120). In both biological models, co-exposure to nanomaterials lead to reduced bioavailability, and G550 graphene nanoplatelets caused a greater reduction in bioavailability or response than the M120 carbon black nanoparticles.	Rodd, A. L. *et al.* Impact of emerging, high-production-volume graphene-based materials on the bioavailability of benzo(a)pyrene to brine shrimp and fish liver cells. Environmental science. Nano 5, 2144–2161 (2018).
graphene oxide	ecotoxicity	In this study, authors investigated the molecular basis for intestinal barrier against toxicity and translocation of graphene oxide (GO) using C. elegans as a model animal. Using C. elegans as an *in vivo* assay system, authors found that several developmental genes required for the control of intestinal development regulated both the intestinal permeability and the GO toxicity. With the focus on PKC-3, authors raised two intestinal signaling cascades, PKC-3-SEC-8-WTS-1 and PKC-3-ISP-1/SOD-3. authors' results will strengthen authors' understanding the molecular basis for developmental machinery of intestinal barrier against GO toxicity and translocation in animals.	Ren, M. *et al.* Developmental basis for intestinal barrier against the toxicity of graphene oxide. Particle and fibre toxicology 15, 26–26 (2018).
graphene oxide, few-layer graphene	toxicity	The mechanism of toxicity of two GBNs (few-layer-graphene, FLG, and graphene oxide, GO) towards human HaCaT skin keratinocytes was investigated. This study shows that FLG and GO induce a cytotoxic effect due to a sustained mitochondrial depolarization. This seems to be mediated by a significant cellular ROS production, caused by the activation of flavoprotein-based oxidative enzymes, such as NADH dehydrogenase and xanthine oxidase.	Pelin, M. *et al.* Graphene and graphene oxide induce ROS production in human HaCaT skin keratinocytes: the role of xanthine oxidase and NADH dehydrogenase. Nanoscale 10, 11820–11830 (2018).
graphene oxide	combined ecotoxicity	This work aims to assess the toxicity of heavy metal ions (Cu(II), Cd(II), and Zn(II)) on Daphnia magna (D. magna) in the presence of GO. GO nanoparticles remarkably reduced the concentrations of heavy metal ions by adsorption and decreased the metal accumulation in D. magna. The results revealed the role of GO nanoparticles in the mitigated toxicity of heavy metal ions in the aquatic environment.	Ni, L. F. & Li, Y. Role of graphene oxide in mitigated toxicity of heavy metal ions on Daphnia magna. RSC ADVANCES 8, 41358–41367 (2018).
graphene, graphene oxide	ecotoxicity	The present study investigates the chronic toxicity of graphene (G) and graphene oxide (GO) in activated sludge. G and GO effects started at concentrations as low as 5.26 and 3.64 mg/L. The ammonia, phosphate and COD removals reached steady state after 8 days. AOB and PAO abundances started to recover after 8 days. GO and G impacted differently the microbial populations in the sludge. GO exhibited higher toxicity then G to microbial communities.	Nguyen, H. N. & Rodrigues, D. F. Chronic toxicity of graphene and graphene oxide in sequencing batch bioreactors: A comparative investigation. Journal of hazardous materials 343, 200–207 (2018).

graphene oxide	toxicity	Here authors produced endotoxin-free GO by a modified Hummers' method and asked whether primary human neutrophils stimulated to produce neutrophil extracellular traps or activated to undergo degranulation are capable of digesting GO. these studies have shown that neutrophils can digest GO and that the biodegraded GO is non-toxic for human lung cells.	Mukherjee, S. P. *et al.* Graphene oxide is degraded by neutrophils and the degradation products are non-genotoxic. Nanoscale 10, 1180–1188 (2018).
graphene oxide	toxicity	Here, authors synthesized GO sheets with differing lateral dimensions and showed by using an array of analytical and imaging techniques, including transmission and scanning electron microscopy, confocal microscopy, and time-of-flight secondary ion mass spectroscopy (ToF-SIMS), that GO elicited the formation of neutrophil extracellular traps (NETs). The present studies have revealed that endotoxin-free GO triggers size-dependent responses in primary human neutrophils, and authors' work has identified key elements of the signaling pathway upstream of NET release in GO-exposed cells.	Mukherjee, S. P. *et al.* Graphene Oxide Elicits Membrane Lipid Changes and Neutrophil Extracellular Trap Formation. CHEM 4, 334–358 (2018).
graphene oxide	toxicity	Here authors compared the effects of two carbon-based nanomaterials, single-walled CNTs (SWCNTs) and graphene oxide (GO), on primary human monocyte-derived macrophages. GO, on the other hand, did not elicit chemokine responses.	Mukherjee, S. P. *et al.* Macrophage sensing of single-walled carbon nanotubes via Toll-like receptors. SCIENTIFIC REPORTS 8, (2018).
graphene oxide	ecotoxicity	The toxic action of graphene oxide (GO) and the micropollutants contained in a biologically-treated wastewater were studied alone and in combination. For the toxicity assays, the unicellular green alga Chlamydomonas reinhardtii was used and the toxic mechanism was assessed. Cells exposed to GO–wastewater mixtures were considerably less affected with lower or non-significant damage in comparison with GO or wastewater alone.	Martin-de-Lucia, I. *et al.* Combined toxicity of graphene oxide and wastewater to the green alga Chlamydomonas reinhardtii. ENVIRONMENTAL SCIENCE-NANO 5, 1729–1744 (2018).
graphene oxide	ecotoxicity	Authors present preliminary water quality guideline values for graphene oxide NMs in freshwaters. Data include 10 species from 7 phyla (bacteria and fungi were not included). The most sensitive organism was found to be the freshwater shrimp Palaemon pandaliformis. The derived guideline values for 99, 95, 90, and 80% species protection were 350, 600, 830, and 1300 µg/L, respectively.	Markovic, M. *et al.* Ecotoxicology of manufactured graphene oxide nanomaterials and derivation of preliminary guideline values for freshwater environments. Environmental toxicology and chemistry 37, 1340–1348 (2018).
pristine graphene	toxicity	The multiple effect of pristine graphene (pG) toxicity on cardiovascular developmental defects was assessed using zebrafish as a model. the exposure to pG was found to be a potential risk factor to cardiovascular system of zebrafish embryos.	Manjunatha, B., Park, S. H., Kim, K., Kundapur, R. R. & Lee, S. J. Pristine graphene induces cardiovascular defects in zebrafish (Danio rerio) embryogenesis. Environmental pollution (Barking, Essex : 1987) 243, 246–254 (2018).

graphene oxide	toxicity	This study investigates the interaction of GO with the ubiquitin–proteasome system, one of the essential machineries in the cellular metabolism, using a combination of experimental and computational approaches. The experimental results show that GO could adsorb the 20S proteasome, causing a dose-dependent suppression of the proteolytic activity of proteasome. This adverse effect eventually disturbed other important cellular activities relevant to cell cycle and survival.	Ma, X. *et al.* Inhibition of the proteasome activity by graphene oxide contributes to its cytotoxicity. Nanotoxicology 12, 185–200 (2018).
graphene oxide	ecotoxicity	This study evaluated the comprehensive toxicological effects of GO on Daphnia magna, a key species in fresh water ecosystem. The results revealed nonsevere acute toxicities, including immobility (72 h EC50: 44.3 mg/L) and mortality (72 h LC50: 45.4 mg/L), of GO on D. magna. These results hence suggest that GO could accumulate and induce significant oxidative stress in the gut of D. magna, while D. daphnia can also relieve the acute toxicity by depurating GO.	Lv, X. *et al.* A mechanism study on toxicity of graphene oxide to Daphnia magna: Direct link between bioaccumulation and oxidative stress. Environmental pollution (Barking, Essex : 1987) 234, 953–959 (2018).
graphene oxide	ecotoxicity	In the present study, the acute toxicity of GO, Phe, Cd^{2+}, GO-Phe, and GO-Cd^{2+} to Artemia salina were systemically assessed and compared for the first time. The sublethal toxicity of GO at ambient concentration are confirmed. The impacts of GO on toxicities of Phe or Cd^{2+} appear concentration-dependent. The formation and bioaccumulation of Phe/Cd^{2+}-GO complexes determine the toxicity.	Lu, J. *et al.* Graphene oxide in the marine environment: Toxicity to Artemia salina with and without the presence of Phe and Cd(2). Chemosphere 211, 390–396 (2018).
graphene oxide	ecotoxicity	To investigate the chronic toxicity of graphene oxide (GO) and its functionalized products (GO-carboxyl, GO-imidazole and GO-polyethylene glycol), a two-generation study was conducted using the aquatic model species Daphnia magna. Chronic exposure to GO, GO-carboxyl, and GO-imidazole had no adverse effect on body length or offspring number in the daphnid F0 generation, however, this exposure paradigm led to significant growth or reproduction inhibition in the following generation.	Liu, Y. *et al.* Comparative toxicity of pristine graphene oxide and its carboxyl, imidazole or polyethylene glycol functionalized products to Daphnia magna: A two generation study. Environmental pollution (Barking, Essex : 1987) 237, 218–227 (2018).
graphene, graphene oxide	combined ecotoxicity	Herein, the effects of graphene (GN) and graphene oxide (GO, an important oxidized derivative of graphene) on copper (Cu) toxicity to Daphnia magna were systematically investigated. The results indicated that GN remarkably increased the Cu accumulation in D. magna and enhanced the oxidative stress injury caused by Cu, whereas did not significantly alter D. magna acute mortality within the tested Cu concentrations (0–200 μg L^{-1}). On the contrary, GO significantly decreased the Cu accumulation in D. magna and alleviated the oxidative stress injury caused by Cu. Meanwhile, the presence of GO significantly reduced the mortality of D. magna when Cu concentration exceeded 50 μg L^{-1}.	Liu, Y. Y., Fan, W. H., Xu, Z. Z., Peng, W. H. & Luo, S. L. Comparative effects of graphene and graphene oxide on copper toxicity to &ITDaphnia magna&IT: Role of surface oxygenic functional groups. ENVIRONMENTAL POLLUTION 236, 962–970 (2018).

reduced graphene oxide	toxicity	Epithelial-mesenchymal transition (EMT) has profound effect on development of pulmonary fibrosis. Herein, authors evaluated the EMT effect of rGO samples on A549 cells. Firstly, rGO penetrated through the A549 cells membrane into the cytosol by endocytosis and located in late endosome and/or lysosomes observed via transmission electron microscopy (TEM), and were well tolerant by cells. Secondly, rGO promoted the cell migration and invasion capacities at lower doses (below 10 μg/ml), but significantly inhibited the capacities at 20 μg/ml. Moreover, rGO-induced EMT were evidenced by decreased expression of epithelial marker like E-cadherin, β-catenin, Smad4 and increased expression of mesenchymal markers like Vimentin, VEGF-B, TWIST1. Based on authors' findings, it is supposed that rGO can effectively induce EMT through altering epithelial–mesenchymal transition markers in A549 cells.	Liao, Y. Y. *et al.* Reduced graphene oxide triggered epithelial-mesenchymal transition in A549 cells. SCIENTIFIC REPORTS 8, (2018).
graphene oxide, reduced graphene oxide	toxicity	Conflicting results are found in the literature about the biocompatibility and cytoxicity of GOs and rGOs under *in vitro* and *in vivo* conditions. This is partly related to the researchers employing various oxidation times, different types, and different concentrations of oxidants for the synthesis, producing GOs with structure and reactivity as well as impurity level that differ from one study to another. The diversity in the structural properties has a large impact on the cell-GO interactions. In addition, the various chemical oxidizers and reducing agents used to prepare GOs and rGOS can generate metallic impurities and organic contaminations, thus altering their interactions with cells, tissues and organs, and resulting in cellular damage and apoptosis.	Liao, C., Li, Y. & Tjong, S. C. Graphene Nanomaterials: Synthesis, Biocompatibility, and Cytotoxicity. International journal of molecular sciences 19, (2018).
graphene oxide, reduced graphene oxide	ecotoxicity	The present study found that graphene oxide (GO), GO quantum dots (GOQDs) and reduced GO (rGO) translocated from wheat stems to grains and formed large nanomaterial aggregates. GOQD and rGO changed the proteomic and metabolomic profiles more strongly than GO, suggesting that graphene materials with a small size and a low oxidation content are clearly more detrimental to grain quality.	Li, X. K. *et al.* Effects of the size and oxidation of graphene oxide on crop quality and specific molecular pathways. CARBON 140, 352–361 (2018).
graphene oxide	combined ecotoxicity	The present work revealed that GO significantly enhanced the accumulation of PAHs by 26.4–92.5% in rice. GO further promoted increased aryl hydrocarbon receptor (AhR) and cytochrome P450 levels, which are induced by PAHs. The altered proteins were mainly associated with oxidative stress and transmembrane transport. Amino acid metabolism was the primary metabolic pathway influenced by GO and PAHs. Arabinose and pentanoic acid were positively associated with the uptake of PAHs and oxidative stress, respectively, during co-exposure to GO.	Li, X. K., Mu, L. & Hu, X. G. Integrating proteomics, metabolomics and typical analysis to investigate the uptake and oxidative stress of graphene oxide and polycyclic aromatic hydrocarbons. ENVIRONMENTAL SCIENCE-NANO 5, 115–129 (2018).

graphene oxide	ecotoxicity	To explore the effect of graphene on plants, three-week-old, tissue-cultured 'Gala' apple plants (Malus domestica) were treated with different concentrations (0, 0.1, 1, 10 mg/L) of graphene oxide (GO) and examined after 40 days. Collectively, the results indicate that treatment of 'Gala' apple plants with 0.1 mg/L GO had a positive effect on root formation but a negative effect on root growth. This response may be related to the negative impact of GO on cellular structure and function.	Li, F. H. *et al.* The effect of graphene oxide on adventitious root formation and growth in apple. PLANT PHYSIOLOGY AND BIOCHEMISTRY 129, 122–129 (2018).
pristine graphene	toxicity	The results suggest that some reported immunostimulatory effects of graphene preparations may have resulted from contaminants in the formulations.	Lebre, F., Hanlon, D., Boland, J. B., Coleman, J. & Lavelle, E. C. Exfoliation in Endotoxin-Free Albumin Generates Pristine Graphene with Reduced Inflammatory Properties. ADVANCED BIOSYSTEMS 2, (2018).
black phosphorus	toxicity	Here, the cytotoxicity of black phosphorus prepared by two different synthesis methods, *i.e.* vapour growth transport and high pressure conversion, are investigated. Authors found black phosphorus synthesized by vapour growth transport to have higher toxicity effects compared to black phosphorus produced via high pressure conversion. .Wefindthatthe toxicity ofphosphorus materials is likely to be influenced by their degree of exfoliation andextent of oxidation.	Latiff, N. M., Mayorga-Martinez, C. C., Sofer, Z., Fisher, A. C. & Pumera, M. Cytotoxicity of phosphorus allotropes (black, violet, red). APPLIED MATERIALS TODAY 13, 310–319 (2018).
pristine graphene	toxicity	The aim of the present study was to evaluate the cytotoxicity of pristine graphene monolayer and its utility as a scaffold for murine fibroblast L929 cell line. Graphene was found to have no cytotoxicity on L929 fibroblasts and increased cell adhesion and proliferation within 24 h of culture. The area of cells growing on graphene was comparable to the area of fibroblasts cultured on glass. Taken together, authors' results indicate that pristine graphene monolayer is non-toxic for murine subcutaneous connective tissue fibroblasts	Lasocka, I. *et al.* Biocompatibility of pristine graphene monolayer: Scaffold for fibroblasts. Toxicology *in vitro* : an international journal published in association with BIBRA 48, 276–285 (2018).
graphene oxide	toxicity	Here authors performed a mechanistic *in vitro* study on the placental uptake and biological effects of four non-labelled GO with varying physicochemical properties using the human trophoblast cell line BeWo. Although GO did not elicit major acute adverse effects on BeWo trophoblast cells, the pronounced cellular internalization as well as the potential adverse effects on hormone release and barrier integrity warrants further studies on the long-term consequences of GO on placental functionality in order to understand potential embryo-fetotoxic risks.	Kucki, M. *et al.* Impact of graphene oxide on human placental trophoblast viability, functionality and barrier integrity. 2D Materials 5, 035014 (2018).

graphene oxide	toxicity	This study conducted a short-term graphene oxide inhalation toxicity analysis using a nose-only inhalation exposure system and male Sprague–Dawley rats. No significant body or organ weight changes were noted after the short-term exposure or during the recovery period. Similarly, no significant systemic effects of toxicological importance were noted in the hematological assays, bronchoalveolar lavage fluid (BAL) inflammatory markers, BAL fluid cytokines, or blood biochemical assays following the graphene oxide exposure or during the post-exposure observation period. Moreover, no significant differences were observed in the BAL cell differentials. Histopathological examination of the liver and kidneys did not reveal any significant test-article-relevant histopathological lesions.	Kim, Y. H. *et al.* Short-term inhalation study of graphene oxide nanoplates. Nanotoxicology 12, 224–238 (2018).
graphene oxide	toxicity	Our studies collectively indicated that GO accumulation in reproductive organs, suppression of spermatogenesis, and the alteration of fatty acid metabolism play critical roles in understanding mechanisms of toxicity in C. elegans.	Kim, Y. *et al.* Graphene oxide nano-bio interaction induces inhibition of spermatogenesis and disturbance of fatty acid metabolism in the nematode Caenorhabditis elegans. Toxicology 410, 83–95 (2018).
graphene oxide	toxicity	Here, authors have evaluated the cytotoxicity of ammonia-modified GO (GO-NH2) and pristine GO particles in human lung cancer cells, A549 and embryonic stem cells, Lep3 exposed to different particles concentrations (0.1, 1, 10, 20, and 50 µg/ml) for different times (24 and 48h). These results suggested that both GO particles exert different degree of cytotoxicity which is time, dose and cell dependent. In general, ammonia-modified GO particles are more toxic than the pristine GO	Keremidarska-Markova, M. *et al.* Cytotoxicity Evaluation of Ammonia-Modified Graphene Oxide Particles in Lung Cancer Cells and Embryonic Stem Cells. ADVANCES IN CONDENSED MATTER PHYSICS 2018, (2018).
MoS2	toxicity	Herein, authors focus on the current demands of 2D TMDs and produce high-quality, few-layered and defect-free MoS2 nanosheets, exfoliated and dispersed in pure water, stabilized up to three weeks. Hence, authors studied the impact of this material on human cells by investigating its interactions with three cell lines: two tumoral, MCF7 (breast cancer) and U937 (leukemia), and one normal, HaCaT (epithelium). Authors observed novel and intriguing results, exhibiting evident cytotoxic effect induced in the tumor cell lines, absent in the normal cells in the tested conditions.	Kaur, J. *et al.* Biological interactions of biocompatible and water-dispersed MoS2 nanosheets with bacteria and human cells. SCIENTIFIC REPORTS 8, 16386–16386 (2018).
graphene oxide	ecotoxicity	The purpose of this study was to evaluate the different medium composition and lighting effects on the acute ecotoxicity of graphene oxide on crustacean Daphnia magna. An increase of the toxic effect with incubation time was observed. Effective concentrations EC50 after 48 h of incubation were nearly two times lower than that after 24 h. It was found that in media with lower hardness the toxicity of graphene oxide increases when compared with harder water.	Kalinowski, R. *et al.* Effects of environmental factors on graphene oxide ecotoxicity towards crustacean Daphnia magna. DESALINATION AND WATER TREATMENT 117, 249–256 (2018).

reduced graphene oxide	ecotoxicity	The aim of this study was to compare the toxicity effects of carbon nanomaterials (CNMs), namely fullerene (C60), reduced graphene oxide (rGO) and multi-walled carbon nanotubes (MWCNTs), on a mini-ecosystem of rice grown in a loamy potted soil. authors' results indicate that different CNMs indeed resulted in environmental toxicity to rice and soil bacterial community in the rhizosphere and suggest that CNMs themselves and their incorporated products should be reasonably used to control their release/discharge into the environment to prevent their toxic effects on living organisms and the potential risks to food safety.	Hao, Y. *et al.* Carbon nanomaterials alter plant physiology and soil bacterial community composition in a rice-soil-bacterial ecosystem. Environmental pollution (Barking, Essex : 1987) 232, 123–136 (2018).
graphene oxide	ecotoxicity	In this study, authors reported that Acidithiobacillus sp., isolated from sewages, were used to bioleach Cu2+ and Zn2+ from sewages sludge in the presence of graphene. The negative effect on the growth of Acidithiobacillus sp. and dose-dependent were observed in presence of graphene. thevstudy confirmed that the direct contacts between graphene and cell at 1 mg L^{-1} graphene caused cell membrane disruption, while Acidithiobacillus sp. grew better by forming dense biofilms around the suspended graphene at a 50 mg L^{-1}. LIVE/DEAD staining further demonstrated that almost no live cells were detected at 1 mg L^{-1} graphene	Guo, S., Lin, J., Wang, Q., Megharaj, M. & Chen, Z. The toxicity of graphene and its impacting on bioleaching of metal ions from sewages sludge by Acidithiobacillus sp. Chemosphere 195, 90–97 (2018).
graphene oxide	toxicity	Here, the cytotoxicity of three sizes of commercially available GO was investigated on six cell lines, as values of NOAEL/LOAEL. The effectiveness of four viability assays was also evaluated. The overall toxicity of GO greatly varied between cell lines; The six cell lines were also tested to evaluate their response to varying GO flake sizes: the suspension/phagocytic cells showed little variation in viability, while a difference was observed for the adherent/non-phagocytic cell lines.	Gies, V. & Zou, S. Systematic toxicity investigation of graphene oxide: evaluation of assay selection, cell type, exposure period and flake size. Toxicology research 7, 93–101 (2018).
graphene oxide	ecotoxicity	The current study was performed to explore the potential impacts of nano-garphene oxide (NGO) at various concentrations (100–800 µg mL^{-1}) on morphological, physiological and biochemical responses of Plantago major L. calli cultures under normal and polyethylene glycol-induced drought stress conditions. Overall, the results demonstrated that NGO can positively affect the performance of P. major L. calli cells when applied at specific concentrations, and provide useful inputs into the further studies on phytotoxicity assessment of NGO.	Ghorbanpour, M., Farahani, A. H. K. & Hadian, J. Potential toxicity of nano-graphene oxide on callus cell of Plantago major L. under polyethylene glycol-induced dehydration. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 148, 910–922 (2018).

graphene	ecotoxicity	Carbonaceous nanomaterials (CNMs) can affect agricultural soil prokaryotic communities, but how the effects vary with the crop growth stage is unknown. To investigate this, soybean plants were cultivated in soils amended with 0, 0.1, 100, or 1000 mg kg^{-1} of carbon black, multiwalled carbon nanotubes (MWCNTs), or graphene. None of the three CNMs affected rhizosphere soil prokaryotic communities at day 0, but they caused mild effects at the vegetative stage (only the low dose of MWCNTs) and relatively stronger effects at the reproductive stage (almost all treatments except the high dose of MWCNTs). Overall, low doses of specific CNMs may have a high impact on soil rhizosphere prokaryotic communities and their associated functions at the soybean reproductive stage, a crucial stage of soybean development for seed yield.	1.Ge, Y. *et al.* Carbonaceous Nanomaterials Have Higher Effects on Soybean Rhizosphere Prokaryotic Communities During the Reproductive Growth Phase than During Vegetative Growth. ENVIRONMENTAL SCIENCE & TECHNOLOGY 52, 6636–6646 (2018).
graphene oxide	ecotoxicity	The aim of the current study was to find the linkage among the surface adsorption, colloidal stability and combined toxicity of graphene oxide (GO) and divalent heavy metal cations (denoted as Me(II)). Based on authors' study, it is found that the combined effects of GO and heavy metal cations on the survival of bacteria are related to the surface adsorption and GO colloidal stability. The binding of metal ions to GO increases the zeta potential and size of GO aggregates and decreases the sharpness of GO edges. As a result, the combined toxicity of GO and metal ions towards bacteria decreases significantly.	Gao, Y. *et al.* Graphene oxide interactions with co-existing heavy metal cations: adsorption, colloidal properties and joint toxicity. ENVIRONMENTAL SCIENCE-NANO 5, 362–371 (2018).
graphene	toxicity	To conduct cytotoxicity and mutagenicity assessments, exfoliated graphene (EGr) dispersed in Tween-20® was diluted in cell culture medium. Authors' results demonstrate that NR8383 cell viability significantly decreased after 24 h exposure to EGr, which was taken up by the cells and caused death at concentrations ≥10 µg/mL. EGr did not appreciably induce mutagenesis.	Fujita, K. *et al.* Assessment of cytotoxicity and mutagenicity of exfoliated graphene. Toxicology *in vitro* : an international journal published in association with BIBRA 52, 195–202 (2018).
graphene	toxicity	The aim of this study was to evaluate the mechanism of graphene toxicity in different tissues of Danio rerio, considering different parameters of stress. Animals were injected intraperitoneally (i.p.) with 10 µL of suspensions containing different graphene concentrations (5 and 50 mg/L); The results showed pathological effects in all tissues, excluding the intestine, after exposure to both concentrations. Overall, these results indicate that graphene induces different grades of toxicological effects that are dependent on the analysed organ, with distinct pathological effects on some and oxidative effects on others. However, the brain and gills seem to be the primary target organs for graphene toxicity.	Fernandes, A. L. *et al.* Assessment of the effects of graphene exposure in Danio rerio: A molecular, biochemical and histological approach to investigating mechanisms of toxicity. Chemosphere 210, 458–466 (2018).

graphene oxide	toxicity	In this work, authors systematically investigated the toxic responses of commercially available GO on a rat pheochromocytoma-derived PC12 cell line, which was an ideal *in vitro* model to study the neurotoxicity of GO. Authors found that cell viability was decreased in a dose- and time-dependent manner following GO exposure. Additionally, GO triggered an increased autophagic response and the impairment of autophagic flux in PC12 cells, which was determined to be associated with defects in lysosome degradation capability.	Feng, X. *et al.* Graphene oxide induces p62/SQSTM-dependent apoptosis through the impairment of autophagic flux and lysosomal dysfunction in PC12 cells. Acta biomaterialia 81, 278–292 (2018).
graphene oxide	toxicity	To unveil the underlying mechanisms of microRNAs (miRNAs) and potential target genes involved in GO cytotoxicity, authors firstly compiled GO-related miRNAs and genes in human cancer cell lines treated with GO from public databases and published works. Functional investigations displayed that miRNAs might be involved in the control of apoptosis through disruption of cell adhesion in response to cytotoxicity.	Farahani, M. *et al.* Deciphering the transcription factor-microRNA-target gene regulatory network associated with graphene oxide cytotoxicity. Nanotoxicology 12, 1014–1026 (2018).
graphene oxide	ecotoxicity	The presented research focuses on reproductive dysfunction and cellular changes in Acheta domesticus after exposure to GO nanoparticles in food (concentrations of 20 and 200 $\mu g \cdot g^{-1}$ of food) throughout their entire life cycle. The results showed that long-term exposure to GO caused a significant decrease in the reproductive capabilities of the animals. Moreover, the next generation of A. domesticus had a lower cell vitality compared to their parental generation. It is possible that graphene oxide can cause multigenerational harmful effects.	Dziewiecka, M. *et al.* Reduced fecundity and cellular changes in Acheta domesticus after multigenerational exposure to graphene oxide nanoparticles in food. SCIENCE OF THE TOTAL ENVIRONMENT 635, 947–955 (2018).
carbon nitride	toxicity	Heptazine and triazine-based carbon nitrides. To fill in this gap in the current research work, authors conducted *in vitro* cytotoxicity studies on them. Seven concentrations of the nanomaterials were incubated for 24 h with A549 cells to test their toxicity. The toxicity effects were found to be dose-dependent for both materials, and t-C3N4 was observed to be more toxic than h-C3N4. As compared to graphene oxide (GO), t-C3N4 and h-C3N4 demonstrated lower toxicity.	Dong, Q. *et al.* Triazine- and Heptazine-Based Carbon Nitrides: Toxicity. ACS APPLIED NANO MATERIALS 1, 4442–4449 (2018).
graphene oxide	ecotoxicity	In Caenorhabditis elegans, mutation of mlt-7 causes the deficits in epidermal barrier. Using the nematodes with epidermal-specific RNA interference (RNAi) knockdown of mlt-7 as a genetic tool, authors found that epidermal-specific RNAi knockdown of mlt-7 resulted in a susceptibility to graphene oxide (GO) toxicity, and enhanced GO accumulation in the body. authors' data highlights the importance of maintaining normal epidermal barrier for nematodes against the GO toxicity.	Ding, X., Rui, Q. & Wang, D. Functional disruption in epidermal barrier enhances toxicity and accumulation of graphene oxide. Ecotoxicology and environmental safety 163, 456–464 (2018).

graphene oxide	ecotoxicity	Authors employed Caenorhabditis elegans to examine the possible influence of a deficit in the epidermal barrier caused by RNA interference (RNAi) knockdown of unc-52 encoding a perlecan protein on the toxicity of graphene oxide (GO). authors' data further highlight the important function of the epidermal barrier against toxicity of environmental ENMs.	Ding, X. C. *et al.* Toxicity of Graphene Oxide in Nematodes with a Deficit in the Epidermal Barrier Caused by RNA Interference Knockdown of unc-52. ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS 5, 622–628 (2018).
few layer graphene	toxicity	The present study was aimed at providing some elucidations on the specific molecular signaling induced by low doses of a well characterized FLG material in macrophages. Exposure to low doses of FLG resulted in no significant decrease of macrophage viability. Nevertheless, it elicited a marked oxidative stress. The latter triggered significant inflammatory responses, increasing Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) secretion as well as nitric oxide (NO) production, leading to autophagy via endoplasmic reticulum (ER) stress. Authors' work highlight for the first time for this type graphene an interplay between oxidative stress and ER stress-mediated autophagy. It also suggests that such a pathway could protect the cells from exaggerated inflammation.	Di Cristo, L., Mc Carthy, S., Paton, K., Movia, D. & Prina-Mello, A. Interplay between oxidative stress and endoplasmic reticulum stress mediated-autophagy in unfunctionalised few-layer graphene-exposed macrophages. 2D MATERIALS 5, (2018).
graphene oxide, reduced graphene oxide	toxicity	Herein, the toxicity of three distinct GFNs; GO, hydrazine reduced GO (H.rGO) and AA.rGO, prepared through diverse chemical routes are studied. All GFNs induced high levels of alveolar cell toxicity. Interaction of AA.rGO with the A549 human lung epithelial carcinoma cell line resulted in increased leakage of lactate dehydrogenase, indicative of diminished cell membrane integrity. The uncharacteristic shape of the AA.rGO may be responsible for this proliferated release of the essential protein. The presented data therefore demonstrates that modification of synthetic processes significantly alter the biological activities of GFNs.	Dervin, S., Murphy, J., Aviles, R., Pillai, S. C. & Garvey, M. An *in vitro* cytotoxicity assessment of graphene nanosheets on alveolar cells. APPLIED SURFACE SCIENCE 434, 1274–1284 (2018).
graphene	toxicity	The aim of the present study was to determine the effects of graphene or MWCNT [as pure, carboxyl (COOH) functionalized, and amide (NH2) functionalized] on cytotoxicity, intracellular levels of reactive oxygen species, apoptosis, gene expression changes, and gene mutation induction in L5178Y/Tk+/−3.7.2C mouse lymphoma cell line. Although some adverse effects were observed at concentrations of 350 and 450 μg/ml, which are excessive and not environmentally relevant levels, no marked effects were detected at concentrations of 250 μg/ml and lower.	Demir, E. & Marcos, R. Toxic and genotoxic effects of graphene and multi-walled carbon nanotubes. Journal of toxicology and environmental health. Part A 81, 645–660 (2018).

single-layer graphene, multi layer graphene	toxicity	Commercial CNP candidates (ACS Material, USA) were selected, covering single layer (SLG) or multi layer graphene (MLG), carboxyl graphene, single layer graphene oxide, and graphite oxide. As *in vitro* screening models both, primary rat alveolar macrophages (AM) and MRC-5 human lung fibroblast cells were analysed on membrane damage (LDH release) and metabolic activity (AlamarBlue test). Interestingly, the two SLG induced marked concentration-dependent membrane damage in AM after 24 h of incubation whereas no such effect was observed for MRC-5 cells. In conclusion, SLG showed a (geno)toxic potential *in vitro* in AM, but not in lung fibroblasts. Again, SLG but not MLG exhibited some inflammogenic potential.	Creutzenberg, O. H., Ziemann, C., Schaudien, D., Oliveira, H. & Farcal, L. The PLATOX project: Combining *in vitro* and *in vivo* investigations to generate valid toxicity data for risk assessment of graphene nanoplatelets. TOXICOLOGY LETTERS 295 MA-P17-06, S205–S205 (2018).
MoS2	toxicity	Contamination is avoided here through preparing MoS2 atomically thin film via direct sulfurization of molybdenum thin film on quartz plate, which permits a direct assessment of its toxicity without any contamination. Six different types of cells, including normal, cancer, and immortal cells, are cultured in the media containing MoS2 thin film on quartz plates or dispersed MoS2 microparticles and their viability is evaluated. Allergy testing on skin of guinea pigs is also conducted to understand their effect on animal skins. authors' results of coculturing cells in medium of different concentration of dispersed MoS2 microparticles show that the MoS2 microparticles did not induce any toxic behaviors at the low concentration of 0.016 mg mL−1	Chen, W. B. *et al.* Direct Assessment of the Toxicity of Molybdenum Disulfide Atomically Thin Film and Microparticles via Cytotoxicity and Patch Testing. SMALL 14, (2018).
graphene oxide	ecotoxicity	In this study, authors compared the toxicity of graphene oxide (GO) to naked oats (Avena sativa L.) in hydroponic and soil cultures. Serious toxicity of GO was only observed in hydroponic culture.	Chen, L. Y. *et al.* Toxicity of graphene oxide to naked oats (Avena sativa L.) in hydroponic and soil cultures. RSC ADVANCES 8, 15336–15343 (2018).
graphene oxide	ecotoxicity	In the present study, authors investigated the phytotoxicity of unfunctionalized graphene oxide (GO) and amine-functionalized graphene oxide (G-NH2) on wheat (Triticum aestivum) in the concentration range from 125 to 2000 μg/mL after 9 days of hydroponic culture. authors' results found that the incubation with both nanomaterials did not affect the final seed germination rate	Chen, J., Yang, L., Li, S. & Ding, W. Various Physiological Response to Graphene Oxide and Amine-Functionalized Graphene Oxide in Wheat (Triticum aestivum). Molecules (Basel, Switzerland) 23, (2018).

graphene oxide	ecotoxicity	In the present study, the graphene oxide effect from aquatic ecosystems was assessed considering the interaction with humic acid on 9 organisms: Raphidocelis subcapitata (green algae), Lemna minor (aquatic plant), Lactuca sativa (lettuce), Daphnia magna (planktonic microcrustacean), Artemia salina (brine shrimp), Chironomus sancticaroli (Chironomidae), Hydra attenuata (freshwater polyp), and Caenorhabditis elegans and Panagrolaimus sp. (nematodes). he safest scenario associated with the predicted no-effect concentration values for graphene oxide in the aquatic compartment were estimated as 20 to 100 $\mu g\,L^{-1}$ (in the absence of humic acid) and 5 to 23 $\mu g\,L^{-1}$ (in the presence of humic acid).	Castro, V. L. *et al.* Nanoecotoxicity assessment of graphene oxide and its relationship with humic acid. Environmental toxicology and chemistry 37, 1998–2012 (2018).
graphene oxide	ecotoxicity	This study addresses the effects of GO on pollen germination and pollen tube elongation in the model species Nicotiana tabacum and in the non-model species Corylus avellana. *In vitro* germination experiments were conducted without or with GO (control and treated samples, respectively) at concentrations of 25, 50 and 100 $\mu g\ mL^{-1}$. Authors showed that GO impaired the pollen performance in N. tabacum and C. avellana at the highest concentration tested and most of this is derived from the acidic properties of the material.	Carniel, F. C. *et al.* Graphene oxide impairs the pollen performance of Nicotiana tabacum and Corylus avellana suggesting potential negative effects on the sexual reproduction of seed plants. ENVIRONMENTAL SCIENCE-NANO 5, 1608–1617 (2018).
graphene oxide	toxicity	Authors realized the exposure of boar spermatozoa to graphene oxide (GO) at concentration of 0.5, 1, 5, 10 and 50 µg/mL in an *in vitro* system able to promote the capacitation. authors found that the highest GO concentration (5, 10 and 50 µg/mL) are toxic for spermatozoa, while the lowest ones (0.5 and 1 µg/mL) seem to significantly increase the sperm cells fertilizing ability ($p > .05$) in an *in vitro* fertilization experiment. GO is able to interact with spermatozoa membranes and, in particular, it seems to be able to extract the cholesterol, which is a key player in spermatozoa physiology, from plasma membrane of boar spermatozoa incubated under capacitation conditions	Bernabo, N. *et al.* Graphene oxide affects *in vitro* fertilization outcome by interacting with sperm membrane in an animal model. CARBON 129, 428–437 (2018).
graphene nanoplatelets	ecotoxicity	In this study, the behavior of some carbon-based nanomaterials (multi-walled carbon nanotubes and graphene nanoplatelets) in environmental media (sea water, soil, and airborne fi ne particulate) were evaluated by using the infl uence on nanomaterial physicochemical properties (size, zeta potential, surface chemistry, morphology and sedimentation) and on the toxicity of bacterium (Gram-positive and Gram-negative bacteria). authors' results indicated that the toxicity depended on the type of environmental media and their concentration, and the physicochemical properties of the carbon-based nanomaterials changed when compared to the results obtained in controlled conditions.	Baysal, A., Saygin, H. & Ustabasi, G. S. Influence of environmental media on carbon nanotubes and graphene nanoplatelets towards bacterial toxicity. ARCHIVES OF ENVIRONMENTAL PROTECTION 44, 85–98 (2018).

graphene oxide nanosheets	toxicity	This study aims to investigate the influence of fabricated nano graphene oxide (NGO) sheets on the secondary and quaternary structural alterations of human hemoglobin (Hb) and cytotoxicity against lymphocyte cells. Cellular and molecular assays revealed that NGOs lead to ROS formation, cell cycle arrest, and apoptosis through the BAX and BCL2 pathway. These data reveal that NGOs can induce some protein structural changes and stimulate cytotoxicity against normal cell targets.	Babadaei, M. M. N. *et al.* Biophysical, bioinformatical, cellular, and molecular investigations on the effects of graphene oxide nanosheets on the hemoglobin structure and lymphocyte cell cytotoxicity. International journal of nanomedicine 13, 6871–6884 (2018).
graphene oxide nanoplatelets	toxicity	This study assessed the potential toxicity of graphene oxide nanoplatelets (GONs) in an *in vivo* animal model. Normal saline (control group) or GONs (3–6 layers, lateral dimension=5–10 μm, and thickness=0.8–2 nm) at dose rate of 50, 150, or 500 mg/kg were intraperitoneally injected into adult male Wistar rats (n=5) every 48 hours during 1 week to receive each animal a total of four doses. In conclusion, this study shows that GONs without functionalization are toxic.	Amrollahi-Sharifabadi, M. *et al. in vivo* toxicological evaluation of graphene oxide nanoplatelets for clinical application. International journal of nanomedicine 13, 4757–4769 (2018).
graphene oxide, reduced graphene oxide	toxicity	A comprehensive assessment of biological interactions and toxicological outcomes of rGO-nZVI and its parent materials, *i.e.*, GO, rGO, and nZVI on BEAS-2B cells included cellular uptake, cell viability, cell membrane integrity, reactive oxygen species (ROS) generation, and cell cycle analyses. The toxic behavior of rGO-nZVI nanohybrids was found to be in between that of rGO/GO (most toxic) and nZVI (least toxic); however, it was majorly governed by rGO/GO toxicity and its mechanisms.	Aich, N. *et al. In vitro* Pulmonary Toxicity of Reduced Graphene Oxide-Nano Zero Valent Iron Nanohybrids and Comparison with Parent Nanomaterial Attributes. ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY 257 MA–223, 12797–12806 (2018).
graphene nanoplatelets	toxicity	Macrophages were exposed to GNPs at different concentrations of 0, 25, 50, or 100 μg/ml for 1, 3, or 6 h. Following exposure, no cytotoxicity was observed, while GNPs readily associated with macrophages in a concentration-dependent manner. A number of metabolites were found in common between cells exposed to the CD36 receptor ligand, SSO, and GNPs suggesting both CD36-dependent and independent responses to GNP exposure.	Adamson, S. X. F., Wang, R. X., Wu, W. Z., Cooper, B. & Shannahan, J. Metabolomic insights of macrophage responses to graphene nanoplatelets: Role of scavenger receptor CD36. PLOS ONE 13, (2018).
graphene oxide, reduced graphene oxide, MoS2	toxicity	In this study, authors use a dye-leakage assay to quantitatively assess the disruption of a model phospholipid bilayer membrane (*i.e.*, lipid vesicles) by five emerging 2D nanomaterials: graphene oxide (GO), reduced graphene oxide (rGO), molybdenum disulfide (MoS2), copper oxide (CuO), and iron oxide (α-Fe2O3). For GO, the most disruptive nanomaterial, authors show that the extent of membrane integrity loss was dependent on total surface area, not edge length, which is consistent with a lipid-extraction mechanism and inconsistent with a piercing mechanism.	Zucker, I. *et al.* Loss of Phospholipid Membrane Integrity Induced by Two-Dimensional Nanomaterials. ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS 4, 404–409 (2017).

graphene oxide	ecotoxicity	Using Saccharomyces cerevisiae as an experimental model, the potential toxicity of graphene oxide (GO) was evaluated following exposure to 0–600 mg L^{-1} for 24 h. The results showed that cell proliferation was observably inhibited and the IC50 value was 352.704 mg L^{-1}. Mortality showed a concentration-dependent increase, and was 19.3% at 600 mg L^{-1}. The results presented so far indicate that GO has the potential to cause adverse effects on organisms when released into the environment.	Zhu, S., Luo, F., Zhu, B. & Wang, G.-X. Toxicological effects of graphene oxide on Saccharomyces cerevisiae. Toxicology research 6, 535–543 (2017).
graphene oxide	ecotoxicity	Using Artemia salina as an experimental model, the potential risks of graphene oxide (GO) to marine ecosystems were investigated. Authors' results show that GO significantly decreased ($p < 0.01$) the hatchability of capsulated and decapsulated cysts following exposure to 400 and 600 mg/L for 36 h. There was a concentration-dependent increase in mortality and decrease in swimming speed of larvae, and instar II larvae showed a greater sensitivity compared with instar I and instar III larvae. Finally, the uptake result indicated that GO was ingested and concentrated in the gut, and was visible within the primary body cavity and yolk.	Zhu, S., Luo, F., Chen, W., Zhu, B. & Wang, G. Toxicity evaluation of graphene oxide on cysts and three larval stages of Artemia salina. The Science of the total environment 595, 101–109 (2017).
graphene oxide nanosheets	ecotoxicity	Herein, rice was exposed to graphene oxide (GO) nanosheets at 0.01–1.0 mg/L for 7 days under hydroponic exposure, followed by a 7-day post exposure (GO-free). Taken together, the results of the present study showed that alterations to phenylalanine metabolism, secondary metabolism, and heme peroxidases reflected the systemic stress and recovery patterns in rice roots in response to GO nanosheets. Oxidative stress, root development, cell wall biosynthesis, phytohormones, and gene transcription were found to be affected by GO.	Zhou, Q. X. & Hu, X. G. Systemic Stress and Recovery Patterns of Rice Roots in Response to Graphene Oxide Nanosheets. ENVIRONMENTAL SCIENCE & TECHNOLOGY 51, 2022–2030 (2017).
graphene oxide	toxicity	Using assay system of Caenorhabditis elegans, authors investigated the potential involvement of canonical Wnt/β-catenin signaling pathway in the regulation of response to GO. Authors' results demonstrated that long-term exposure to GO could cause the damage on the functions of both primary and secondary targeted organs by dysregulating the Wnt/β-catenin signaling pathway.	Zhi, L. T. *et al.* Graphene oxide induces canonical Wnt/beta-catenin signaling-dependent toxicity in Caenorhabditis elegans. CARBON 113, 122–131 (2017).
graphene oxide, reduced graphene oxide, multi-layer graphene	ecotoxicity	Authors systematically investigated the toxicity mechanism of three graphene-family materials (GFNs), graphene oxide (GO), reduced graphene oxide (rGO) and multi-layer graphene (MG), to algae (Chlorella pyrenoidosa). For GO, shading effect (~16%), oxidative stress-induced membrane damage, and nutrient depletion (~53%) were responsible for the observed toxicity. rGO and MG showed no shading effect on algal growth due to their poor dispersibility while nutrient depletion led to 35% and 27% of the total toxicity, respectively. Membrane damage induced by both oxidative stress and physical penetration/extraction could be dominant mechanisms for rGO and MG.	Zhao, J., Cao, X., Wang, Z., Dai, Y. & Xing, B. Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae. Water research 111, 18–27 (2017).

graphene oxide	toxicity	The present study established that the development of zebrafish embryos exposed to trace concentrations (1–100 μg/L) of GO was impaired because of DNA modification, protein carbonylation and excessive generation of reactive oxygen species (ROS), especially the superoxide radical.	Zhang, X., Zhou, Q., Zou, W. & Hu, X. Molecular Mechanisms of Developmental Toxicity Induced by Graphene Oxide at Predicted Environmental Concentrations. Environmental science & technology 51, 7861–7871 (2017).
graphene oxide	ecotoxicity	Here, authors report that GO (at 10 to 160 mg/L) induced significant inhibitory effects on the growth of different unicellular organisms, including eukaryotes (*i.e.* Saccharomyces cerevisiae, Candida albicans, and Komagataella pastoris) and prokaryotes (Pseudomonas fluorescens).	Yu, Q. *et al.* Graphene oxide significantly inhibits cell growth at sublethal concentrations by causing extracellular iron deficiency. Nanotoxicology 11, 1102–1114 (2017).
graphene oxide, reduced graphene oxide	toxicity	This study both assessed the short term effect of GONPs and rGONPs on THP-1 cells and long term effects on THP-1a differentiating from GFNs exposed THP-1 cells. GFNs with different oxidation degrees resulted in the toxicity effects of monocytes via different signaling pathways. Interestingly, the different cellular locations of GFNs resulted in different damage on the endocytosis and phagocytosis of THP-1a. Furthermore, the amount of rGONPs entering cells was higher than that of GONPs, which resulted in more damage in F-actin.	Yan, J. *et al.* Consecutive evaluation of graphene oxide and reduced graphene oxide nanoplatelets immunotoxicity on monocytes. Colloids and surfaces. B, Biointerfaces 153, 300–309 (2017).
graphene oxide	ecotoxicity	In this study, authors determined the role and value of mir-247 in the detection of GO toxicity using *in vivo* assay system of C. elegans. In nematodes, mir-247 acted in the neurons to regulate GO toxicity. Neuronal overexpression of mir-247 induced a susceptibility to GO toxicity. In wild-type nematodes, authors detected the toxicity of GO at concentrations more than 100 μg L^{-1}. In contrast, authors could observe the toxicity in nematodes exposed to GO at concentrations more than 10 μg L^{-1} in transgenic strain overexpressing neuronal mir-247.	1.Xiao, G. S., Zhi, L. T., Ding, X. C., Rui, Q. & Wang, D. Y. Value of mir-247 in warning of graphene oxide toxicity in nematode Caenorhabditis elegans. RSC ADVANCES 7, 52694–52701 (2017).
reduced graphene oxide	toxicity	Reduced graphene films with layered structure have been prepared by the improved thermal reduction method at low heating temperature. The reducing agent-free and low temperature process also offers a green and effective approach to the in-situ coating of rGO film on various substrates. The results of cytotoxicity experiments indicate that the rGO films are biocompatible with mammalian cells,	Wang, X. *et al.* Reduced Graphene Oxide Paper: Fabrication by a Green Thermal Reduction Method and Preliminary Study of its *In vitro* Cytotoxicity. JOURNAL OF NANO RESEARCH 45, 199–207 (2017).

graphene	toxicity	This study evaluated the toxicity effects of graphene on the airway epithelial cell line BEAS-2B, which represents the first barrier of the human body to interact with airborne graphene particles. results showed that graphene can induce the cellular Ca2+ by phospholipase C (PLC) associated pathway by activating epidermal growth factor receptor (EGFR), and that raphene exposure may exacerbate lung function and other related diseases such as lung cancer, COPD, and cardiovascular diseases.	Tsai, S. M. *et al.* Graphene-induced apoptosis in lung epithelial cells through EGFR. JOURNAL OF NANOPARTICLE RESEARCH 19, (2017).
graphene oxide, MoS2, WS2, boron nitride	toxicity	The effects of Graphene oxide (GO), molybdenum sulfide (MoS2), tungsten sulfide (WS2), and boron nitride (BN) on human adipose-derived mesenchymal stem cells (hADMSCs). model two-dimensional materials were coated on cell-culture substrates by a drop-casting method. Acute toxicity was not observed with any of the four different 2D materials at a low concentration range (<5 μg/ml). Interestingly, the 2D material-modified substrates exhibited a higher cell adhesion, spreading, and proliferation when compared with a non-treated (NT) substrate. Remarkably, in the case of differentiation, the MoS2-, WS2-, and BN-modified substrates exhibited a better performance in terms of guiding the adipogenesis of hADMSCs when compared with both NT and GO-modified substrates. In contrast, the osteogenesis was found to be most efficiently induced by the GO-coated substrate (50 μg/mL) among all 2D-material coated substrates. In summary, 2D materials could act as favorable sources for controlling the stem cell growth and differentiation, which might be highly advantageous in both biomedical research and therapy.	Suhito, I. R., Han, Y., Kim, D.-S., Son, H. & Kim, T.-H. Effects of two-dimensional materials on human mesenchymal stem cell behaviors. Biochemical and biophysical research communications 493, 578–584 (2017).
graphene oxide	ecotoxicity	Graphene oxide exposure caused apoptotic and necrotic stages in zebrafish gill cells. Graphene oxide induced reactive oxygen generation in zebrafish gill cells. Gill and liver tissues suffered injuries after graphene oxide chronic exposure. Zebrafish blood cells did not present DNA damages after graphene oxide exposure.	Souza, J. P., Baretta, J. F., Santos, F., Paino, I. M. M. & Zucolotto, V. Toxicological effects of graphene oxide on adult zebrafish (Danio rerio). Aquatic toxicology (Amsterdam, Netherlands) 186, 11–18 (2017).
graphene oxide	ecotoxicity	This study evaluated the effects of GO exposure (at 5, 10, 50 or 100 mg/L) during six consecutive days on mortality, hatching, spontaneous movement, heart rate, morphology, locomotion behavior, acetylcholinesterase (AChE) activity, dopamine levels and relative gene expression of developmental neurology-related genes using zebrafish larvae. GO exposure changed cardiac, locomotor and morphological parameters *in vivo*. GO toxicity showed non-dose-dependent effect.	Soares, J. C. *et al.* Developmental neurotoxic effects of graphene oxide exposure in zebrafish larvae (Danio rerio). Colloids and surfaces. B, Biointerfaces 157, 335–346 (2017).

graphene oxide	toxicity	Using *in vivo* assay system of Caenorhabditis elegans, authors here found that antimicrobial proteins of LYS-1, LYS-8, SPP-1, DOD-6, and F55G11.4 were activated by graphene oxide (GO) exposure. These antimicrobial proteins functioned as molecular targets of transcriptional factor DAF-16 in insulin signaling pathway and acted in intestine to regulate the response to GO. Therefore, authors' results demonstrate the crucial protection role of antimicrobial proteins for animals in response to environmental ENMs' exposure. The elucidated different signaling cascades mediated by antimicrobial proteins provide important molecular targets for future toxicity assessment and chemical modification of GO.	Ren, M., Zhao, L., Lv, X. & Wang, D. Antimicrobial proteins in the response to graphene oxide in Caenorhabditis elegans. Nanotoxicology 11, 578–590 (2017).
pristine graphene	toxicity	Here, by using live cell imaging techniques, authors investigate the effect of pristine graphene on the viability as well as stress of both nonneuronal and neuronal cells under physiological conditions. Authors find that graphene promotes cell adhesion and proliferation. Furthermore, authors find that graphene has no detectable adverse effect on mitochondrial membrane potential and morphology, or autophagy levels in the cell, indicating that graphene does not induce cell stress.	Rastogi, S. K., Raghavan, G., Yang, G. & Cohen-Karni, T. Effect of Graphene on Nonneuronal and Neuronal Cell Viability and Stress. Nano letters 17, 3297–3301 (2017).
graphene oxide	toxicity	The aim of this study was to investigate the biocompatibility of GO coated collagen membranes on human dental pulp stem cells (DPSCs) focusing on biomaterial cytotoxicity, ability to promote DPSCs differentiation process and to control inflammation event induction. GO coated membranes are not toxic for DPSCs, induce a faster DPSCs differentiation into odontoblasts/osteoblasts and may represent good alternative to conventional membranes thus ensuring more efficient bone formation and improving the clinical performance.	Radunovic, M. *et al.* Graphene oxide enrichment of collagen membranes improves DPSCs differentiation and controls inflammation occurrence. Journal of biomedical materials research. Part A 105, 2312–2320 (2017).
graphene oxide	toxicity	In this study, using *in vivo* assay system of Caenorhabditis elegans, authors investigated the function of ERK signaling in response to graphene oxide (GO) exposure and the underlying molecular mechanism. GO exposure increased the expression of MEK-2/MEK and MPK-1/ERK in the ERK signaling pathway.	Qu, M., Li, Y., Wu, Q., Xia, Y. & Wang, D. Neuronal ERK signaling in response to graphene oxide in nematode Caenorhabditis elegans. Nanotoxicology 11, 520–533 (2017).
graphene	toxicity	The cytotoxic and genotoxic potential for f-G was dependent on p53 status. The f-G exposures presented a lower cytotoxic and genotoxic hazard to B-lymphoblastoid cells with functional p53 than the hazard it presented to p53-deficeint B-lymphoblastoid cells. Albeit, the f-G exposures did not increase chromatin loss, or gene mutation, and induced chromosome damage only at the highest dose tested.	Petibone, D. M. *et al.* p53-competent cells and p53-deficient cells display different susceptibility to oxygen functionalized graphene cytotoxicity and genotoxicity. Journal of applied toxicology : JAT 37, 1333–1345 (2017).

graphene oxide	toxicity	In the current paper the detailed toxicological studies on single and four-layer graphene oxide (GO) nanoflakes is presented. The cytotoxicity assay confirmed comparable, dose dependent cytotoxicity of single and four layers GO flakes. The differences between these two nanomaterials became more distinct during cell proliferation study and ROS detection. Namely, markedly stronger inhibition of cell proliferation and higher ROS generation by one-layer GO-PEG than four-layer GO-PEG were observed. These findings emphasize the role of number of layer and functionalization in GO toxicological characteristics.	Peruzynska, M. *et al.* Comparative *in vitro* study of single and four layergraphene oxide nanoflakes - Cytotoxicity and cellular uptake. Toxicology *in vitro* : an international journal published in association with BIBRA 41, 205–213 (2017).
few layer graphene, graphene oxide	toxicity	The present study was carried out on HaCaT keratinocytes, an *in vitro* model of skin toxicity, on which the effects of few layer graphene, and three samples of graphene oxide (GOs, a research-grade GO1, and two commercial GOs, GO2 and GO3) were evaluated. Even though no significant effects were observed after 24 h, after 72 h the less oxidized compound (FLG) was the less cytotoxic. By contrast, the largest and most oxidized compound, GO3, was the most cytotoxic. These results suggest that only high concentrations and long exposure times to FLG and GOs could impair mitochondrial activity associated with plasma membrane damage, suggesting low cytotoxic effects at the skin level.	Pelin, M. *et al.* Differential cytotoxic effects of graphene and graphene oxide on skin keratinocytes. Scientific reports 7, 40572–40572 (2017).
graphene	toxicity	Liquid phase exfoliation of graphite in six different animal sera and evaluation of its toxicity are reported here. a nanotoxicity study of this graphene fully dispersed to human embryonic kidney cells, human lung cancer cells, and nematodes (Caenorhabditis elegans) showed no acute toxicity for up to 7 days at various doses (50–500 μg/mL), but prolonged exposure at higher doses (300–500 μg/mL, 10–15 days) showed cytotoxicity to cells (~95% death) and reproductive toxicity to C. elegans (5–10% reduction in brood size). The origin of toxicity was found to be due to the highly fragmented smaller graphene sheets (<200 nm), while the larger sheets were nontoxic (50–300 μg/mL dose).	Pattammattel, A. *et al.* Controlling the Graphene-Bio Interface: Dispersions in Animal Sera for Enhanced Stability and Reduced Toxicity. Langmuir : the ACS journal of surfaces and colloids 33, 14184–14194 (2017).
graphene oxide	toxicity	Authors investigated toxicity of Graphene Oxide (GO) in rats following exposure with respect to hepatotoxicity and oxidative stress biomarkers. Four groups of five male rats were orally administered GOs, once a day for five days, with doses of 10, 20 and 40mg/kg GO. The results of this study demonstrate that GO may be hepatotoxic, and its toxicity might be mediated through oxidative stress.	Patlolla, A. K., Rondalph, J. & Tchounwou, P. B. Biochemical and Histopathological Evaluation of Graphene Oxide in Sprague-Dawley Rats. Austin journal of environmental toxicology 3, (2017).

graphene oxide	toxicity	This study examined the effects of NGO exposure on male reproductive function of adult Wistar rats. Repeated exposure of NGO for 15 and 30 days resulted in decreased epididymal sperm counts and elevated sperm abnormalities. Percentage of motile sperms was also significantly reduced due to the exposure. Results of the study demonstrated that high-dose of NGO resulted in considerable histological damage to testicular tissue which included atrophy of seminiferous tubules with reduction in germinal epithelium, germ cell loss and vacuolization. Low and mid-doses of NGO were not associated with sperm dysfunction or testis damage. Withdrawal of treatment for 30 days demonstrated significant recovery potential. Histology of epididymis and male fertility potential were not affected due to the NGO exposure.	Nirmal, N. K., Awasthi, K. K. & John, P. J. Effects of Nano-Graphene Oxide on Testis, Epididymis and Fertility of Wistar Rats. Basic & clinical pharmacology & toxicology 121, 202–210 (2017).
graphene	ecotoxicity	This study investigates the acute toxicity of graphene to sludge microbial communities. Results also showed that the graphene acute toxicity led to significant reduction of the microbial community metabolic activity, which in return reduced BOD, nitrogen and phosphorous removals. Additionally, the presence of graphene led to significant changes in the sludge microbial community structure. graphene can be more deleterious to the wastewater treatment process than other carbon-based nanomaterials.	Nguyen, H. N., Castro-Wallace, S. L. & Rodrigues, D. F. Acute toxicity of graphene nanoplatelets on biological wastewater treatment process. ENVIRONMENTAL SCIENCE-NANO 4, 160–169 (2017).
few layer graphene, graphene oxide	toxicity, method	In this work, authors present a laser-based optical detection methodology for noninvasive detection and pharmacokinetics analysis of GBNs directly in blood flow in mice using *in vivo* photoacoustic (PA) flow cytometry (PAFC). Overall, PAFC provided unique data crucial for understanding GBN toxicity through real-time detection of GBNs using their intrinsic light absorption contrast.	Nedosekin, D. A. *et al. in vivo* noninvasive analysis of graphene nanomaterial pharmacokinetics using photoacoustic flow cytometry. Journal of applied toxicology : JAT 37, 1297–1304 (2017).
Pristine graphene, graphene oxide	toxicity	Authors chemically increased the oxidation level of the pristine graphene and compared the corresponding toxicological effects along with those for the graphene oxide. authors' results showed a dose-dependent trend in the cytotoxicity profile, where pristine graphene was the most cytotoxic, with decreasing toxicity observed with increasing oxygen content.	Majeed, W. *et al.* The role of surface chemistry in the cytotoxicity profile of graphene. Journal of applied toxicology : JAT 37, 462–470 (2017).
few layer graphene	ecotoxicity	This study focused on using carbon-14-labeled few-layer graphene (FLG) to determine whether the size of graphene plays a role in its uptake, depuration, and biodistribution in adult zebrafish. While the L-FLG mainly accumulated in the gut of adult zebrafish, the S-FLG was found in both the gut and liver after exposure with or without NOM. Strikingly, the S-FLG was able to pass through the intestinal wall and enter the intestinal epithelial cells and blood. The presence of NOM increased the quantity of S-FLG in these cells. Exposure to L-FLG or S-FLG also had a significantly different impact on the intestinal microbial community structure.	Lu, K. *et al.* Biological Uptake, Distribution, and Depuration of Radio-Labeled Graphene in Adult Zebrafish: Effects of Graphene Size and Natural Organic Matter. ACS NANO 11, 2872–2885 (2017).

graphene oxide	toxicity	Authors exposed HEK293T cells and zebrafish embryos to different concentrations of GO for 24 h, and transcriptional profiles of BER pathway genes, DNA damage, and cell viability were analyzed both *in vitro* and *in vivo*. the exposure to elevated GO concentration could cause DNA damage to HEK293T cells and zebrafish embryos	Lu, C.-J. *et al.* Graphene oxide nanosheets induce DNA damage and activate the base excision repair (BER) signaling pathway both *in vitro* and *in vivo*. Chemosphere 184, 795–805 (2017).
MoS2, boron nitride	toxicity	Cytotoxicities of sheetlike MoS2 and BN nanomaterials on human hepatoma HepG2 cells were systematically investigated at different toxic end points. Results showed that MoS2 and BN nanomaterials decreased cell viability at 30 μg/mL and induced adverse effects on intracellular ROS generation (≥2 μg/mL), mitochondrial depolarization (≥4 μg/mL), and membrane integrity (≥8 μg/mL for MoS2 and ≥2 μg/mL for BN).	Liu, S. *et al.* Cytotoxicity and Efflux Pump Inhibition Induced by Molybdenum Disulfide and Boron Nitride Nanomaterials with Sheetlike Structure. ENVIRONMENTAL SCIENCE & TECHNOLOGY 51, 10834–10842 (2017).
graphene oxide	ecotoxicity	Authors investigated the influence of graphene oxide (GO) on the growth, chlorophyll content and structure of white moss Leucobryum glaucum. Authors' results indicated that GO did not alter the fresh weight of L. glaucum, but inhibited the dry weight gain seriously, resulting in higher water-holding rates. authors' results collectively suggested that graphene had the potential environmental risk to moss plants, and the release of graphene into the environment should be strictly restricted.	Lin, X. W. *et al.* Toxicity of graphene oxide to white moss Leucobryum glaucum. RSC ADVANCES 7, 50287–50293 (2017).
pristine graphene	toxicity	The objective of this study was twofold: (i) to test different carbon-based nanomaterials (CBNs) [pristine graphene and multi-walled carbon nanotubes (MWCNTs)] for the presence of endotoxin and develop strategies for depyrogenation, and (ii) to compare the immune response exhibited by macrophages after exposure to native CBNs versus depyrogenated CBNs. The results of this study reaffirm that assessment of endotoxin and other bacterial contamination is critical when evaluating the cellular toxicity of nanomaterials.	Lahiani, M. H., Gokulan, K., Williams, K., Khodakovskaya, M. V. & Khare, S. Graphene and carbon nanotubes activate different cell surface receptors on macrophages before and after deactivation of endotoxins. Journal of applied toxicology : JAT 37, 1305–1316 (2017).
graphene oxide, reduced graphene oxide	toxicity	Here authors aimed to assess the potential toxic effects of GO and rGO to marine organisms by using *in vitro* assays with mussel (Mytilus galloprovincialis) hemocytes. Cells were exposed to a wide range of concentrations (up to 100 mg/L) of GO (with and without polyvinylpyrrolidone-PVP as stabilizing agent: GO and GO-PVP) and rGO with PVP (rGO-PVP) to assess cytotoxicity and cell membrane integrity. In conclusion, GO, GO-PVP and rGO-PVP are not highly toxic to mussel cells but they cause membrane damage and their toxicity is ROS-mediated.	Katsumiti, A., Tomovska, R. & Cajaraville, M. P. Intracellular localization and toxicity of graphene oxide and reduced graphene oxide nanoplatelets to mussel hemocytes *in vitro*. Aquatic toxicology (Amsterdam, Netherlands) 188, 138–147 (2017).

graphene oxide, reduced graphene oxide	toxicity	In this study, authors focused on the biological effects of graphene oxide (GO) and reduced graphene oxide (rGO) materials on PC12 cells, a type of traditional neural cell line. Authors found that GO and rGO exerted significant toxic effects on PC12 cells in a dose- and time-dependent manner. Moreover, apoptosis appeared to be a response to toxicity. In conclusion, authors' results show that GO has more potent toxic effects than rGO and that apoptosis and cell cycle arrest are the main toxicity responses to GO and rGO treatments, which are likely due to ERK pathway regulation.	Kang, Y. *et al.* Graphene oxide and reduced graphene oxide induced neural pheochromocytoma-derived PC12 cell lines apoptosis and cell cycle alterations via the ERK signaling pathways. International journal of nanomedicine 12, 5501–5510 (2017).
reduced graphene, graphene oxide, reduced graphene oxide	toxicity	In this study, authors explore the biocompatibility of graphene-related materials with chicken embryo red blood cells (RBC). The hemolysis assay was employed to evaluate the *in vitro* blood compatibility of reduced graphene, graphene oxide, and reduced graphene oxide. GN, GO, and rGO incubated with chicken embryo RBC caused damage to the structure of RBC and induced dose-dependent hemolysis. Treatments with all forms of graphene led to structural damage of cell membranes and formation of knizocytes and echinocytes. However, there were significant differences between the negative impact of the studied graphene forms, indicating that hydrophobic, reduced graphene nanoparticles (GN and rGO) are more toxic than those of the hydrophilic, oxidized form (GO).	Jaworski, S. *et al.* Interaction of different forms of graphene with chicken embryo red blood cells. Environmental science and pollution research international 24, 21671–21679 (2017).
graphene oxide nanosheets	toxicity	Parental zebrafish were exposed to GO nanosheets at concentrations of 0.01–1 μg/L. GO did not trigger obvious neurotoxicity in parental zebrafish, whereas remarkable neurotoxicity occurred in the offspring, which exhibited a loss of dopaminergic neurons and reductions in acetylcholinesterase activity. I	Hu, X. G., Wei, Z. & Mu, L. Graphene oxide nanosheets at trace concentrations elicit neurotoxicity in the offspring of zebrafish. CARBON 117, 182–191 (2017).
graphene oxide	ecotoxicity	This study examined the antibacterial activity of graphene oxide (GO) before and after solar transformation under two reaction scenarios. GO was directly phototransformed under simulated sunlight or indirectly photolyzed by photochemically generated hydroxyl radical (˙OH). The results indicate that compared to parent GO, directly phototransformed GO showed increased toxicity to bacteria, while the indirectly phototransformed GO became less toxic.	Hou, W. C., Lee, P. L., Chou, Y. C. & Wang, Y. S. Antibacterial property of graphene oxide: the role of phototransformation. ENVIRONMENTAL SCIENCE-NANO 4, 647–657 (2017).
graphene	ecotoxicity	The effects of graphene (at 0, 5, 50, 100, and 200 mg/L concentrations) on antioxidant enzyme activity, chlorophyll content and malondialdehyde (MDA) content in rice seedlings were studied. Results indicated the potential toxicity of graphene in rice seedlings and suggested the possible utility for 5 mg/L graphene to enhance growth in rice seedlings.	1.He, Y. *et al.* Effect of Graphene on Antioxidant Activity and Chlorophyll Content in Rice Seedlings. JOURNAL OF BIOBASED MATERIALS AND BIOENERGY 11, 510–515 (2017).

graphene oxide	ecotoxicity	The effect of different concentrations of GO on Picochlorum sp. during the different growth phases was examined. The results showed that the toxicity of GO increases with increasing its concentration. The lowest concentration (0.5 mg L^{-1}) was found to improve the algae growth and pigment content of Picochlorum sp. In contrast, higher GO concentrations had a negative consequence on the growth of algae and photosynthetic pigment concentration.	1.Hazeem, L. J. *et al.* Toxicity effect of graphene oxide on growth and photosynthetic pigment of the marine alga Picochlorum sp. during different growth stages. Environmental science and pollution research international 24, 4144–4152 (2017).
graphene oxide, reduced graphene oxide	ecotoxicity	The impact of graphene oxide (GO) and reduced graphene oxide (rGO) on biofilm formation and development in Luria-Bertani (LB) medium using Escherichia coli and Staphylococcus aureus was compared. GO and rGO showed different impacts on bacterial biofilm. Oxidative stress were involved in the toxicity of rGO to biofilm. Toxicity of rGO to biofilm were eliminated in mature phase of bacterial biofilm. EPS protection and oxidation of rGO contributed to the elimination of the toxicity of rGO.	Guo, Z. *et al.* Toxicity and transformation of graphene oxide and reduced graphene oxide in bacteria biofilm. The Science of the total environment 580, 1300–1308 (2017).
few layer graphene	ecotoxicity	The effect of FLG on the photosynthetic benthic diatom Nitzschia palea was assessed making distinction between the impact of a direct contact with FLG and a shading effect of FLG on diatoms. these results suggest that one potential toxicity process of graphene could be a combination of direct and shading effect leading to a strong interaction between biofilm and nanoparticles	Garacci, M. *et al.* Few Layer Graphene sticking by biofilm of freshwater diatom Nitzschia palea as a mitigation to its ecotoxicity. CARBON 113, 139–150 (2017).
few layer graphene	ecotoxicity	The toxicity of few-layer graphene (FLG) was evaluated in different tissues of the shrimp Litopenaeus vannamei following exposure to FLG through a diet for four weeks. results demonstrate that exposure to FLG through the diet induces alterations in the redox state of cells, leading to a subsequent oxidative stress situation. It is therefore clear that nanomaterials presenting these physico-chemical characteristics may be harmful to aquatic biota.	Fernandes, A. L. *et al.* Exposure to few-layer graphene through diet induces oxidative stress and histological changes in the marine shrimp Litopenaeus vannamei. Toxicology research 6, 205–214 (2017).
pristine graphene	toxicity	This study demonstrated that pristine graphene exposed to cultured kidney tubular epithelial cells is capable of inducing DNA fragmentation measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, which is usually associated with cell death. graphene induces cell death through oxidative injury, caspase-mediated and caspase-independent pathways; and endonucleases DNase I and EndoG are important for graphene toxicity.	Fahmi, T. *et al.* Mechanism of graphene-induced cytotoxicity: Role of endonucleases. Journal of applied toxicology : JAT 37, 1325–1332 (2017).

graphene oxide	toxicity	This study was performed to explore the genotoxic and pulmonary toxic potential of different doses of graphene oxide nanosheets' (GOs) in mice. GO potentiate the induction of genotoxicity and pulmonary injury in mice in time and dose dependent manner.	El-Yamany, N. A. *et al.* Graphene oxide nanosheets induced genotoxicity and pulmonary injury in mice. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie 69, 383–392 (2017).
graphene oxide	toxicity	This research assesses the *in vivo* toxicity of pure and manganese ion-contaminated GO that were administrated to Acheta domesticus with food (at 200 mg kg−1 of food) throughout their ten-day adult life. Short-term exposure to GO in food caused as follows: an increase in the parameters of oxidative stress, damage to the DNA, and numerous degenerative changes in the tissues of Acheta domesticus. Both pure and Mn2+ contaminated GO caused the same effects.	Dziewiecka, M. *et al.* Short-term *in vivo* exposure to graphene oxide can cause damage to the gut and testis. JOURNAL OF HAZARDOUS MATERIALS 328, 80–89 (2017).
graphene oxide	toxicity	Graphene oxide (GO), single-layer GO, thickness 0.7-1.2 nm was used in this study. All the assays were carried out at a concentration of 1 mg/mL (stock suspension by ultrasonication) (stable 10 days). In this study the first parameter evaluated was in animal toxicity in acute and chronic responses. The second one was to observe the morphology and histological changes on major organs, like signs of inflammatory areas. it could be concluded that GO of dose less than 0.5 µg/g failed to exhibit systemic toxicity and mutagenesis, while the dose of more than 5.0 µg/g exhibited obvious toxicity and showed potential to cause mutagenesis.	Duran, M., Duran, N., Favaro, W. J., & IOP. *in vivo* nanotoxicological profile of graphene oxide. in 5TH NANOSAFE INTERNATIONAL CONFERENCE ON HEALTH AND SAFETY ISSUES RELATED TO NANOMATERIALS FOR A SOCIALLY RESPONSIBLE APPROACH (NANOSAFE 2016) vol. 838 (2017).
graphene oxide	ecotoxicity	In this work, column experiments were conducted to investigate the transport characteristics of graphene oxide (GO) nanoparticles in limestone media under various electrolytes, solution pH, and humic acid (HA) concentration conditions. Experimental results showed that all the tested factors including electrolyte, pH and humic acid played important roles in controlling the transport of GO in limestone media. The mobility of GO in the limestone media increased with the increasing solution pH and HA concentrations, but the decreasing ionic strength. In comparison to Cl^{-}, S^{2-} in the electrolyte solution enhanced GO mobility in limestone media.	Dong, S. N. *et al.* Retention and transport of graphene oxide in water-saturated limestone media. CHEMOSPHERE 180, 506–512 (2017).

graphene oxide	ecotoxicity	The present study aimed to assess the toxic effects of different concentrations of GO (0.01; 0.10 and 1.00 mg/L) in D. neapolitana physiological (regenerative capacity) and biochemical (energy reserves, metabolic activity and oxidative stress related biomarkers) performance, after 28 days of exposure. The results obtained revealed that the exposure to GO induced negative effects on the regenerative capacity of D. neapolitana, with organisms exposed to higher concentrations regenerating less segments and taking longer periods to completely regenerate. GO also seemed to alter energy-related responses, especially glycogen content, with higher values in polychaetes exposed to GO when exposed to GO. Under GO contamination D. neapolitana presented cellular damage, despite higher activities of antioxidant and biotransformation enzymes in individuals exposed to GO.	De Marchi, L. *et al.* Physiological and biochemical impacts of graphene oxide in polychaetes: The case of Diopatra neapolitana. Comparative biochemistry and physiology. Toxicology & pharmacology : CBP 193, 50–60 (2017).
graphene oxide	ecotoxicity	This study sought to evaluate the toxicological effects of graphene oxide (GO) through tests with Danio rerio (zebrafish) embryos, considering the influence of the base washing treatment and the interaction with natural organic matter. Although the embryo exposure showed no acute toxicity or malformation, the larvae exposed to GO showed a reduction in their overall length and acetylcholinesterase activity.	Clemente, Z., Castro, V. L. S. S., Franqui, L. S., Silva, C. A. & Martinez, D. S. T. Nanotoxicity of graphene oxide: Assessing the influence of oxidation debris in the presence of humic acid. Environmental pollution (Barking, Essex : 1987) 225, 118–128 (2017).
graphene oxide	ectoxicity, method	In this study, 13C-stable isotope labeling of the carbon skeleton of graphene oxide (GO) was applied to investigate the bioaccumulation and toxicity of GO in wheat. GO inhibited the germination of wheat seeds at high concentrations (≥0.4 mg/mL). The mechanism of GO toxicity to wheat may be associated with oxidative stress induced by GO bioaccumulation. The results demonstrate that 13C labeling is a promising method to investigate environmental impacts and fates of carbon nanomaterials in biological systems.	Chen, L. *et al.* Bioaccumulation and Toxicity of 13C-Skeleton Labeled Graphene Oxide in Wheat. Environmental science & technology 51, 10146–10153 (2017).
graphene oxide	toxicity	Using the *in vivo* assay system of C. elegans, authors here investigated the effect of GO exposure on NLG-1-mediated signaling in neurons. In nematodes, GO exposure significantly decreased the NLG-1 expression, and mutation of nlg-1 gene induced a susceptible property to GO toxicity, suggesting the crucial role of NLG-1 in the induction of GO toxicity.	Chen, H., Li, H. & Wang, D. Graphene Oxide Dysregulates Neuroligin/NLG-1-Mediated Molecular Signaling in Interneurons in Caenorhabditis elegans. Scientific reports 7, 41655–41655 (2017).

graphene oxide, reduced graphene oxide	toxicity	The potential hazards of graphene nanomaterials were investigated by exposing the nematode Caenorhabditis elegans to graphene oxide (GO) and reduced graphene oxide (rGO). Comparative analysis of GO vs. rGO effects found that the wingless-type MMTV integration site family (Wnt) pathway and the mitogen-activated protein kinase (MAPK) pathway were evoked in GO- but not in rGO-exposed worms. authors' results highlight the distinct biological and molecular mechanisms of GO and rGO exposure and the role of Wnt-MAPK pathway.	Chatterjee, N. *et al.* A systems toxicology approach reveals the Wnt-MAPK crosstalk pathway mediated reproductive failure in Caenorhabditis elegans exposed to graphene oxide (GO) but not to reduced graphene oxide (rGO). Nanotoxicology 11, 76–86 (2017).
graphene, graphene oxide	ecotoxicity	The present study addresses the bioaccumulation and toxicity seen in Daphnia magna exposed to multiwalled carbon nanotubes (MWCNTs), graphene, and graphene oxide (GO) dispersed in sodium dodecyl benzene sulfonate (SDBS). The results from the present study show bioaccumulation, alterations in swimming velocity, and generation of ROS in D. magna exposed to CNMs.	Cano, A. M. *et al.* Bioaccumulation, stress, and swimming impairment in Daphnia magna exposed to multiwalled carbon nanotubes, graphene, and graphene oxide. Environmental toxicology and chemistry 36, 2199–2204 (2017).
graphene oxide	ecotoxicity	This study focuses on the interaction of selected types of nanomaterials with different Gram-positive and Gram-negative bacteria, filamentous fungi, and yeasts. Tested nanomaterials were on carbon base–nanodiamonds, carbon nanotubes, and graphene oxide. Authors' data shows that antimicrobial potential depends on the interaction between nanomaterials and microorganism. Antimicrobial effect was observed only against Gram-positive bacteria exposed to graphene oxide GO-2 and single-wall carbon nanotubes (SWCNT). Genotoxicity was detected in case of 500 nm boron doped diamond nanopowder. Both forms (liquid and powder) of graphene oxide GO-2 in the highest applied concentration showed the toxic effect on S. Typhimurium.	Brandeburova, P. *et al.* The influence of selected nanomaterials on microorganisms. MONATSHEFTE FUR CHEMIE 148, 525–530 (2017).
graphene oxide, reduced graphene oxide	ecotoxicity	In this study, authors comparatively studied the influence of GO and reduced GO (RGO) on the activity and conformation of lysozyme. GO inhibited lysozyme activity seriously, while RGO nearly had no influence on the enzyme activity. neither GO nor RGO induced the fibrillation of lysozyme after 12 d incubation.	Bai, Y. *et al.* Influence of graphene oxide and reduced graphene oxide on the activity and conformation of lysozyme. Colloids and surfaces. B, Biointerfaces 154, 96–103 (2017).
graphene oxide	ecotoxicity	In authors' study, it was found that GO could induce delay in pupation and eclosion of Drosophila under acute exposure. GO could be ingested by Drosophila but can be easily excreted from the body. There is no obvious accumulation of GO in Drosophila. Thus, in chronic exposure, GO didn't bring obvious toxicity on the Drosophila longevity.	Zou, H. Y. *et al. in vivo* Toxicity Evaluation of Graphene Oxide in Drosophila Melanogaster After Oral Administration. JOURNAL OF NANOSCIENCE AND NANOTECHNOLOGY 16, 7472–7478 (2016).

graphene oxide	toxicity	In this study, authors investigated the possible involvement of Wnt signals in the control of graphene oxide (GO) toxicity using the *in vivo* assay system of Caenorhabditis elegans. In nematodes, the Wnt ligands, CWN-1, CWN-2, and LIN-44, were found to be involved in the control of GO toxicity.	Zhi, L., Ren, M., Qu, M., Zhang, H. & Wang, D. Wnt Ligands Differentially Regulate Toxicity and Translocation of Graphene Oxide through Different Mechanisms in Caenorhabditis elegans. Scientific reports 6, 39261–39261 (2016).
graphene oxide	ecotoxicity	Using *in vivo* assay system of Caenorhabditis elegans, authors found that mutation of genes encoding core p38 mitogen-activated protein kinase (MAPK) signaling pathway caused susceptible property to GO toxicity and enhanced translocation of GO into the body of nematodes. Exposure to GO induced significantly increased expression of genes encoding p38 MAPK-SKN-1/Nrf signaling cascade, which further implies that the identified p38 MAPK-SKN-1/Nrf signaling cascade may encode a protection mechanism for nematodes in intestine to be against GO toxicity.	Zhao, Y. *et al.* p38 MAPK-SKN-1/Nrf signaling cascade is required for intestinal barrier against graphene oxide toxicity in Caenorhabditis elegans. Nanotoxicology 10, 1469–1479 (2016).
graphene oxide	toxicity	Using Caenorhabditis elegans as an *in vivo* assay system, authors' results suggest the toxicity of graphene oxide in reducing reproductive capacity by inducing damage on gonad development. The observed reproductive toxicity of GO on gonad development was due to the combinational effect of germline apoptosis and cell cycle arrest, and DNA damage activation might act as an inducer for this combinational effect.	Zhao, Y., Wu, Q. & Wang, D. An epigenetic signal encoded protection mechanism is activated by graphene oxide to inhibit its induced reproductive toxicity in Caenorhabditis elegans. Biomaterials 79, 15–24 (2016).
graphene	ecotoxicity	In the present study, effects of graphene on plant roots and shoots after 48 h or 30 days of hydroponic culture were evaluated to determine its phytotoxicity. Results showed that although exposure to graphene (250, 500, 1000 and 1500 mg L^{-1}) significantly improved root elongation, root hair production was impaired. authors conclude that graphene has growth-limiting effects on plants, including root hair reduction, oxidative burst, photosynthesis inhibition, and nutritional disorder.	Zhang, P. *et al.* Toxic effects of graphene on the growth and nutritional levels of wheat (Triticum aestivum L.): short- and long-term exposure studies. Journal of hazardous materials 317, 543–551 (2016).
graphene oxide	ecotoxicity	In this study, authors investigated the toxicity of synthesized graphene oxide (GO) against the model industrial organism Pichia pastoris. Authors found that the synthesized GO showed dose-dependent toxicity to P. pastoris, through cell membrane damage and intracellular reactive oxygen species (ROS) accumulation.	Zhang, M. *et al.* Graphene oxide induces plasma membrane damage, reactive oxygen species accumulation and fatty acid profiles change in Pichia pastoris. Ecotoxicology and environmental safety 132, 372–378 (2016).

graphene nanoplatelets	toxicity	Several short-term inhalation toxicity studies (1-day, 5-day and 28-day) for graphene nanomaterials have been conducted. Acute inhalation study of graphene oxide induced minimal toxic response in rat lungs with high concentration (3.76 mg/m^3). Five-day short term inhalation study (STIS) of graphene nanoplatelets with 28 days of recovery also showed minimal toxic effects at a high concentration (3.86 mg/m^3). Twenty-eight day inhalation toxicity study of graphene nanoplatelets with 90-day recovery also showed minimal toxic effects up to 2 mg/m^3.	Yu, I. J. *et al.* Occupational exposure to graphene nanomaterials and inhalation toxicities of graphene nanomaterials. TOXICOLOGY LETTERS 259 MA-S11-2, S21–S21 (2016).
graphene, graphene oxide	toxicity	This report studied graphene and GO on B cell and plasma cell function. Authors revealed that graphene and GO showed distinct effect on B cells and raised the significance of studying on the immune modulation of nanomaterials, particularly the humoral immunity. The influence of G and GO on antibody-mediated response and B cell-mediated response implied that graphene derivatives should be modified to attenuate or even eliminate the adverse effect on immune system.	Xu, S. *et al.* Graphene Oxide Modulates B Cell Surface Phenotype and Impairs Immunoglobulin Secretion in Plasma Cell. Journal of nanoscience and nanotechnology 16, 4205–4215 (2016).
graphene	toxicity	In this study, mice were exposed to graphene for 4 weeks, and high-throughput sequencing was applied to characterize the changes in microbial community and antibiotic resistance genes (ARGs) in mouse gut. Graphene exposure increased the gut microbial diversity of mouse. 1 μg/d graphene had higher influence on gut micorbiota than 10 and 100 μg/d grapheme. Graphene increased abundances of oxidative stress and membrane-damage related genes. Gram-negative bacteria were more tolerant to graphene than Gram-positive bacteria. Graphene exposure increased types and abundances of antibiotic resistance genes.	Xie, Y. *et al.* Influences of graphene on microbial community and antibiotic resistance genes in mouse gut as determined by high-throughput sequencing. Chemosphere 144, 1306–1312 (2016).
graphene oxide	toxicity	In this study, authors investigated the toxicity of graphene oxide (GO) to white rot fungus (Phanerochaete chrysosporium) at the concentrations of 0–4 mg/mL for 7 d. Beyond the toxicity, GO did not alter the activities of P. chrysosporium at low concentrations but led to the complete loss of activity at high concentrations.	Xie, J. *et al.* Toxicity of graphene oxide to white rot fungus Phanerochaete chrysosporium. Chemosphere 151, 324–331 (2016).
graphene oxide	toxicity	In this study, authors first examined lncRNA-dependent regulation of GO toxicity with the aid of C. elegans *in vivo* assay system. Authors identified a limited number of lncRNAs that are dysregulated by GO exposure. Authors propose an lncRNA-miRNA network that may be involved in the control of GO toxicity.	Wu, Q. *et al.* Genome-wide identification and functional analysis of long noncoding RNAs involved in the response to graphene oxide. Biomaterials 102, 277–291 (2016).
MoS2	toxicity	In this study, authors utilized metabolomics technology to explore the effects of different concentrations of molybdenum disulfide nanosheets on Escherichia coli for the first time. The results showed that with increasing concentration of molybdenum disulfide nanosheets, the survival rate of Escherichia coli was decreased.	Wu, N. *et al.* Investigating the Influence of MoS2 Nanosheets on E. coli from Metabolomics Level. PloS one 11, e0167245–e0167245 (2016).

pristine graphene, graphene oxide, reduced graphene oxide	toxicity	In the present work, the toxicity of three forms of graphene: pristine graphene (pG), graphene oxide (GO), and reduced graphene oxide (rGO) was investigated using a chicken embryo model in concentrations of 50, 500, and 5000 μg/ml. Survival of embryos decreased significantly after treatment with all types of graphene, but not in a dose-dependent manner. The body weights were only slightly affected by the highest doses of graphene, while the organ weights were not different among treatment groups. In all experimental groups, atypical hepatocyte ultrastructure and mitochondrial damage were observed. The concentration of the marker of DNA damage 8-OHdG in the liver significantly decreased after pG and rGO treatments.	1.Szmidt, M. *et al.* Toxicity of different forms of graphene in a chicken embryo model. Environmental science and pollution research international 23, 19940–19948 (2016).
graphene oxide	toxicity	Here, authors used confocal and live-cell fluorescence microscopy, as well as scanning electron microscopy, of rat basophilic leukemia (RBL) cells to demonstrate profound plasma membrane (PM) ruffling and shedding induced by GO. Graphene oxide (GO) induces ruffling and shedding of mammalian cell plasma membranes GO-induced plasma membrane responses induce loss of contact inhibition in RBL cells GO-treated plasma membranes undergo nuanced structural and functional changes	Sun, C. *et al.* Graphene Oxide Nanosheets Stimulate Ruffling and Shedding of Mammalian Cell Plasma Membranes. CHEM 1, (2016).
few layer graphene	toxicity	Herein, using human primary umbilical vein endothelial cells as model of vascular transport, authors investigated the basic mechanism underlying the biological behavior of graphene. mechanistic toxicity studies revealed that FLG exerted cellular toxicity employing an oxidative stress paradigm in HUVEC cells, which severely altered critical cell parameters including cytoskeletal dysfunction, reduction in metabolic activity, compromised plasma membrane integrity, elevated levels of intracellular ROS, lipid peroxidation, oxidized glutathione, ionized calcium and depolarization of mitochondrial membrane potential.	Sasidharan, A., Swaroop, S., Chandran, P., Nair, S. & Koyakutty, M. Cellular and molecular mechanistic insight into the DNA-damaging potential of few-layer graphene in human primary endothelial cells. Nanomedicine : nanotechnology, biology, and medicine 12, 1347–1355 (2016).
graphene	ecotoxicity	In this paper, authors report on the toxicity of three carbon nanomaterials (fullerene-soot, multiwall carbon nanotubes, and graphene). Two standardized toxicity bioassays, the immobilization of the invertebrate Daphnia magna and the bioluminescence inhibition of the marine bacteria Vibrio fischeri, have been used. Toxicity to D. magna was as follows: fullerene soot > multiwall carbon nanotubes > graphene. These results were proportional to the size of aggregates, smaller aggregates being the most toxic.	Sanchis, J. *et al.* New Insights on the Influence of Organic Co-Contaminants on the Aquatic Toxicology of Carbon Nanomaterials. ENVIRONMENTAL SCIENCE & TECHNOLOGY 50, 961–969 (2016).
graphene	toxicity	Authors have studied and analyzed different aspects and origins of cytotoxicity of diverse varieties of sp2 hybridized carbon materials. Cytotoxicity results using Caco-2 cells confirmed that cell survival rates vary with different types of carbon and nanographene caused the minimal cell survival amongst all the samples studied.	Saha, D. *et al.* A study on the cytotoxicity of carbon-based materials. Materials science & engineering. C, Materials for biological applications 68, 101–108 (2016).

reduced graphene oxide	toxicity	This study aimed to elucidate the nano-biointeractions of PEGylated reduced graphene oxide (PrGO) and reduced graphene oxide (rGO) with that of lung alveolar epithelial cells (A549). This study highlights the possible adverse toxic effect of PrGO and rGO upon inhalation and persistence of these particles in the lungs.	1.Reshma, S. C., Syama, S. & Mohanan, P. V. Nano-biointeractions of PEGylated and bare reduced graphene oxide on lung alveolar epithelial cells: A comparative *in vitro* study. Colloids and surfaces. B, Biointerfaces 140, 104–116 (2016).
graphene oxide	toxicity	It was found that graphene oxide (GO) at concentrations of 0.01 μg/L–1 μg/L induced Parkinson's disease-like symptoms in zebrafish larvae. Moreover, it was also shown that GO was able to translocate from the water environment to the brain and localize to the nucleus of the diencephalon, thereby inducing structural and morphological damage in the mitochondria. Cell apoptosis and senescence were triggered via oxidative stress	Ren, C., Hu, X., Li, X. & Zhou, Q. Ultra-trace graphene oxide in a water environment triggers Parkinson's disease-like symptoms and metabolic disturbance in zebrafish larvae. Biomaterials 93, 83–94 (2016).
graphene oxide	toxicity	This work describes the ability of s-GO to alter synapses and induce glial cell reaction. This might compromise neuronal signaling and CNS functions and seems crucially dependent on the GO sheet dimensions since larger flakes were found unequivocally cytotoxic. In authors' experiments, 6 days of exposure of cultures to equal amounts of dispersed l-GO induced unequivocal hippocampal cell loss, both neuroglia and neurons, thus hampering any further evaluation of membrane/flake interactions.	1.Rauti, R. *et al.* Graphene Oxide Nanosheets Reshape Synaptic Function in Cultured Brain Networks. ACS Nano 10, 4459–4471 (2016).
graphene oxide	combined ecotoxicity	GO significantly enhanced the bioaccumulation of PFOS in blood, kidney, liver, gill, intestine, and muscle of carp, and the corresponding bioaccumulation factor (BAF) was in the range of 2026–53513 L/kg. However, FA could facilitate the flocculation of GO in the intestine and also accelerate excretion of GO–PFOS complex. Thus, in the presence of FA, PFOS absorption was reduced and the promotion effect of GO on PFOS accumulation was remitted.	Qiang, L. W., Chen, M., Zhu, L. Y., Wu, W. & Wang, Q. Facilitated Bioaccumulation of Perfluorooctanesulfonate in Common Carp (Cyprinus carpio) by Graphene Oxide and Remission Mechanism of Fulvic Acid. ENVIRONMENTAL SCIENCE & TECHNOLOGY 50, 11627–11636 (2016).
graphene oxide	toxicity	To determine which chemical fragments of GO are responsible for this toxicity, GOs containing variable redox-active groups on the surface were generated and compared. The results reveal that endoperoxides play a decisive role in GO-induced oxidative stress.	Pieper, H. *et al.* Endoperoxides Revealed as Origin of the Toxicity of Graphene Oxide. Angewandte Chemie (International ed. in English) 55, 405–407 (2016).

graphene oxide	toxicity	The present study investigated the response of kidneys in male Sprague-Dawley rats following exposure to 0, 10, 20 and 40 mg/kg GO for five days. The results showed that administration of GOs significantly increased the activities of superoxide dismutase, catalase and glutathione peroxidase in a dose-dependent manner in the kidneys compared with control group. Taken together, the results of this study demonstrate that GO is nephrotoxic and its toxicity may be mediated through oxidative stress.	Patlolla, A. K., Randolph, J., Kumari, S. A. & Tchounwou, P. B. Toxicity Evaluation of Graphene Oxide in Kidneys of Sprague-Dawley Rats. International journal of environmental research and public health 13, 380–380 (2016).
few layer graphene	ecotoxicity	MLGs composed of 2–20 graphene layers, were tested on two mammalian cell models and on X. laevis as an important *in vivo* and environmental model organism. MLG showed to be substantially not toxic towards cellular models and X. larvae used in this study.	Muzi, L. *et al.* Examiningthe impact of multi-layer graphene using cellular and amphibian models. 2D MATERIALS 3, (2016).
few layer graphene	ecotoxicity	Here authors compare the inhibition of Xenopus laevis larvae growth after *in vivo* exposure to different carbon nanoparticles for 12 days using different dose metrics and clearly show that surface area is the most relevant descriptor of toxicity for different types of carbon allotropes.	Mottier, A. *et al.* Surface Area of Carbon Nanoparticles: A Dose Metric for a More Realistic Ecotoxicological Assessment. Nano letters 16, 3514–3518 (2016).
reduced graphene oxide	toxicity	In the present study, reduced graphene oxide (rGO) was functionalized with PEG, and its effects on key components of the blood–brain barrier, such as astrocytes and endothelial cells, were analyzed in culture and in an *in vivo* rat model. The *in vitro* studies demonstrated concentration-dependent toxicity. The highest concentration (100 µg/mL) of non-PEGylated rGO had a lower toxic influence on cell viability in primary cultures of astrocytes and rat brain endothelial cells, while PEGylated rGO induced deleterious effects and cell death.	Mendonça, M. C. P. *et al.* PEGylation of Reduced Graphene Oxide Induces Toxicity in Cells of the Blood-Brain Barrier: An *in vitro* and *in vivo* Study. MOLECULAR PHARMACEUTICS 13, 3913–3924 (2016).
reduced graphene oxide	toxicity	Our purpose was to trace whether the rGO inside the hippocampus triggered toxic alterations in this brain region and in target organs (blood, liver and kidney) of rats at various time points (15 min, 1, 3 h and 7 days). The toxic effects seemed to be peripheral and transitory in the short-term analysis after systemic administration of rGO. The effects were self-limited and non-significant even at 7 days post-rGO administration.	Mendonca, M. C. P. *et al.* Reduced graphene oxide: nanotoxicological profile in rats. JOURNAL OF NANOBIOTECHNOLOGY 14, (2016).
pristine graphene	toxicity	Here the toxicity, uptake and catabolic response of primary human macrophages to pristine graphene (PG) and pristine single walled carbon nanotubes (pSWCNT) are explored. In conclusion, these two nanomaterials, with similar surface chemistries but unique geometries, differ significantly in their uptake mechanisms and subsequently induced lysosomal and autophagic catabolic pathways in human primary macrophages.	McIntyre, J. *et al.* A comparison of catabolic pathways induced in primary macrophages by pristine single walled carbon nanotubes and pristine graphene. RSC ADVANCES 6, 65299–65310 (2016).

few layer graphene	ecotoxicity	14C-labeled few layer graphene (FLG) was dispersed in artificial freshwater and uptake of FLG by Limnodrilus hoffmeisteri, an oligochaete, was assessed. The data provide the first evidence that the proteins secreted by Limnodrilus hoffmeisteri after exposure to FLG can coat FLG, thus increasing the aqueous stability of FLG, decreasing its size, and changing its bioaccumulation potential.	Mao, L. *et al.* Exposure of few layer graphene to Limnodrilus hoffineisteri modifies the graphene and changes its bioaccumulation by other organisms. CARBON 109, 566–574 (2016).
graphene oxide	toxicity	The cytotoxicity of graphene oxide has been evaluated with an SRB assay with living mammalian cell lines, human breast cancer cell line MCF-7 and monkey normal kidney cell line Vero. The estimated cell viabilities are greater than 80% over a 10–80 μg mL^{-1} concentration.	Maktedar, S. S., Avashthi, G. & Singh, M. Understanding the significance of O-doped graphene towards biomedical applications. RSC ADVANCES 6, 114264–114275 (2016).
graphene oxide	ecotoxicity	A comprehensive evaluation on the biosafety of graphene oxide (GO) was developed by combining 16S rRNA sequencing, gene expression detection, histology and a scanning electron microscope assay on fish. GO does not affect the diversity and composition of gut microbiota, but down-regulates gene expression in fish liver, suggesting that it poses a potential risk to aquatic ecosystems. In summary, although GO did not affect the composition and diversity of gut bacterial microbiota, the expression of most genes was significantly down-regulated in the liver.	Ma, K. Y., Zhang, S. P., Ye, B. Q., Ouyang, J. Y. & Yue, G. H. A new view of graphene oxide biosafety in a water environment using an eatable fish as a model. RSC ADVANCES 6, 29619–29623 (2016).
graphene	toxicity	In the present study, graphene, as the model nanomaterial, was used to test its potential effects on the cell proliferation based on multiple representative cell lines, including HepG2, A549, MCF-7, and HeLa cells. No influences on cell apoptosis were observed in graphene-treated cells when compared to the negative controls, proving the low cytotoxicity of this emerging nanomaterial.	Liu, W. *et al.* Graphene Enhances Cellular Proliferation through Activating the Epidermal Growth Factor Receptor. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 64, 5909–5918 (2016).
graphene, graphene oxide	toxicity	In this study, authors analyzed the potential effects of graphene and GO at relatively low concentrations on cellular xenobiotic defense system mediated by efflux transporters. This study demonstrates that low levels of graphene and GO are not environmentally safe since they can significantly make cell more susceptible to other xenobiotics, and this chemosensitizing activity should be considered in the risk assessment of graphene and GO.	Liu, S. *et al.* Low levels of graphene and graphene oxide inhibit cellular xenobiotic defense system mediated by efflux transporters. Nanotoxicology 10, 597–606 (2016).
graphene oxide	toxicity	Rat tail veins were injected with 2.5, 5, or 10 mg/kg GO for seven days and behavioral patterns, pathology, and tissue morphology were assessed. Data show that behaviors were not altered according to an open field test and a functional observational battery test, but histopathological analysis indicated that GO caused inflammation of the lung, liver, and spleen. GO also reduced cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL). No other organs were modified.	Li, Y. *et al.* Sub-Acute Toxicity Study of Graphene Oxide in the Sprague-Dawley Rat. International journal of environmental research and public health 13, (2016).

graphene oxide	toxicity	Effect of carbon nanomaterials (graphene oxide and carbon nanotube) in the processing of abasic sites DNA damage by APE1 is shown here for the first time. Carbon nanomaterials are found to create conformational destabilization in both the adsorbed DNA and the enzyme, which is an interesting finding. Inhibition of APE1 activity was found to be more for multiwall carbon nanotube than graphene oxide.	Kumari, R., Mondal, T., Bhowmick, A. K. & Das, P. Impeded repair of abasic site damaged lesions in DNA adsorbed over functionalized multiwalled carbon nanotube and graphene oxide. MUTATION RESEARCH-GENETIC TOXICOLOGY AND ENVIRONMENTAL MUTAGENESIS 803, 39–46 (2016).
graphene oxide	toxicity	A detailed analysis of kidney function, histopathology, and ultrastructure was performed, along with the *in vitro* responses of two highly specialized GFB cells (glomerular endothelial cells and podocytes) following exposure to GO.This study provided a previously unreported understanding of the interaction between thin GO sheets with different components of the GFB *in vitro* and *in vivo* to highlight that the glomerular excretion of significant amounts of GO did not induce any signs of acute nephrotoxicity or glomerular barrier dysfunction.	Jasim, D. A. *et al.* The Effects of Extensive Glomerular Filtration of Thin Graphene Oxide Sheets on Kidney Physiology. ACS NANO 10, 10753–10767 (2016).
graphene oxide	ecotoxicity	Herein, single-cell Chlorella vulgaris was exposed to graphene oxide (GO). The inhibition of cell division and chlorophyll a biosynthesis and the enhancement of reactive oxygen species (ROS) and cell plasmolysis were observed under GO exposure; these adverse effects returned to normal levels when the cells were placed in fresh medium, suggesting that the tested effects were recoverable.	Hu, X. G., Gao, Y. & Fang, Z. Integrating metabolic analysis with biological endpoints provides insight into nanotoxicological mechanisms of graphene oxide: From effect onset to cessation. CARBON 109, 65–73 (2016).
graphene oxide, reduced graphene oxide	toxicity	The present study analyzed the dose-dependent cyto- and genotoxicity of graphene oxide and reduced graphene oxide on spermatogonial stem cells (SSCs). Authors conclude that a high concentration of graphene can be toxic to SSCs.	Hashemi, E. *et al.* Synthesis and cyto-genotoxicity evaluation of graphene on mice spermatogonial stem cells. Colloids and surfaces. B, Biointerfaces 146, 770–776 (2016).
graphene	ecotoxicity	Authors exposed dry grassland soil for 1 year to 1 mg g^{-1} of either natural nanostructured material (biochar), industrial carbon black, three types of multiwalled carbon nanotubes (MWCNTs), or graphene. After a 1-year exposure, compared to the no amendment control, some treatments reduced soil DNA (*e.g.*, biochar, all three MWCNT types, and graphene; $P < 0.05$) and altered bacterial communities (*e.g.*, biochar, carbon black, narrow MWCNTs, and graphene); however, there were no significant differences across the amended treatments. These findings suggest that ECNMs may moderately affect dry soil microbial communities but that the effects are similar to those from natural and industrial carbonaceous materials, even after 1-year exposure.	Ge, Y. *et al.* Long-Term Effects of Multiwalled Carbon Nanotubes and Graphene on Microbial Communities in Dry Soil. Environmental science & technology 50, 3965–3974 (2016).

graphene	ecotoxicity	This study assessed the acute and chronic toxicity of GN to Daphnia magna by comparing the toxic effects of GN with those of three other typical carbon nanomaterials (CNMs), fullerene (C60), single-walled carbon nanotube (SWCNT), and multi-walled carbon nanotube (MWCNT). The toxicity of GN was significantly higher than that of the other three CNMs, although GN bioaccumulation in D. magna was relatively lower.	Fan, W. H. *et al.* The mechanism of chronic toxicity to Daphnia magna induced by graphene suspended in a water column. ENVIRONMENTAL SCIENCE-NANO 3, 1405–1415 (2016).
graphene oxide	ecotoxicity	The toxicity of pure and manganese ion contaminated graphene oxide (GO) was measured. Oxidative stress was measured in A. domesticus 1, 24, 48, 72 h after GO injection. Neither pure GO nor GO contaminated with manganese were neutral to the organism. CAT and GSTPx activity increased in the 1st and 24th hour after GO injection. In the following days, increasing HSP 70 levels were noticed.	Dziewięcka, M. *et al.* Evaluation of *in vivo* graphene oxide toxicity for Acheta domesticus in relation to nanomaterial purity and time passed from the exposure. JOURNAL OF HAZARDOUS MATERIALS 305, 30–40 (2016).
reduced graphene oxide	ecotoxicity	In this study, the impact of reduced graphene oxide (RGO) on the microalgae Scenedesmus obliquus was evaluated to determine its phytotoxicity. RGO impaired the extra- and intra-cellular morphology and increased oxidative stress and thus inhibited algal growth and photosynthesis.	Du, S. *et al.* Reduced graphene oxide induces cytotoxicity and inhibits photosynthetic performance of the green alga Scenedesmus obliquus. Chemosphere 164, 499–507 (2016).
graphene oxide	toxicity	The bioluminescence inhibition assays with marine Photobacterium phosphoreum and recombinant Escherichia coli strains were varied in minimal toxic concentrations and EC50 values but led to well-correlated biotoxicity evaluation for the most active compounds, which were ranked as Cu > (MgO, CuO) > (fullerenol, graphene oxide).	Deryabin, D. G. *et al.* Comparative sensitivity of the luminescent Photobacterium phosphoreum, Escherichia coli, and Bacillus subtilis strains to toxic effects of carbon-based nanomaterials and metal nanoparticles. MICROBIOLOGY 85, 198–206 (2016).
graphene	toxicity	TRG injected in the brain together with a retroviral vector expressing GFP to label dividing progenitor cells in the core of the adult olfactory bulb (OB) did not alter de novo neurogenesis, neuronal and astrocyte survival nor did it produce a microglial response.	Defteralı, Ç. *et al.* Thermally reduced graphene is a permissive material for neurons and astrocytes and de novo neurogenesis in the adult olfactory bulb *in vivo*. Biomaterials 82, 84–93 (2016).
graphene oxide	ecotoxicity	The toxicity effect of graphene oxide (GO) on the microbial functions involved in the biological wastewater treatment process is studied, using Pseudomonas putida and salicylic acid (SA) as bacterial and pollutant models. This study reveals that the presence of GO in simulated urban and industrial wastewaters (SUW and SIW, respectively) has a negative effect on the bacterial growth and viability of Pseudomonas putida. The growth of P. putida is inhibited by the presence of GO concentrations higher than 0.05 mg mL^{-1}, this effect being noticeable in both simulated media	Combarros, R. G., Collado, S. & Díaz, M. Toxicity of graphene oxide on growth and metabolism of Pseudomonas putida. Journal of hazardous materials 310, 246–252 (2016).

graphene oxide	toxicity	In the present study, authors performed a comprehensive study of the size- and dose-dependent toxicity of GOs in the presence or absence of Pluronic F-127 on THP-1 cells. Authors' findings suggest that SLGOs and MLGOs can produce severe cytotoxicity in a dose- and size-dependent manner and induce an immune response, as evidenced by phagocytosis and cytokine expression. Furthermore, the degree of cytotoxicity differed among the various sizes and layer numbers of SLGOs and MLGOs, possibly due to differences in dispersion and protein adsorption.	Cho, Y. C., Pak, P. J., Joo, Y. H., Lee, H.-S. & Chung, N. *In vitro* and *in vivo* comparison of the immunotoxicity of single- and multi-layered graphene oxides with or without pluronic F-127. Scientific reports 6, 38884–38884 (2016).
graphene oxide	toxicity	In this study, acute toxicity, oxidative stress and immunotoxicity of GO were investigated in zebrafish. No obvious acute toxicity was observed when zebrafish were exposed to 1, 5, 10 or 50 mg/L GO for 14 days. Graphene oxide induces oxidative stress in adult Zebrafish. Graphene oxide induces expression of inflammatory cytokines in adult zebrafish. Graphene oxide induces histopathological lesions in adult zebrafish.	Chen, M. *et al.* Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. Aquatic toxicology (Amsterdam, Netherlands) 174, 54–60 (2016).
graphene, graphene oxide	toxicity	Authors used five different representatives of GFNs-pristine (GNP-Prist), carboxylated (GNP-COOH) and aminated (GNP-NH_2) graphene nanoplatelets as well as single layer (SLGO) and few layer (FLGO) graphene oxide. The order of DNA damage is GNP-pristine ≥ GNP-COOH > GNP-NH_2 ≥ FLGO > SLGO. The GFNs possibly caused DNA damage by affecting NER and NHEJ repair systems. Global hypermethylation in SLGO/FLGO whereas hypomethylation in GNPs treatment. Possibly DNMT3B and MBD1 genes regulate GFN's induced global DNA methylation status.	Chatterjee, N., Yang, J. & Choi, J. Differential genotoxic and epigenotoxic effects of graphene family nanomaterials (GFNs) in human bronchial epithelial cells. Mutation research. Genetic toxicology and environmental mutagenesis 798–799, 1–10 (2016).
graphene oxide	toxicity	Authors tested antibacterial activity and cytotoxicity toward peripheral blood mononuclear cells of 3 different GO samples. A size-dependent growth inhibition of Escherichia coli (DH5 α) in suspension was found, which proved that this effect depends strongly on the protocol followed for exposure. Hemocompatibility was confirmed by exposing peripheral blood mononuclear cells to materials for 24 hours; viability and apoptosis tests were also carried out.	Campos-Delgado, J. *et al.* Effect of graphene oxide on bacteria and peripheral blood mononuclear cells. JOURNAL OF APPLIED BIOMATERIALS & FUNCTIONAL MATERIALS 14, E423–E430 (2016).
few layer graphene, graphene oxide	toxicity	Here authors conducted a comprehensive analysis of the effects of chronic and acute exposure of rat primary cortical neurons to few-layer pristine graphene (GR) and monolayer graphene oxide (GO) flakes. authors' results show that, although graphene exposure does not impact neuron viability, it does nevertheless have important effects on neuronal transmission and network functionality.	Bramini, M. *et al.* Graphene Oxide Nanosheets Disrupt Lipid Composition, Ca2+ Homeostasis, and Synaptic Transmission in Primary Cortical Neurons. ACS Nano 10, 7154–7171 (2016).
graphene oxide	toxicity	Here authors demonstrate that highly purified and thoroughly washed GO neither inhibited nor stimulated the growth of E. coli, ATCC25922; E. coli NCIMB11943 and S. aureus ATCC25923 at concentrations of up to 1 mg ml^{-1}.	Barbolina, I. *et al.* Purity of graphene oxide determines its antibacterial activity. 2D MATERIALS 3, (2016).

boron nitride	toxicity	The aim of authors' study is to investigate serum boron levels using ICP-MS after implantation of different ratios of nano-hBN–HA composites in rat femurs. Authors demonstrated that neither short-term nor long-term implantation of hBN–HA composite resulted in statistically increased serum boron levels in experimental groups compared to healthy group.	Atila, A. *et al.* Study of the boron levels in serum after implantation of different ratios nano-hexagonal boron nitride-hydroxy apatite in rat femurs. Materials science & engineering. C, Materials for biological applications 58, 1082–1089 (2016).
reduced graphene oxide	toxicity	Here, authors show that the presence of functionalized single walled carbon nanotubes (SWCNT-COOH) and reduced graphene oxide at concentrations of 1–25 µg/mL do not affect sperm viability. However, SWCNT-COOH generate significant reactive superoxide species at a higher concentration (25 µg/mL), while reduced graphene oxide does not initiate reactive species in human sperm. Further, authors demonstrate that exposure to these nanomaterials does not hinder the sperm sorting process, and microfluidic sorting systems can select the sperm that show low oxidative stress post-exposure	Asghar, W. *et al.* Toxicology Study of Single-walled Carbon Nanotubes and Reduced Graphene Oxide in Human Sperm. Scientific reports 6, 30270–30270 (2016).
graphene oxide	toxicity	With the aid of a HiSeq 2000 sequencing technique, authors first examined the dysregulated mRNAs in GO-exposed nematodes and identified 970 up-regulated and 995 down-regulated mRNAs induced by GO exposure. Both gene ontology analysis and KEGG pathway analysis implied that the dysregulated mRNAs may mediate many important biological processes. Some dysregulated genes encoding the JNK signaling pathway were proven to be involved in the control of GO toxicity.	Zhao, Y. L., Wu, Q. L. & Wang, D. Y. A microRNAs-mRNAs network involved in the control of graphene oxide toxicity in Caenorhabditis elegans. RSC ADVANCES 5, 92394–92405 (2015).
graphene oxide	ecotoxicity	Authors investigated the possible safety property and translocation of graphene oxide (GO) in the range of µg/L in Arabidopsis. GO exposure did not obviously influence germination, seed development, shoot and root development of seedlings, and flowering time.	Zhao, S., Wang, Q., Zhao, Y., Rui, Q. & Wang, D. Toxicity and translocation of graphene oxide in Arabidopsis thaliana. Environmental toxicology and pharmacology 39, 145–156 (2015).
graphene oxide	toxicity	Authors evaluated the cytotoxicity of three GO samples with various oxidation degrees on mouse embryo fibroblasts (MEFs). The morphology, viability and apoptosis results indicate that the GO with lower degree of oxidation displayed stronger toxicity on MEFs. Meanwhile, three GO samples induced dramatic enhancement of ROS levels in cells, where the less oxidized GO stimulated higher intracellular ROS production.	Zhang, W. *et al.* Deciphering the underlying mechanisms of oxidation-state dependent cytotoxicity of graphene oxide on mammalian cells. Toxicology letters 237, 61–71 (2015).

reduced graphene oxide	toxicity	Herein, authors explored the short- and long-term effects of orally administered rGO on mouse behaviors, including general locomotor activity level, balance and neuromuscular coordination, exploratory and anxiety behaviors, and learning and memory abilities. These findings demonstrated that exposure to a high dosage of rGO nanosheets via oral administration caused a short-term decrease in locomotor activity and neuromuscular coordination but did not affect anxiety-like, exploratory, or spatial learning and memory behaviors.	Zhang, D. *et al.* The short- and long-term effects of orally administered high-dose reduced graphene oxide nanosheets on mouse behaviors. Biomaterials 68, 100–113 (2015).
reduced graphene oxide	toxicity	Here authors report the influence of rGO exposure on female mouse reproductive ability and offspring development. malformed fetuses were found among rGO-injected dam litters. All mice had abortions when injected with low (6.25 mg/kg) or intermediate (12.5 mg/kg) doses at a late gestational stage (~20 days); the majority of pregnant mice died when injected with the high dose of rGO at this stage of pregnancy. Interestingly, all surviving rGO-injected mouse mothers gave birth to another litter of healthy pups.	Xu, S., Zhang, Z. & Chu, M. Long-term toxicity of reduced graphene oxide nanosheets: Effects on female mouse reproductive ability and offspring development. Biomaterials 54, 188–200 (2015).
reduced graphene oxide	toxicity	The aim of authors' study was to evaluate the platelet activation and thrombogenic properties of an acellular tissue scaffold that was surface modified with reduced graphene oxide (rGO). Cytotoxicity indicates that the rGO can damage cells in direct contact but have no effect on the viability of fibroblasts in indirect contact.	Wilczek, P., Major, R., Lipinska, L., Lackner, J. & Mzyk, A. Thrombogenicity and biocompatibility studies of reduced graphene oxide modified acellular pulmonary valve tissue. Materials science & engineering. C, Materials for biological applications 53, 310–321 (2015).
graphene nanoplatelets	toxicity, method	Here, authors developed an *in vitro* methodology called the constrained drop surfactometer (CDS) to quantitatively study PS inhibition by airborne CNM. Authors show that airborne multiwalled carbon nanotubes and graphene nanoplatelets induce a concentration-dependent PS inhibition under physiologically relevant conditions.	Valle, R. P., Wu, T. & Zuo, Y. Y. Biophysical Influence of Airborne Carbon Nanomaterials on Natural Pulmonary Surfactant. ACS NANO 9, 5413–5421 (2015).
graphene oxide	combined ecotoxicity	The combined effects of graphene oxide (GO) and Cd^{2+} solution on Microcystis aeruginosa were investigated. GO at low concentrations exhibited no significant toxicity. The presence of GO at low concentrations significantly enhanced Cd^{2+} toxicity as the 96 h half maximal effective concentration of the Cd^{2+} reduced from 0.51 ± 0.01 to 0.474 ± 0.01 mg/L. However, concentrations of GO above 5 mg/L did not significantly increase the toxicity of the Cd^{2+}/GO system.	Tang, Y. *et al.* Combined effects of graphene oxide and Cd on the photosynthetic capacity and survival of Microcystis aeruginosa. The Science of the total environment 532, 154–161 (2015).
graphene oxide	toxicity	Authors evaluate the compatibility of GO with two different oxidation levels following implantation in subcutaneous and intraperitoneal tissue sites. A reduction in the degree of GO oxidation results in faster immune cell infiltration, uptake, and clearance following both subcutaneous and peritoneal implantation.	Sydlik, S. A., Jhunjhunwala, S., Webber, M. J., Anderson, D. G. & Langer, R. *in vivo* compatibility of graphene oxide with differing oxidation states. ACS nano 9, 3866–3874 (2015).

graphene oxide	toxicity	Authors performed an experiment investigating diamond, graphene oxide and graphite nanoparticles, which were repeatedly administrated intraperitoneally into Wistar rats for four weeks. none of the tested nanoparticles affected the health of animals.	Strojny, B. *et al.* Long Term Influence of Carbon Nanoparticles on Health and Liver Status in Rats. PloS one 10, e0144821–e0144821 (2015).
MoS2	toxicity	Herein, authors report the cytotoxicity of the MoS2 nanosheets based on the cytotoxic assay results and electrical impedance analysis using rat pheochromocytoma cells (PC12) and rat adrenal medulla endothelial cells (RAMEC). authors' results indicated that the MoS2 nanosheets synthesized in authors' work are safe	Shah, P., Narayanan, T. N., Li, C.-Z. & Alwarappan, S. Probing the biocompatibility of MoS2 nanosheets by cytotoxicity assay and electrical impedance spectroscopy. Nanotechnology 26, 315102–315102 (2015).
few layer graphene	toxicity	Authors present a comprehensive 3-month report on the acute and chronic toxicity of intravenously administered (20 mg kg−1) few-layer graphene (FLG) and, its carboxylated (FLG-COOH) and PEGylated (FLG-PEG) derivatives in Swiss albino mice. FLG and FLG-COOH accumulated within organs induced significant cellular and structural damages to lungs, liver, spleen, and kidney, ranging from mild congestion to necrosis, fibrosis and glomerular filtration dysfunction, without appreciable clearance.	Sasidharan, A. *et al.* Comparative *in vivo* toxicity, organ biodistribution and immune response of pristine, carboxylated and PEGylated few-layer graphene sheets in Swiss albino mice: A threemonth study. CARBON 95, 511–524 (2015).
graphene	ecotoxicity	This study evaluated the impact of graphene on the structure, abundance and function of the soil bacterial community based on quantitative real-time polymerase chain reaction (qPCR), pyrosequencing and soil enzyme activities. Graphene transiently promoted the enzyme activities and soil bacterial biomass. A transiently significant shift in soil bacterial community structure was caused. The effect of graphene on soil bacterial community was time dependent. Graphene significantly suppressed Nitrospira and Planctomyces. Graphene promoted some bacteria populations degrading organic pollutants.	Ren, W. J., Ren, G. D., Teng, Y., Li, Z. G. & Li, L. N. Time-dependent effect of graphene on the structure, abundance, and function of the soil bacterial community. JOURNAL OF HAZARDOUS MATERIALS 297, 286–294 (2015).
graphene oxide	toxicity	Authors found that GO flakes have a very strong haemolytic activity increasing with the GO flakes size reduction. This activity was almost absent when the plasma protein corona was absorbed on the GO flakes surfaces.	Papi, M. *et al.* Plasma protein corona reduces the haemolytic activity of graphene oxide nano and micro flakes. RSC ADVANCES 5, 81638–81641 (2015).
graphene oxide nanosheets	toxicity	In the present work, size is proposed as the main reason for the differences in the biological responses to GONS and GOQD. GOQD induced more obvious biological effects than GONS, including cellular uptake, cell division, cell permeability, and oxidative stress.	Ouyang, S., Hu, X. & Zhou, Q. Envelopment-Internalization Synergistic Effects and Metabolic Mechanisms of Graphene Oxide on Single-Cell Chlorella vulgaris Are Dependent on the Nanomaterial Particle Size. ACS applied materials & interfaces 7, 18104–18112 (2015).

graphene oxide, reduced graphene oxide	toxicity	The biological effects induced *in vitro* by Graphene-oxide (GO) thermally reduced graphene oxide (TRGO) and nitrogen-doped graphene (N–Gr) on human dental follicle stem cells, were evaluated. Graphene oxide showed the lowest cytotoxic effect, followed by the nitrogen-doped graphene. Thermally reduced graphene oxide exhibited high cytotoxic effects.	Olteanu, D. *et al.* Cytotoxicity assessment of graphene-based nanomaterials on human dental follicle stem cells. COLLOIDS AND SURFACES B-BIOINTERFACES 136, 791–798 (2015).
graphene oxide	ecotoxicity	In this study authors evaluated the effects of graphene oxide (GO) on green algae Raphidocelis subcapitata. The toxic effects from GO, as observed in algal density and autofluorescence, started at concentrations from 20 and 10 μg mL^{-1}, respectively.	1.Nogueira, P. F. M., Nakabayashi, D. & Zucolotto, V. The effects of graphene oxide on green algae Raphidocelis subcapitata. Aquatic toxicology (Amsterdam, Netherlands) 166, 29–35 (2015).
graphene oxide	toxicity	Authors examined egg fertilisation in purple sea urchin (Paracentrotus lividus) after sperm exposure to carbon-based nanomaterials, carbon black (CB) and graphene oxide (GO), from 0.0001 mg/L to 1.0 mg/L. CB exposure of P. lividus sperm reduces egg fertilisation already at 0.0001 mg/L. GO exposure of P. lividus sperm does not affect egg fertilisation at up to 1 mg/L. CB and GO exposure of sperm induce developmental anomalies in gastrulae and plutei. CB and GO exposure of sperm reduce cholinesterase activities in gastrulae.	Mesarič, T. *et al.* Sperm exposure to carbon-based nanomaterials causes abnormalities in early development of purple sea urchin (Paracentrotus lividus). AQUATIC TOXICOLOGY 163, 158–166 (2015).
graphene oxide	toxicity	Authors investigated the effects of three different carbon-based nanomaterials on brine shrimp (Artemia salina) larvae. The larvae were exposed to different concentrations of carbon black, graphene oxide, and multiwall carbon nanotubes for 48 h. Carbon-based nanomaterials adsorb onto the body surface of A. salina larvae. Surface adsorption results in concentration–dependent inhibition of larval swimming. Carbon-based nanomaterials induce no significant mortality of A. salina larvae.	Mesarič, T. *et al.* High surface adsorption properties of carbon-based nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses in Artemia salina larvae. Aquatic toxicology (Amsterdam, Netherlands) 163, 121–129 (2015).
graphene oxide	toxicity	Authors synthesized nanographene oxide (nGO) and engineered the surface with polyethylene glycol (PEG), bovine serum albumin (BSA), and poly(ether imide) (PEI). In contrast to pristine nGO, decoration with PEG and BSA hindered endocytosis and improved their benignancy toward macrophages.	Luo, N., Ni, D., Yue, H., Wei, W. & Ma, G. Surface-engineered graphene navigate divergent biological outcomes toward macrophages. ACS applied materials & interfaces 7, 5239–5247 (2015).

graphene	ecotoxicity	The effects of graphene on the germination and growth of rice seeds were studied. Seeds were treated with graphene solutions at different concentrations. Significant inhibitions on the stem length and fresh weight of over ground part were observed at concentration of 50 mg/L. In addition, all the indexes were inhibited at concentrations of 100 mg/L and 200 mg/L. It indicates that graphene certainly inhibit the morphogenesis of rice seedlings.	Liu, S. *et al.* Effects of Graphene on Germination and Seedling Morphology in Rice. Journal of nanoscience and nanotechnology 15, 2695–2701 (2015).
graphene oxide	toxicity	The exposure to a single dose of 2.1 mg kg^{-1} (single-high-dose exposure) small size GO or large size GO caused macrophage nodule formation in the lungs of the mice, and the exposure to seven repeated doses of 0.3 mg kg^{-1} (multiple-low-dose exposure) large size GO also induced small macrophage nodule formation, serious lymphocyte infiltration around the bronchioles in the lungs of the mice, and even death of the mice. Nephritic inflammatory reactions were also observed after the multiple-low-dose exposure to large size GO. However, no obvious lung toxicity but hepatic inflammatory infiltration was observed after the exposure to multiple-low-dose small size GO. GO accumulation in the macrophage nodules was verified by Raman mapping. The lower toxicity of the multiple-low-dose exposure to s-GO than that of the multiple-low-dose exposure to l-GO implicates that GO with a smaller size could be more benign to mice.	1.Liu, J. H. *et al.* Biocompatibility of graphene oxide intravenously administrated in mice-effects of dose, size and exposure protocols. TOXICOLOGY RESEARCH 4, 83–91 (2015).
graphene oxide nanosheets	toxicity	Toxicology testing of GO nanosheets against Paecilomyces catenlannulatus (P. catenlannulatus) was performed by measuring the efflux of cytoplasmic materials of P. catenlannulatus. The results showed that the damage of cell membrane of P. catenlannulatus was attributed to the direct contact of the P. catenlannulatus with the extremely sharp edges of GO nanosheets, which resulted in the P. catenlannulatus inactivation. The less resistant to the damage of cell membrane was observed with increasing of GO concentration and contact time.	Li, X. Y., Li, F. B., Gao, Z. M. & Fang, L. J. Toxicology of Graphene Oxide Nanosheets Against Paecilomyces catenlannulatus. BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 95, 25–30 (2015).
graphene, graphene oxide, reduced graphene oxide	toxicity, method	Here authors present a suite of high-throughput methods to study nanotoxicity in intact animals using Caenorhabditis elegans as a model. At the population level, authors' system measures food consumption of thousands of animals to evaluate population fitness. At the organism level, authors' automated system analyzes hundreds of individual animals for body length, locomotion speed, and lifespan.	1.Jung, S.-K. *et al.* Multi-endpoint, high-throughput study of nanomaterial toxicity in Caenorhabditis elegans. Environmental science & technology 49, 2477–2485 (2015).
graphene oxide	toxicity	Herein, the nanotoxicology of typical graphene oxide (GO) and carboxyl single-walled carbon nanotubes (C-SWCNT) was compared. The results showed that cell division of Chlorella vulgaris was promoted at 24 h and then inhibited at 96 h after nanomaterial exposure. At 96 h, GO and C-SWCNT inhibited the rates of cell division by 0.08–15% and 0.8–28.3%, respectively.	1.Hu, X. G., Ouyang, S. H., Mu, L., An, J. & Zhou, Q. Effects of Graphene Oxide and Oxidized Carbon Nanotubes on the Cellular Division, Microstructure, Uptake, Oxidative Stress, and Metabolic Profiles. ENVIRONMENTAL SCIENCE & TECHNOLOGY 49, 10825–10833 (2015).

graphene oxide	toxicity	Using CGMD simulation and experimental measurement, authors illustrate the interactions between the PS monolayer films and the GO nanosheets. Authors report the retention and adverse biophysical impact of GO on the PS film. The GO nanosheets induce pores on the monolayer film, and affect its biophysical properties. Remarkably, GO nanosheets increase the compressibility of PS films, which indicates the biophysical inhibition of PS.	Hu, Q. *et al.* Effects of graphene oxide nanosheets on the ultrastructure and biophysical properties of the pulmonary surfactant film. Nanoscale 7, 18025–18029 (2015).
graphene oxide	ecotoxicity	This study examined the toxicity of GO with protozoa Euglena gracilis as test organism. The 96 h EC50 value of graphene oxide in Euglena gracilis was 3.76 ± 0.74 mg L^{-1}. Graphene oxide exerted oxidative stress to the protozoa. Graphene oxide could inhibit the acquisition of nutrition and light.	Hu, C. *et al.* Ecotoxicological effects of graphene oxide on the protozoan Euglena gracilis. Chemosphere 128, 184–190 (2015).
graphene oxide	toxicity	GO sheets were incubated with plasma from human subjects with different diseases/conditions, including hypofibrinogenemia, blood cancer, thalassemia major, thalassemia minor, rheumatism, fauvism, hypercholesterolemia, diabetes, and pregnancy. Identical sheets coated with varying protein corona decorations exhibited significantly different cellular toxicity, apoptosis, and uptake, reactive oxygen species production, lipid peroxidation and nitrogen oxide levels.	Hajipour, M. J. *et al.* Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. Nanoscale 7, 8978–8994 (2015).
graphene oxide	toxicity	In this work, authors studied the potential developmental toxicity of GO when they entered the body of maternal mice and their offspring by oral exposure with two doses. It can be concluded that GO showed many negative effects on the development of mice in the lactation period.	1.Fu, C. *et al.* Effects of graphene oxide on the development of offspring mice in lactation period. Biomaterials 40, 23–31 (2015).
boron nitride	toxicity	The Cosmetic Ingredient Review Expert Panel (Panel) assessed the safety of boron nitride which functions in cosmetics as a slip modifier. The Panel reviewed available chemistry, animal data, and clinical data and concluded that this ingredient is safe in the present practices of use and concentration in cosmetic formulations.	Fiume, M. M. *et al.* Safety Assessment of Boron Nitride as Used in Cosmetics. International journal of toxicology 34, 53S-60S (2015).
graphene oxide	toxicity	Through all of these experimental assays, such as cytotoxicity, genotoxicity, mutagenicity, ecotoxicity and *in vivo* toxicity assays, the GO appeared as a potential new drug (~50- 100 µg/mL) for several diseases' *in vivo* treatment with very low limitations.	Duran, N. *et al.* Interlab study on nanotoxicology of representative graphene oxide. in 4TH INTERNATIONAL CONFERENCE ON SAFE PRODUCTION AND USE OF NANOMATERIALS (NANOSAFE2014) vol. 617 (2015).
graphene oxide	ecotoxicity	Authors investigated the effects of GO on soil microbial activity in a 59-day soil incubation study. authors' results indicate that soil enzyme activity can be lowered by the entry of GO into soils in short term but it can be recovered afterwards.	Chung, H. *et al.* Effects of graphene oxides on soil enzyme activity and microbial biomass. SCIENCE OF THE TOTAL ENVIRONMENT 514, 307–313 (2015).

graphene oxide	ecotoxicity	During zebrafish embryogenesis, GO induced a significant hatching delay and cardiac edema. The intensive interactions of GO with the chorion induces damage to chorion protuberances, excessive generation of $HO^{\bullet}$, and changes in protein secondary structure. Humic acid (HA) mitigated the mitochondrial damage and oxidative stress induced by GO. This work reveals a feasible antidotal mechanism for GO in the presence of NOM and avoids overestimating the risks of GO in the natural environment.	Chen, Y., Ren, C., Ouyang, S., Hu, X. & Zhou, Q. Mitigation in Multiple Effects of Graphene Oxide Toxicity in Zebrafish Embryogenesis Driven by Humic Acid. Environmental science & technology 49, 10147–10154 (2015).
graphene nanoplatelets, graphene oxide	toxicity	The GFNs used in this study are graphene nanoplatelets ([GNPs]–pristine, carboxylate [COOH] and amide [NH_2]) and graphene oxides (single layer [SLGO] and few layers [FLGO]). The human bronchial epithelial cells (Beas2B cells) as *in vitro* system and the nematode Caenorhabditis elegans as *in vivo* system were used to profile the toxicity response of GFNs. GNPs exhibited higher toxicity than GOs in Beas2B cells, and among the GNPs the order of toxicity was pristine>NH_2>COOH. Although the order of toxicity of the GNPs was maintained in C. elegans reproductive toxicity, but GOs were found to be more toxic in the worms than GNPs. In both systems, SLGO exhibited profoundly greater dose dependency than FLGO.	Chatterjee, N. *et al.* Screening of toxic potential of graphene family nanomaterials using *in vitro* and alternative *in vivo* toxicity testing systems. Environmental health and toxicology 30, e2015007–e2015007 (2015).
graphene oxide	toxicity, method	In an effort to understand graphene-induced bacterial inactivation, authors studied the interaction of GO with bacterial (Escherichia coli) cell membranes using atomic force microscopy (AFM). authors' force spectroscopy results suggest that physicochemical interactions do not underlie the primary mode of action of GO in bacteria.	Castrillon, S. R. V., Perreault, F., de Faria, A. F. & Elimelech, M. Interaction of Graphene Oxide with Bacterial Cell Membranes: Insights from Force Spectroscopy. ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS 2, 112–117 (2015).
graphene oxide	toxicity	In this study, authors report the impact of GO, with and without the addition of bovine serum albumin, on healthy (Chinese hamster ovary) and a cancer (mouse hepatoma MH-22A) cells viability and the estimation of the intracellular distribution of GO inside the cells *in vitro*. The GO influence on cell morphology changes, cell structure, cells colony growth dynamics and GO accumulation inside the cells was higher in the case of mouse hepatoma MH-22A cells.	Batiuskaite, D., Grinceviciute, N. & Snitka, V. Impact of graphene oxide on viability of Chinese hamster ovary and mouse hepatoma MH-22A cells. Toxicology *in vitro* : an international journal published in association with BIBRA 29, 1195–1200 (2015).

graphene oxide	toxicity	Biodistribution study of the NGO sheets (intravenously injected into male mice at dose of ~2000 μg/mL or 4 mg/kg of body weight) showed a high graphene uptake in testis. the NGO sheets affected the important parameters of the spermatozoa including their viability, morphology, kinetics and chromosomes. In addition, the NGO sheets accumulated in the testis could increase the ROS level of semen of the mice. Then, the semen of the NGO-treated mice (containing the damaged spermatozoa and a medium with high ROS level) disturbed the pregnant functionality of female mice (including fertility, gestation ability, hormone secretion, and multi-production capability) and finally postnatal viability of the delivered pups.	Akhavan, O., Ghaderi, E., Hashemi, E. & Akbari, E. Dose-dependent effects of nanoscale graphene oxide on reproduction capability of mammals. Carbon 95, 309–317 (2015).
graphene	toxicity	In this review, authors present a summary of some very recent studies on this important subject with both experimental and theoretical approaches. The molecular interactions of graphene with proteins, DNAs, and cell membranes (both bacteria and mammalian cells) are discussed in detail. Graphene can have significant disruptions to protein and DNA structures due to the strong π–π stacking interactions, and also damage the integrity of cell membranes (both bacteria and human cells). More interestingly, two types of molecular mechanisms for the graphene-induced degradation of cell membranes have been identified, one by severe insertion and cutting, and the other by destructive extraction of lipid molecules.	Zhou, R. & Gao, H. Cytotoxicity of graphene: recent advances and future perspective. Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology 6, 452–474 (2014).
graphene nanoplatelets	toxicity	The biologic/cytotoxic effects of dispersed nanographene platelets (NGPs) on human osteosarcoma cells (MG63 cell line) were studied. By studying intrinsic reasons of cell viability inhibition (cell cycle and cell apoptosis), the morphology influence of cell microstructure, the cytotoxicity mechanism and the cell functional activity, authors found that NGPs exhibited cytotoxic behavior on MG63 cell to some degree and its cytotoxicity displayed a positive dose-dependent effect with 10 μg mL^{-1} as a criticalconcentrations.	Zhang, X. *et al.* Cell response of nanographene platelets to human osteoblast-like MG63 cells. Journal of biomedical materials research. Part A 102, 732–742 (2014).
graphene nanoflakes	toxicity, method	Graphene nanoflake toxicity was analyzed using cell-based electrochemical impedance biosensing with interdigitated indium tin oxide (ITO) electrodes installed in a custom-built mini- incubator positioned on an inverted optical microscope. The increased toxicity of smaller Graphene nanoflakes (30 nm) as measured by electrochemical impedance sensing and optical monitoring of treated cells was consistent with the biological assay results.	Yoon, O. J., Kim, I., Sohn, I. Y., Kieu, T. T. & Lee, N.-E. Toxicity of graphene nanoflakes evaluated by cell-based electrochemical impedance biosensing. Journal of biomedical materials research. Part A 102, 2288–2294 (2014).
graphene oxide	toxicity	Authors used Caenorhabditis elegans to investigate the microRNAs (miRNAs) control of GO toxicity. With the aid of SOLiD sequencing, authors identified 23 up-regulated and 8 down-regulated miRNAs in GO-exposed nematodes. authors raise a hypothesis here that GO may reduce the lifespan of animals through influencing the functions of insulin/IGF signaling, TOR signaling, and germline signaling pathways controlled by miRNAs.	Wu, Q., Zhao, Y., Zhao, G. & Wang, D. microRNAs control of *in vivo* toxicity from graphene oxide in Caenorhabditis elegans. Nanomedicine : nanotechnology, biology, and medicine 10, 1401–1410 (2014).

graphene oxide	toxicity	Authors employed an *in vivo* Caenorhabditis elegans assay system to identify molecular signals involved in the control of the translocation and toxicity of GO. Authors identified 7 genes whose mutations altered both the translocation and toxicity of GO. authors hypothesize that both intestinal permeability and defecation behavior may have crucial roles in controlling the functions of the identified molecular signals. The molecular signals may further contribute to the control of transgenerational toxic effects of GO. authors' results provide an important insight into understanding the molecular basis for the *in vivo* translocation and toxicity of GO.	Wu, Q., Zhao, Y., Li, Y. & Wang, D. Molecular signals regulating translocation and toxicity of graphene oxide in the nematode Caenorhabditis elegans. Nanoscale 6, 11204–11212 (2014).
graphene oxide	toxicity	Here, authors show that chronic GO exposure not only caused damage on the function of both primary and secondary targeted organs but also induced severe accumulation of pathogenic microbial food (OP50) in the intestine of Caenorhabditis elegans,	Wu, Q., Zhao, Y., Fang, J. & Wang, D. Immune response is required for the control of *in vivo* translocation and chronic toxicity of graphene oxide. Nanoscale 6, 5894–5906 (2014).
graphene oxide	toxicity	This study investigated the possible toxicity of graphene oxide and its mechanisms on multiple myeloma cells (RPMI 8226 cells). In summary, graphene oxide is dose-dependently cytotoxic to cultured RPMI 8226 cells, and its toxicity is closely associated with increased oxidative stress.	Wang, Y. *et al. In vitro* toxicity evaluation of graphene oxide on human RPMI 8226 cells. Bio-medical materials and engineering 24, 2007–2013 (2014).
graphene oxide	ecotoxicity	In the present study, authors investigated the toxicity and translocation of graphene oxide (GO) in the μg L^{-1} range in Arabidopsis plants under both normal and stress conditions. Exposure to GO for 4 weeks did not cause adverse effects on the development of Arabidopsis seedlings. In contrast, the combined exposure to GO and PEG 6000 (20%) or NaCl (200 mM) resulted in a more severe loss of morphology, decrease in fresh weight or root length, and increase in root-to-shoot ratio in Arabidopsis seedlings compared with exposure to stress alone. Authors hypothesize that, under stress conditions, GO may induce oxidative stress and membrane ion leakage, which may in turn induce GO translocation from the roots to the leaves.	Wang, Q. Q., Zhao, S. Q., Zhao, Y. L., Rui, Q. & Wang, D. Y. Toxicity and translocation of graphene oxide in Arabidopsis plants under stress conditions. RSC ADVANCES 4, 60891–60901 (2014).
MoS2, WS2, WSe2, graphene oxide	toxicity	Authors investigated the cytotoxicity of three common exfoliated TMDs (exTMDs), namely MoS2, WS2, and WSe2, and compared their toxicological effects with graphene oxides and halogenated graphenes. it was concluded that MoS2 and WS2 nanosheets induced very low cytotoxicity to A549 cells, even at high concentrations. On the other hand, WSe2 exhibited dose-dependent toxicological effects on A549 cells, reducing cell viability to 31.8 % at the maximum concentration of 400 μg mL^{-1}. In comparison with graphene oxides and halogenated graphenes, MoS2 and WS2 were much less hazardous, whereas WSe2 showed similar degree of cytotoxicity.	Teo, W. Z., Chng, E. L. K., Sofer, Z. & Pumera, M. Cytotoxicity of exfoliated transition-metal dichalcogenides (MoS2, WS2 , and WSe2) is lower than that of graphene and its analogues. Chemistry (Weinheim an der Bergstrasse, Germany) 20, 9627–9632 (2014).

graphene oxide	toxicity	This work suggests that graphene and other nano carbon particles produced by the pyrolysis of bio-products in air are non-toxic to humans.	Saxena, M. & Sarkar, S. Involuntary graphene intake with food and medicine. RSC ADVANCES 4, 30162–30167 (2014).
pristine graphene	toxicity	In the present work, effects of pristine graphene (pG) on the development of a living organism, with an emphasis on morphological and molecular states of the brain, were investigated using a chicken embryo model. Administration of pG to chicken embryos reduced survival rate but did not affect body weight, organ weights, or blood serum biochemical indices. pG affected the ultrastructure of the brain and likely affected DNA synthesis in the brain, indicating its toxicity, which ought to be considered prior to any medical applications.	Sawosz, E. *et al.* Toxicity of pristine graphene in experiments in a chicken embryo model. International journal of nanomedicine 9, 3913–3922 (2014).
pristine graphene	toxicity	The ecotoxicity of pristine graphene was investigated in model marine organisms. Acute toxicity effects were detected in V. fischeri and D. tertiolecta. Acute toxicity was directed in (particles)size-dependent manner. No acute toxicity was detected in A. salina. An altered pattern of oxidative stress biomarkers was detected in exposed A. salina	Pretti, C. *et al.* Ecotoxicity of pristine graphene to marine organisms. Ecotoxicology and environmental safety 101, 138–145 (2014).
graphene oxide, reduced graphene oxide	toxicity	This study was aimed to investigate the toxic effects of 3 nanomaterials, *i.e.* multi-walled carbon nanotubes (MWCNTs), graphene oxide (GO), and reduced graphene oxide (RGO), on zebrafish embryos. MWCNTs, GO, and RGO were all toxic to zebrafish embryos to influence embryos hatching and larvae length.	Liu, X. T. *et al.* Toxicity of multi-walled carbon nanotubes, graphene oxide, and reduced graphene oxide to zebrafish embryos. Biomedical and environmental sciences : BES 27, 676–683 (2014).
graphene oxide	toxicity	In the present study, authors employed SOLiD sequencing technique to investigate the molecular control of *in vitro* GO toxicity in GLC-82 pulmonary adenocarcinoma cells by microRNAs (miRNAs). In GLC-82 cells, GO exposure at concentrations more than 50 mg/L resulted in severe reduction in cell viability, induction of lactate dehydrogenase leakage, reactive oxygen species production and apoptosis, and dysregulation of cell cycle.	Li, Y. *et al.* Response of microRNAs to *in vitro* treatment with graphene oxide. ACS nano 8, 2100–2110 (2014).
graphene oxide	ecotoxicity	Authors examined the toxicity of four carbon-based nanomaterials (unmodified) by using carbon quantum dots (CQDs), graphene quantum dots (GQDs), graphene oxide (GO), and single-walled carbon nanotubes (SWCNTs) to cultivate bean sprout. Results showed that the toxicity of these four carbon nanomaterials increases with the increasing of concentration and cultivating time	Li, X. *et al.* The effect of pristine carbon-based nanomaterial on the growth of green gram sprouts and pH of water. Nanoscale research letters 9, 583–583 (2014).

graphene oxide	toxicity	In this study, authors assessed the *in vivo* behavior and toxicology of nanoscale graphene oxide (NGO) in mice after intravenous injection. The influence of a polyethylene glycol (PEG) coating on the distribution and toxicity of the NGO was also investigated. The results show that NGO is mainly retained in the liver, lung, and spleen. Retention in the lung is partially due to NGO aggregation. The PEG coating reduces the retention of NGO in the liver, lung, and spleen and promotes the clearance of NGO from these organs, but NGO and NGO-PEG are still present after 3 months. The PEG coating effectively reduces the early weight loss caused by NGO and alleviates NGO-induced acute tissue injuries, which can include damage to the liver, lung, and kidney, and chronic hepatic and lung fibrosis.	Li, B. *et al.* Influence of polyethylene glycol coating on biodistribution and toxicity of nanoscale graphene oxide in mice after intravenous injection. International journal of nanomedicine 9, 4697–4707 (2014).
graphene nanoplatelets, graphene oxide	toxicity	This study aimed to assess the toxicity of graphene oxide (GO) and carboxyl graphene (CXYG) nanoplatelets to non-mammalian species using the fish cell line PLHC-1 as *in vitro* model. GO and CXYG nanoplatelets caused physical injury of the plasma membrane. GO and CXYG accumulated in the cytosol and interacted with cellular organelles. PLHC-1 cells exposed to GO/CXYG demonstrated high ROS levels but low cytotoxicity. ROS formation was related with GO/CXYG-induced structural damage of mitochondria.	Lammel, T. & Navas, J. M. Graphene nanoplatelets spontaneously translocate into the cytosol and physically interact with cellular organelles in the fish cell line PLHC-1. Aquatic toxicology (Amsterdam, Netherlands) 150, 55–65 (2014).
graphene oxide	toxicity	The distribution and cytotoxicity of graphene oxide (GO) and TiO2-graphene oxide composite (TiO2-GO composite) were evaluated in A549 cells. Cell viability and cell ultrastructure were measured. Authors' results indicated that GO could enter A549 cells and located in the cytoplasm and nucleus without causing any cell damage.	Jin, C. *et al.* Distribution of Graphene Oxide and TiO2-Graphene Oxide Composite in A549 Cells. BIOLOGICAL TRACE ELEMENT RESEARCH 159, 393–398 (2014).
graphene	toxicity, method	Authors herein report a metabolomics approach to investigate the metabolic responses on graphene treated HepG2. authors' findings demonstrated that metabolomics would be an efficient platform to understand the molecular mechanism of cytotoxicity of graphene.	Jiao, G. Z. *et al.* Metabolomics study on the cytotoxicity of graphene. RSC ADVANCES 4, 44712–44717 (2014).
graphene nanosheets	toxicity	Two engineered E. coli bacteria strains of DPD2794 and TV1061 were incubated with aqueous dispersion of three carbon allotropes (multi-walled carbon nanotubes (MWCNTs), graphene nanosheets and carbon black nanopowders) with different concentrations. The carbon black nanopowder showed the highest cytotoxicity towards the bacterial bioreporter cells out of three tested carbon-based nanomaterials	Jia, K., Marks, R. S. & Ionescu, R. E. Influence of carbon-based nanomaterials on lux-bioreporter Escherichia coli. Talanta 126, 208–213 (2014).

graphene oxide	combined ecotoxicity	This work revealed that GO greatly amplifies the phytotoxicity of arsenic (As), a widespread contaminant, in wheat, for example, causing a decrease in biomass and root numbers and increasing oxidative stress, which are thought to be regulated by its metabolisms. Compared with As or GO alone, GO combined with As inhibited the metabolism of carbohydrates, enhanced amino acid and secondary metabolism and disrupted fatty acid metabolism and the urea cycle	Hu, X. *et al.* Graphene oxide amplifies the phytotoxicity of arsenic in wheat. Scientific reports 4, 6122–6122 (2014).
graphene oxide	toxicity	In this work, authors assess the toxicity of pristine GO (p-GO) and functionalized GO (GO-COOH and GO-PEI) to primary human peripheral blood T lymphocytes and human serum albumin (HSA). authors' results indicate that p-GO and GO-COOH have good biocompatibility to T lymphocytes at the concentration below 25 μg mL^{-1}, but notable cytotoxicity above 50 μg mL^{-1}.	Ding, Z., Zhang, Z., Ma, H. & Chen, Y. *In vitro* hemocompatibility and toxic mechanism of graphene oxide on human peripheral blood T lymphocytes and serum albumin. ACS applied materials & interfaces 6, 19797–19807 (2014).
graphene oxide	toxicity	This study was carried out by varying both graphene oxide (GO) concentration (10 μg/mL, 50 μg/mL, 100 μg/mL) and flakes sizes of 1320 nm and 130 nm. A 24-h cytotoxicity test showed, for A549, a loss in the viability, while the test exhibits overall a positive increase in the viability for CaCo2 and Vero. A 24-h comet assay shows a marked GO genotoxicity: for micrometer-sized GO flakes the genotoxicity is in positive correlation with the concentration, while for nanometer-sized GO flakes there was a high degree of genotoxicity at the lowest concentration tested.	De Marzi, L. *et al.* Flake size-dependent cyto and genotoxic evaluation of graphene oxide on *in vitro* A549, CaCo2 and vero cell lines. Journal of biological regulators and homeostatic agents 28, 281–289 (2014).
graphene, graphene oxide	toxicity	In this study, the cytoxicity effects of highly hydrogenated graphene (HHG) and its graphene oxide (GO) counterpart on the basis of *in vitro* toxicological assessments are reported and the effects correlated with the physiochemical properties of the tested nanomaterials. Upon 24 h exposure to the nanomaterials, a dose-dependent cellular cytotoxic effect was exhibited and the HHG was observed to be more cytotoxic than its GO control.	Chng, E. L. K., Sofer, Z. & Pumera, M. Cytotoxicity profile of highly hydrogenated graphene. Chemistry (Weinheim an der Bergstrasse, Germany) 20, 6366–6373 (2014).
graphene oxide nanoribbons, graphene oxide nanoplatelets	toxicity	In this study, authors examined the cytotoxicity of graphene-oxide nanoribbons (GONRs; ~310 × 5000 nm) and graphene-oxide nanoplatelets (GONPs; 100 × 100 nm)-n vitro assessments revealed that the GONRs exhibited a much stronger cytotoxicity over the GONPs, and authors correlated that observation with characterization data that showed GONRs to have a greater amount of carbonyl groups as well as greater length.	Chng, E. L. K., Chua, C. K. & Pumera, M. Graphene oxide nanoribbons exhibit significantly greater toxicity than graphene oxide nanoplatelets. Nanoscale 6, 10792–10797 (2014).

graphene oxide, reduced graphene oxide	toxicity	Authors performed a comprehensive study about biological interaction of grapheme nanomaterials by using OMICS in graphene oxide (GO) and reduced graphene oxide (rGO) treated HepG2 cells. Similar toxic responses (cytotoxicity, DNA damage, oxidative stress) with differential dose dependency were observed for both GO and rGO but they exhibited distinct mechanism. n brief, the distinct biological and molecular mechanisms of GO/rGO were attributed to their differential surface oxidation status.	Chatterjee, N., Eom, H.-J. & Choi, J. A systems toxicology approach to the surface functionality control of graphene-cell interactions. Biomaterials 35, 1109–1127 (2014).
graphene oxide	ecotoxicity	This study investigates the impact of different single-bilayer graphene oxide sheet (hereafter 'graphene oxide', GO; size: 0.5–5 μm) concentrations (0, 100, 200, 400, 800 and 1600 mg L^{-1}) and underlying potential mechanisms in germinating faba bean (Vicia faba L.) seedlings. Graphene oxide (GO) impacted Vicia faba both positively and negatively. GO (1600 > 200 > 100 mg GO L− 1) elevated oxidative stress but lowered its metabolism. GO (800 > 400 mg GO L− 1) enhanced H2O2 scavenging and improved V. faba health status. V. faba-root-polypeptide patterns substantiated GO-positive and -negative impacts. Results imply 800 > 400 mg GO L^{-1}-safe nature.	Anjum, N. A. *et al.* Single-bilayer graphene oxide sheet impacts and underlying potential mechanism assessment in germinating faba bean (Vicia faba L.). The Science of the total environment 472, 834–841 (2014).
graphene oxide	toxicity	Herein authors reported the effects of GOs with and without polyvinylpyrrolidone (PVP) coating on human immune cells such as dendritic cells (DCs), T lymphocytes and macrophages. Authors conclude that modification of PVP can obviously improve the immunological biocompatibility of GO *in vitro*, and the PVP-GO sheets can enhance the immunologic function of lymphocytes to some extent.	Zhi, X. *et al.* The immunotoxicity of graphene oxides and the effect of PVP-coating. Biomaterials 34, 5254–5261 (2013).
graphene oxide nanosheets	toxicity	In this study, authors synthesized uniform ultrasmall graphene oxide nanosheets with high yield by a convenient way of modified Hummers' method. the as-prepared ultrasmall GO nanosheets exhibited lower cytotoxicity and higher cellular uptake amount compared to the random large sized GO nanosheets.	Zhang, H. *et al.* Uniform ultrasmall graphene oxide nanosheets with low cytotoxicity and high cellular uptake. ACS applied materials & interfaces 5, 1761–1767 (2013).
graphene nanoflakes	toxicity, method	The mini-cell culture system integrated with the nanocomposite electrochemical transducer was used to carry out a toxicity analysis of G nanoflakes under size-, concentration- and time-dependent influences. The results also showed that the increased tendency in measured H2O2 concentration as the concentration of the G nanoflakes increased was consistent with the results of the toxicity analysis data obtained by optical bioassays. Also, authors confirmed increased cytotoxicity for smaller G size at the same concentration.	Yoon, O. J., Kim, C. H., Sohn, I. Y. & Lee, N. E. Toxicity analysis of graphene nanoflakes by cell-based electrochemical sensing using an electrode modified with nanocomposite of graphene and Nafion. SENSORS AND ACTUATORS B-CHEMICAL 188, 454–461 (2013).

graphene oxide	toxicity	In the present study, authors examined the potential adverse effects of GO and the underlying mechanism using nematode Caenorhabditis elegans as the assay system. Authors compared the *in vivo* effects of GO between acute exposure and prolonged exposure and found that prolonged exposure to 0.5–100 mg L^{-1} of GO caused damage on functions of both primary (intestine) and secondary (neuron and reproductive organ) targeted organs. authors' data suggest that prolonged exposure to GO may cause potential risk to environmental organisms after release into the environment. GO toxicity may be due to the combinational effects of oxidative stress in the intestinal barrier, enhanced permeability of the biological barrier, and suppressed defecation behavior in C. elegans.	Wu, Q. *et al.* Contributions of altered permeability of intestinal barrier and defecation behavior to toxicity formation from graphene oxide in nematode Caenorhabditis elegans. Nanoscale 5, 9934–9943 (2013).
graphene nanosheets	toxicity	In this study, authors compare the immune response resulting from GNS and MWCNT exposure. In conclusion, the use of GNS or MWCNT as nanocarriers for drug delivery may result in Th2 immune responses that are mediated through the IL-33/ST2 axis and therefore may promote adverse allergic reactions.	Wang, X., Podila, R., Shannahan, J. H., Rao, A. M. & Brown, J. M. Intravenously delivered graphene nanosheets and multiwalled carbon nanotubes induce site-specific Th2 inflammatory responses via the IL-33/ST2 axis. International journal of nanomedicine 8, 1733–1748 (2013).
graphene oxide	toxicity	In this paper, the cytotoxicity and genotoxicity of GO to human lung fibroblast (HLF) cells have been assessed. The results indicated that cytotoxicity and genotoxicity of GO to HLF cells were concentration dependent, and the genotoxicity induced by GO was more severe than the cytotoxicity to HLF cells.	Wang, A. *et al.* Role of surface charge and oxidative stress in cytotoxicity and genotoxicity of graphene oxide towards human lung fibroblast cells. Journal of applied toxicology : JAT 33, 1156–1164 (2013).
graphene oxide	toxicity	In this study, authors first evaluated the toxicity of acid-functionalized single-walled carbon nanotubes and graphene oxides, and found that both carbon nanomaterials induced adverse effects in murine peritoneal macrophages, and GOs were more potent than AF-SWCNTs.	Wan, B. *et al.* Single-walled carbon nanotubes and graphene oxides induce autophagosome accumulation and lysosome impairment in primarily cultured murine peritoneal macrophages. Toxicology letters 221, 118–127 (2013).
graphene oxide	toxicity	The combined data reveal that interaction of GO with TLR4 is the predominant molecular mechanism underlying GO-induced macrophagic necrosis; also, cytoskeletal damage and oxidative stress contribute to decreased viability and function of macrophages upon GO treatment.	Qu, G. *et al.* Graphene oxide induces toll-like receptor 4 (TLR4)-dependent necrosis in macrophages. ACS nano 7, 5732–5745 (2013).

graphene oxide	toxicity	In the current study, the biological influence of graphene oxide (GO) on macrophages was closely investigated. The study demonstrated that GO could provoke apoptosis of erythroid cells through oxidative stress in E14.5 fetal liver erythroid cells and *in vivo* administration of GO-diminished erythroid population in spleen, associated with disordered erythropoiesis in mice.	Qu, G. B., Wang, X. Y., Wang, Z., Liu, S. J. & Jiang, G. B. Cytotoxicity of quantum dots and graphene oxide to erythroid cells and macrophages. NANOSCALE RESEARCH LETTERS 8, (2013).
graphene oxide	toxicity	The effects of two carbon-based nanomaterials, nano-sized carbon black (nCB), and single-layer graphene oxide (GO) on settlement of Amphibalanus amphitrite (Cirripedia, Crustacea) cypris larvae (cyprids) were assessed after 24, 48, and 72 h of exposure. Additionally, the effects of these nanomaterials on the mortality and swimming behaviour of the nauplius larvae (nauplii) of the same organism were determined after 24 and 48 h. Single-layer GO, on the contrary, showed lower antisettlement effects and was more active in altering the survival and inhibiting the swimming behaviour of the nauplii.	Mesaric, T. *et al.* Effects of nano carbon black and single-layer graphene oxide on settlement, survival and swimming behaviour of Amphibalanus amphitrite larvae. CHEMISTRY AND ECOLOGY 29, 643–652 (2013).
graphene	toxicity	Male Wistar rats were exposed head-nose to atmospheres of t0.1, 0.5, or 2.5 mg/m^3 for multi-walled carbon nanotubes and 0.5, 2.5, or 10 mg/m^3 for graphene, graphite nanoplatelets and low-surface carbon black for 6 hours per day on 5 consecutive days. In order to compare the inhalation toxicity. No adverse effects were observed after inhalation exposure to 10 mg/m^3 graphite nanoplatelets or relatively low specific surface area carbon black. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m^3 for multi-walled carbon nanotubes and 10 mg/m^3 for graphene.	Ma-Hock, L. *et al.* Comparative inhalation toxicity of multi-walled carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black. Particle and fibre toxicology 10, 23–23 (2013).
graphene oxide	toxicity	GO interfered with DNA replication and induced mutagenesis at molecular level. GO treatments at concentrations of 10 and 100 μg/mL altered gene expression patterns at cellular level and 101 differentially expressed genes mediated DNA-damage control, cell apoptosis, cell cycle and metabolism. Intravenous injection of GO at 4 mg/kg for 5 consecutive days clearly induced formation of micronucleated polychromic erythrocytes in mice and its mutagenesis potential appeared to be comparable to cyclophosphamide, a classic mutagen. In conclusion, GO can induce mutagenesis both *in vitro* and *in vivo*, thus extra consideration is required for its biomedical applications.	Liu, Y. *et al.* Graphene oxide can induce *in vitro* and *in vivo* mutagenesis. Scientific reports 3, 3469–3469 (2013).

graphene, graphene oxide	toxicity	Graphene oxide (GO) and carboxyl graphene (CXYG) nanoplatelet suspensions were obtained in water and culture medium. Cytotoxicity of GO and CXYG nanoplatelets was assessed in Hep G2 cells. GO and CXYG nanoplatelets caused dose- and time-dependent cytotoxicity in Hep G2 cells with plasma membrane damage and induction of oxidative stress. Moreover, they exerted no toxicity when applied at very low concentrations (< 4 μg/ml).	Lammel, T., Boisseaux, P., Fernández-Cruz, M.-L. & Navas, J. M. Internalization and cytotoxicity of graphene oxide and carboxyl graphene nanoplatelets in the human hepatocellular carcinoma cell line Hep G2. Particle and fibre toxicology 10, 27–27 (2013).
graphene oxide, reduced graphene oxide	toxicity	Authors have investigated the *in vitro* short-term cellular toxicity associated with graphene derivatives (GD): graphene oxide and reduced graphene oxide. Collectively, authors' results indicate that GO exhibited a mild acute cytotoxic action on both epithelial and macrophage cells, as shown by the quantitative viability tests. An important parameter determining the biological effects of GO is its two-dimensional shape. ROS generation upon GO exposure in the epithelial and macrophage cells may contribute to the short-term cytotoxicity.	Horvath, L. *et al.* Evaluation of the toxicity of graphene derivatives on cells of the lung luminal surface. CARBON 64, 45–60 (2013).
graphene oxide	toxicity	A green, simple and non-toxic method for preparing graphene using biomass of Pseudomonas aeruginosa as the reducing reagent is proposed. the biocompatibility of the M-rGO was investigated using primary mouse embryonic fibroblast (PMEF) cells. The present study suggests that the M-rGO has significant biocompatibility for PMEF cells, even at a high concentration of 100 μg ml^{-1}.	Gurunathan, S., Han, J. W., Eppakayala, V. & Kim, J.-H. Biocompatibility of microbially reduced graphene oxide in primary mouse embryonic fibroblast cells. Colloids and surfaces. B, Biointerfaces 105, 58–66 (2013).
graphene	ecotoxicity	14C-labeled graphene was spiked to artificial freshwater and the uptake and depuration of graphene by Daphnia magna were assessed. After exposure for 24 h to a 250 μg/L solution of graphene, the graphene concentration in the organism was nearly 1% of the organism dry mass. Addition of algae and humic acid to water during the depuration period resulted in release of a significant fraction (>90%) of the accumulated graphene, but some still remained in the organism. Accumulated graphene in adult Daphnia was likely transferred to the neonates.	Guo, X. K. *et al.* Biological Uptake and Depuration of Radio-labeled Graphene by Daphnia magna. ENVIRONMENTAL SCIENCE & TECHNOLOGY 47, 12524–12531 (2013).
graphene oxide	toxicity	Here, an *in vitro* toxicological assessment of graphene oxide (GO) and reduced graphene oxide (RGO) and in correlation with their physiochemical properties is reported. It is concluded that although size of the GO sheet plays a role, the functional group density on the GO sheet is one of the key components in mediating cellular cytotoxicity. By controlling the GO reduction and maintaining the solubility, it is possible to minimize the toxicity of GO .	Das, S. *et al.* Oxygenated Functional Group Density on Graphene Oxide: Its Effect on Cell Toxicity. PARTICLE & PARTICLE SYSTEMS CHARACTERIZATION 30, 148–157 (2013).

graphene oxide	toxicity	The cytoxicity effects of the four GOs prepared by different oxidative methods, the influence of the differing oxidative treatment on the toxicological behavior of GOs have been investigated in adherent lung epithelial cells. From the viability data, it is evident that there is a strong dose-dependent cytotoxic response resulting from the four GO nanomaterials tested after a 24 h exposure, and it is suggested that there is a correlation between the amounts of oxygen content/functional groups of GOs with their toxicological behavior towards the A549 cells.	Chng, E. L. K. & Pumera, M. The toxicity of graphene oxides: dependence on the oxidative methods used. Chemistry (Weinheim an der Bergstrasse, Germany) 19, 8227–8235 (2013).
graphene	toxicity	This study was set up to explore potential influence of graphene on T87 cells. Fragmented nuclei, membrane damage, and mitochondrial dysfunction were observed. ROS increased, ROS are key mediators in the cell death signaling pathway. Translocation of graphene into cells and an endocytosis-like structure was observed.	Begum, P. & Fugetsu, B. Induction of cell death by graphene in Arabidopsis thaliana (Columbia ecotype) T87 cell suspensions. Journal of hazardous materials 260, 1032–1041 (2013).
graphene nanoribbons, reduced graphene oxide	toxicity	Human mesenchymal stem cells (hMSCs) were isolated from umbilical cord blood and used for checking the concentration- and time-dependent cyto- and geno-toxic effects of the rGONRs and reduced graphene oxide sheets (rGOSs). The cell viability assay indicated significant cytotoxic effects of 10 μg/mL rGONRs after 1 h exposure time, while the rGOSs exhibited the same cytotoxicity at concentration of 100 μg/mL after 96 h. authors' results demonstrated that, the rGONRs could penetrate into the cells and cause DNA fragmentations as well as chromosomal aberrations, even at low concentration of 1.0 μg/mL after short exposure time of 1 h.	Akhavan, O., Ghaderi, E., Emamy, H. & Akhavan, F. Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. CARBON 54, 419–431 (2013).
graphene oxide	ecotoxicity	In the present study, authors investigate the acute toxicity, *i.e.* short-term and high load, effect of GO on the microbial functions related to the biological wastewater treatment process. GO causes acute toxicity on activated sludge. Significant drop in microbial metabolic activity. Reduced degradation of organic matter and nutrients. Deterioration in terms of effluent quality and sludge dewatering. Accumulation of GO inside sludge flocs and reactive oxygen species production.	Ahmed, F. & Rodrigues, D. F. Investigation of acute effects of graphene oxide on wastewater microbial community: a case study. Journal of hazardous materials 256–257, 33–39 (2013).
graphene oxide	toxicity	In this work, the cellular uptake and cytotoxicity of multi-walled carbon nanotubes (MWCNTs), graphene oxide (GO) and nanodiamond (ND) were examined and compared. Among them, ND exhibited the highest cell uptake ratio, MWCNTs showed a medium cell uptake ratio, and GO displayed the lowest cell uptake ratio. However, the cytotoxicity of CNMs was not associated with their cell uptake ratios. ND possessed the highest cell uptake ratio while exhibiting the best biocompatibility with HeLa cells. Although the cell uptake ratio of MWCNTs was apparently higher than that of GO, their cytotoxicity showed no significant difference.	Zhang, X. Y., Hu, W. B., Li, J., Tao, L. & Wei, Y. A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotubes, graphene oxide, and nanodiamond. TOXICOLOGY RESEARCH 1, 62–68 (2012).

graphene oxide	toxicity	In this study, authors explored the underlying chemical mechanism of the stress-induced toxicity of GO/PP with C. elegans as an *in vivo* model. In summary, at the nano-bio interface, authors deciphered the chemical properties of GO with stress-induced nanotoxicity *in vivo*.	Zhang, W. *et al.* Unraveling stress-induced toxicity properties of graphene oxide and the underlying mechanism. Advanced materials (Deerfield Beach, Fla.) 24, 5391–5397 (2012).
graphene nanoplatelets	toxicity	Authors evaluated the toxicity of graphite nanoplatelets (GNPs) in the model organism Caenorhabditis elegans. The GNPs resulted nontoxic by measuring longevity as well as reproductive capability end points.	Zanni, E. *et al.* Graphite nanoplatelets and Caenorhabditis elegans: insights from an *in vivo* model. Nano letters 12, 2740–2744 (2012).
graphene oxide	toxicity, method	In the present study, authors' group exploited the isobaric tagged relative and absolute quantification (iTRAQ)–coupled two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) approach with the purpose of characterizing the cellular functions in response to these nanomaterials at the proteome level. Specifically, the human hepatoma HepG2 cells were selected as the *in vitro* model to study the potential cytotoxicity of oxidized single-walled carbon nanotubes (SWCNTs) and graphene oxide (GO) on the vital organ of liver. only moderate variation of protein levels for the cells treated with GO was observed and functional assays further confirmed that GO was less cytotoxic in comparison to oxidized SWCNTs	Yuan, J. *et al.* Cytotoxicity evaluation of oxidized single-walled carbon nanotubes and graphene oxide on human hepatoma HepG2 cells: an iTRAQ-coupled 2D LC-MS/MS proteome analysis. Toxicological sciences : an official journal of the Society of Toxicology 126, 149–161 (2012).
graphene oxide, reduced graphene oxide	toxicity	The synthesis, characterization, and toxicity of graphene oxide and reduced graphene oxide are reported. The toxicity depends on the type of dispersant and concentration of the nanomaterials in the suspensions. Detailed analysis suggests that graphene oxide functionalized with PEG in the concentration range between 3125 μg/mL and 25 μg/mL exhibits the best biocompatibility with mice fibroblast cells (line L929).	Wojtoniszak, M. *et al.* Synthesis, dispersion, and cytocompatibility of graphene oxide and reduced graphene oxide. Colloids and surfaces. B, Biointerfaces 89, 79–85 (2012).
graphene	ecotoxicity	Authors studied the effects of engineered carbon nanomaterials of various dimensionalities (carbon nanotubes, C60, graphene) on the germination of rice seeds. A pronounced increase in the rate of germination was observed for rice seeds in the presence of some of these carbon nanostructures, in particular the nanotubes.	Nair, R. *et al.* Effect of carbon nanomaterials on the germination and growth of rice plants. Journal of nanoscience and nanotechnology 12, 2212–2220 (2012).
graphene oxide nanosheets	toxicity	Authors systematically studied the cytotoxicity of GO nanosheets via examining the effect of GO on the morphology, viability and differentiation of a human neuroblastoma SH-SY5Y cell line. he results suggested that GO had no obvious cytotoxicity at low concentration (<80 μg mL^{-1}) for 96 h, but the viability of cells exhibited dose- and time-dependent decreases at high concentration (≥80 μg mL^{-1}). Moreover, GO did not induce apoptosis. Very interestingly, GO significantly enhanced the differentiation of SH-SY5Y induced-retinoic acid (RA) by evaluating neurite length and the expression of neuronal marker MAP2.	Lv, M. *et al.* Effect of graphene oxide on undifferentiated and retinoic acid-differentiated SH-SY5Y cells line. Nanoscale 4, 3861–3866 (2012).

pristine graphene	toxicity	This study concentrates upon investigating differential cellular responses to carbon nanoparticles (CNPs) such as pristine graphene flakes, single-walled carbon nanotubes (SWCNTs) and multiwalled CNTs (MWCNTs), in human dermal fibroblasts (HDFs) vs. the L-929 fibroblast cell line. All the CNPs tested here exerted adverse effects on the viability of HDFs even at 15.6 ppm, through intracellular uptake whereas except MWCNTs, they did not show any cytotoxicity against L-929 cells at 250 ppm.	Lee, J. H. *et al.* Cytotoxicity evaluations of pristine graphene and carbon nanotubes in fibroblastic cells. JOURNAL OF THE KOREAN PHYSICAL SOCIETY 61, 873–877 (2012).
graphene	toxicity, method	The toxicity of graphene was tested against an *in vitro* model of the blood brain barrier (BBB) by measuring trans-endothelial-electrical resistance (TEER). A new approach in terms of electrical impedance sensing was also utilized to kinetically analyze the cytotoxicity of graphene nanomaterials towards the BBB model's individual components, rat astrocytes (CRL-2006) and mouse endothelial cells (CRL-2583), in real time by measuring the impedimetric response. Graphene showed little or no toxicity toward both individual cell types as the resistance measurements were similar to those of the control and further, graphene did not interrupt the integrity of the BBB model as a whole showing the biocompatibility of graphene and the broad potential of using these new nanomaterials for biomedical applications.	Hondroulis, E., Zhang, Z. Q., Chen, C. Y. & Li, C. Z. IMPEDANCE BASED NANOTOXICITY ASSESSMENT OF GRAPHENE NANOMATERIALS AT THE CELLULAR AND TISSUE LEVEL. ANALYTICAL LETTERS 45, 272–282 (2012).
graphene oxide	toxicity	This study examined how macrophage, the primary immune cell type engaging microbes, responded to GO treatment. Authors uncovered that incubation of macrophage cell RAW264.7 with GO elicited autophagy in a concentration-dependent manner. Altogether, authors demonstrated that GO treatment of cells simultaneously triggers autophagy and TLR4/TLR9-regulated inflammatory responses, and the autophagy was at least partly regulated by the TLRs pathway.	Chen, L. Q. *et al.* Toxicity of graphene oxide and multi-walled carbon nanotubes against human cells and zebrafish. SCIENCE CHINA-CHEMISTRY 55, 2209–2216 (2012).
reduced graphene oxide	toxicity	Human mesenchymal stem cells (hMSCs), as a fundamental factor in tissue engineering, were isolated from umbilical cord blood (as a recently proposed source for extracting fresh hMSCs) to investigate, for the first time, the size-dependent cyto- and geno-toxic effects of the rGONPs on the cells. the rGONPs showed genotoxic effects on the stem cells through DNA fragmentations and chromosomal aberrations, even at low concentration of 0.1 μg/mL.	Akhavan, O., Ghaderi, E. & Akhavan, A. Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. Biomaterials 33, 8017–8025 (2012).
graphene oxide	toxicity	Authors determined the distribution and biocompatibility of graphene oxide (GO) in mice by using radiotracer technique and a series of biological assays. Results showed that GO was predominantly deposited in the lungs, where it was retained for a long time. No pathological changes were observed in examined organs when mice were exposed to 1 mg kg^{-1} body weight of GO for 14 days. Moreover, GO showed good biocompatibility with red blood cells. Due to its high accumulation and long time retention, significant pathological changes, including inflammation cell infiltration, pulmonary edema and granuloma formation were found at the dosage of 10 mg kg^{-1} body weight.	Zhang, X. Y. *et al.* Distribution and biocompatibility studies of graphene oxide in mice after intravenous administration. CARBON 49, 986–995 (2011).

graphene	toxicity	The iTRAQ-coupled 2D LC–MS/MS approach was applied to analyze the protein profile change of human hepatoma HepG2 cells treated with graphene and single-walled carbon nanotubes (SWCNTs).only moderate variation of protein levels for the cells treated with graphene was observed, which indicated graphene was less toxic	Yuan, J., Gao, H. & Ching, C. B. Comparative protein profile of human hepatoma HepG2 cells treated with graphene and single-walled carbon nanotubes: an iTRAQ-coupled 2D LC-MS/MS proteome analysis. Toxicology letters 207, 213–221 (2011).
graphene oxide	toxicity	A significant concentration and time dependent decrease in cell viability was observed at different concentrations (10–100 µg/ml) by the MTT assay after 24 and 48 h of exposure and significant increase of early and late apoptotic cells was observed as compared to control cells. authors' study demonstrates that GO induces cytotoxicity and apoptosis in human lung cells.	Vallabani, N. V. S. *et al.* Toxicity of graphene in normal human lung cells (BEAS-2B). Journal of biomedical nanotechnology 7, 106–107 (2011).
graphene oxide	toxicity	This study conclusively demonstrates that graphene oxide does not have intrinsic antibacterial, bacteriostatic, and cytotoxic properties in both bacteria and mammalian cells.	Ruiz, O. N. *et al.* Graphene oxide: a nonspecific enhancer of cellular growth. ACS nano 5, 8100–8107 (2011).
graphene	ecotoxicity	The effects of graphene on root and shoot growth, biomass, shape, cell death, and reactive oxygen species (ROS) of cabbage, tomato, red spinach, and lettuce, were investigated using a concentration range from 500 to 2000 mg/L. The results of the combined morphological and physiological analyses indicate that after 20 days of exposure under authors' experimental conditions, graphene significantly inhibited plant growth and biomass compared to a control.	Begurn, P., Ikhtiari, R. & Fugetsu, B. Graphene phytotoxicity in the seedling stage of cabbage, tomato, red spinach, and lettuce. CARBON 49, 3907–3919 (2011).
graphene	toxicity	Both G and SWCNT induce cytotoxic effects, and these effects are concentration- and shape-dependent. Interestingly, at low concentrations, G induced stronger metabolic activity than SWCNT. reactive oxygen species were generated in a concentration- and time-dependent manner after exposure to G, indicating an oxidative stress mechanism. Furthermore, time-dependent caspase 3 activation after exposure to G (10 µg/mL) shows evidence of apoptosis. Altogether these studies suggest different biological activities of the graphitic nanomaterials, with the shape playing a primary role.	Zhang, Y. *et al.* Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells. ACS nano 4, 3181–3186 (2010).
black phosphorus	toxicity	In the present study, the cytotoxicological effects of layered BP on both cell metabolic activity and membrane integrity were investigated. The dose- and time-dependent cytotoxicity of layered BP was assessed against L-929 fibroblasts. Authors' findings indicate that the cytotoxicity of BPs is proportionally dependent on their concentration and exposure time, which is affected by the oxidative stress-mediated enzyme activity reduction and membrane disruption.	Song, S.-J. *et al.* Dose- and Time-Dependent Cytotoxicity of Layered Black Phosphorus in Fibroblastic Cells. Nanomaterials 8, (2018).