

Status of OECD guidelines regarding regulatory compliance of 2D materials: a case study of graphene

Estado de las directrices de la OCDE sobre el cumplimiento normativo de los materiales bidimensionales: un estudio de caso del grafeno

Blanca Suarez-Merino,^{*,†} Luis Mauricio Ortiz Galvez^{*}

ABSTRACT: The regulatory testing of graphene and other two-dimensional (2D) materials presents significant challenges due to the unique physicochemical properties of these materials. Because graphene is both the most widely used 2D nanomaterial and one of the most structurally variable, it provides a critical case study for evaluating the applicability of OECD Test Guidelines. Strengthening this connection is particularly important because OECD-accepted methods underpin regulatory compliance in frameworks such as the EU's REACH regulation and the US Toxic Substances Control Act, meaning that gaps in guideline suitability directly affect market authorization and risk management. Existing OECD Test Guidelines (TGs) and Guidance Documents (GDs) were primarily developed for conventional chemicals and often fall short in addressing the specificities of 2D materials. These arise from their atomically thin, layered structures and quantum confinement in one dimension. This article provides a critical overview of the current status of OECD guideline applicability for graphene and proposes future directions for harmonizing regulatory testing approaches to ensure safety and compliance.

KEYWORDS: 2D materials, graphene, OECD, risk assessment, regulation, test guideline, guidance.

RESUMEN: La evaluación regulatoria del grafeno y otros materiales bidimensionales (2D) presenta desafíos significativos debido a las propiedades fisicoquímicas únicas de estos materiales. Dado que el grafeno es el nanomaterial 2D más utilizado y uno de los más variables estructuralmente, constituye un caso de estudio crucial para evaluar la aplicabilidad de los protocolos de ensayo de la OCDE, utilizados de manera generalizada en ensayos toxicológicos. Reforzar esta conexión es particularmente importante, pues los métodos aceptados por la OCDE respaldan el cumplimiento regulatorio en marcos como el reglamento REACH de la UE y la Ley de Control de Sustancias Tóxicas de EUA, lo cual significa que las deficiencias en la idoneidad de las directrices afectan directamente la autorización de comercialización y la gestión de riesgos. Las Directrices de Ensayo (Test Guidelines (TG), por sus siglas en inglés) y los Documentos de Orientación (Guidance (GD), por sus siglas en inglés) de la OCDE existentes se desarrollaron principalmente para productos químicos convencionales y, a menudo, no abordan adecuadamente las especificidades de los materiales 2D. Estas se deben a sus estructuras atómicamente delgadas y estratificadas, y a su confinamiento cuántico en una sola dimensión. Este artículo ofrece una visión general

Received: May 24, 2025.

Accepted: November 3, 2025.

Published: December 10, 2025.

^{*} TEMAS Solutions GmbH, Laettweg 5, 5212 Hausen, Switzerland.

^{**} Empa, Technology & Society Laboratory, 9014 St. Gallen, Switzerland.

[†] Author for correspondence: Blanca@temasol.org



crítica del estado actual de la aplicabilidad de las directrices de la OCDE para el grafeno y propone futuras direcciones para armonizar los enfoques de las pruebas regulatorias con el fin de garantizar la seguridad y el cumplimiento.

PALABRAS CLAVE: materiales 2D, graphene, OECD, evaluación de riesgos, directrices de ensayo, documentos de orientación.

Introduction

Two-dimensional (2D) materials, led by graphene and its derivatives, represent a rapidly expanding class of advanced nanomaterials with transformative potential across multiple sectors, including electronics, energy storage, coatings, sensors, and biomedical application (Novoselov *et al.*, 2016). Their unique physicochemical properties —atomic thickness, high surface area, exceptional electrical and thermal conductivity, and tunable surface chemistry— differentiate them from traditional materials and enable innovations that were previously unattainable. At the same time, these very characteristics raise new questions about their environmental and human health safety, as conventional risk assessment frameworks were largely developed for soluble, bulk-phase chemicals. In several instances, to meet the demands of diverse applications, they often require functional enhancement. Several modification strategies have been developed to tune their physicochemical, electronic, catalytic, and biological behaviour. The most common approaches include surface functionalization which enhances solubility, dispersion, and compatibility with biological or polymer matrices (Liu *et al.*, 2008), doping to enhance electrocatalytic activity (Voiry *et al.*, 2016), heterostructure to produce alternative materials with complementary properties in a single platform (Geim and Grigorieva, 2013), phase engineering to improve conductivity or catalytic activity (Eggeman *et al.*, 2013), and control over size and thickness through methods like mechanical exfoliation or liquid-phase exfoliation which enables tuning of optical, electronic, and mechanical properties (Li *et al.*, 2014a).

These modification strategies are essential not only for enhancing material performance but also for addressing challenges in dispersion, stability, and reactivity that are critical for safe and effective integration into commercial and biomedical systems (table 1). However, these unique features which make 2D materials attractive also introduce substantial complexity in evaluating its safety and regulatory compliance. For instance, surface functionalization of graphene oxide with polyethylene glycol (PEG) has been shown to reduce *in vivo* toxicity. Yang *et al.* (2013) found that PEGylated nano-graphene oxide exhibited improved dispersion and lower immune activation in mice following oral and intraperitoneal administration, thus enhancing its biocompatibility (Yang *et al.*, 2013). In contrast, oxidative aging of transition metal dichalcogenides (TMDs) like MoS₂ can increase toxicity. Teo *et al.* (2014) demonstrated that aged MoS₂ nanosheets generated higher levels of reactive oxygen species (ROS), leading to greater cytotoxicity *in*

vitro. This highlights how environmental or storage-induced changes in 2D materials can elevate health risks (Teo *et al.*, 2014). Doping strategies can also modulate toxicity. Nitrogen-doped graphene was shown to alter electron density and reduce hemolytic activity, improving blood compatibility. Lammel *et al.* (2013) reported that such modifications led to altered internalization behaviour and reduced cytotoxic effects in human liver (HepG2) cells compared to pristine graphene oxide (Lammel *et al.*, 2013). Regarding MXenes (e.g., $\text{Ti}_3\text{C}_2\text{Tx}$), the nature of surface terminations plays a crucial role. Saraswat *et al.* (2021) found that different terminations (e.g., $-\text{OH}$, $-\text{O}$, $-\text{F}$) induced variable levels of oxidative stress and cell viability *in vitro* and *in vivo*, underscoring the complexity of evaluating toxicity for surface-reactive nanomaterials (Saraswat *et al.*, 2021).

As a conclusion, a growing number of evidence demonstrates that surface chemistry, phase state, and structural features are critical determinants of biological interactions. Graphene, therefore, translates into a heterogeneous group of materials, termed Graphene-Related Materials (GRMs) which are very complex to regulate, these include monolayer graphene, few-layer graphene, graphene oxide (GO), and reduced graphene oxide (rGO), each with distinct structures, functionalities, and toxicological profiles (Fadeel *et al.*, 2018).

GRMs often exhibit variability in layer number, lateral dimensions, surface charge, and defect density —factors that strongly influence biological interactions and toxicity outcomes.

Against this backdrop, the Organisation for Economic Co-operation and Development (OECD) play a central role in developing internationally harmonized test guidelines (TGs) and guidance documents (GDs) for chemical safety assessment. These guidelines are accepted under the Mutual Acceptance of Data (MAD) system, which underpins regulatory compliance across frameworks such as the EU's REACH regulation and the US Toxic Substances Control Act (OECD 1981). However, most OECD TGs were originally designed for soluble or bulk-phase chemicals, assuming homogeneity, clear dose-response relationships, and stable test conditions. Such assumptions are often invalid for graphene, which tends to agglomerate, sediment, and interact with assay components, leading to confounding results.

Several case studies have already demonstrated that OECD TGs for solubility (TG 105), adsorption-desorption (TG 106), inhalation toxicity (TG 403, TG 412), and genotoxicity (TG 487) show limited applicability to graphene and other 2D materials (Wick *et al.*, 2014). Issues such as colloidal stability, dose metrics (mass *vs.* surface area *vs.* particle number), and assay interference remain unresolved within current OECD frameworks. As a result, regulatory testing of graphene demands both the adaptation of existing TGs and the development of new, nano-specific approaches. Ongoing OECD initiatives —such as the Working Party on Manufactured Nanomaterials (WPMN), the Malta Initiative, and guidance on grouping/read-across

for nanomaterials (OECD, 2016b)—highlight growing recognition of these limitations but also the need for targeted progress.

In this context, graphene provides a compelling case study for examining the strengths and gaps of the OECD guideline system. Its diversity of forms, broad commercial uptake, and regulatory significance position it as a model material for assessing how international frameworks must evolve to ensure safety while enabling innovation. This article critically evaluates the current applicability of OECD guidelines to graphene, identifies key shortcomings, and proposes pathways for harmonizing regulatory testing of 2D materials.

Table 1. Representative 2D materials and associated safety assessment challenges.

2D Material	Common uses	Challenges in safety assessment
Graphene (monolayer of carbon atoms)	Electronics, sensors, composites, coatings, drug delivery.	High variability in form (e. g., few-layer graphene, graphene oxide), inconsistent dispersion in testing media, and lack of standardized toxicity data (Fadeel <i>et al.</i> , 2018).
Graphene oxide (GO)	Biomedical devices, water purification, biosensors.	Surface functional groups affect toxicity; difficult to compare across studies due to inconsistent synthesis and purification methods (Seabra <i>et al.</i> , 2014)
Hexagonal boron nitride (h-BN)	Lubricants, thermal management in electronics, insulators.	Chemically inert and structurally similar to graphene, but inhalation studies are limited; behaviour in biological systems is not well understood (Zhi <i>et al.</i> , 2010)
Transition metal dichalcogenides (TMDs) (e. g., MoS ₂ , WS ₂)	Photodetectors, flexible electronics, catalysis.	Stability in biological and environmental systems varies; can release metal ions; optical properties complicate <i>in vitro</i> testing (Chhowalla <i>et al.</i> , 2013)
Black Phosphorus (Phosphorene)	Transistors, batteries, biomedical imaging.	Rapid oxidation in ambient conditions alters its properties; little is known about its long-term environmental fate or degradation products (Sun <i>et al.</i> , 2015).
MXenes (e. g., Ti ₃ C ₂ Tx)	Energy storage (supercapacitors, batteries), EMI shielding.	Surface chemistry highly variable; reactive surfaces can generate ROS; limited understanding of bioaccumulation or chronic effects (Anasori <i>et al.</i> , 2017)
Silicene, germanene, stanene	Theoretical interest for electronics.	Instability in air and water limits testing; mostly conceptual —few reliable toxicological datasets exist (Tao <i>et al.</i> , 2015).

Source: Author's elaboration.

Methodology

This article adopts a critical review and case-study approach to evaluate the applicability of existing OECD Test Guidelines (TGs) and Guidance Documents (GDs) to graphene and other two-dimensional (2D) materials. The analysis was conducted in three steps:

1. **Literature and regulatory review** – A systematic review of peer-reviewed publications (2004–2024) was performed using databases such as Web of Science, Scopus, and PubMed. Search terms included graphene risk assessment, OECD test guidelines nanomaterials, 2D materials toxicity, and regulatory testing graphene. In addition, OECD legal instruments, guidance documents, and workshop reports (including OECD, 1981, 2012, 2016a, 2016b, and recent OECD's Working Party on Manufactured Nanomaterials (WPMN) reports were examined to ensure coverage of both scientific and regulatory perspectives.
2. **Comparative analysis of guidelines** – The selected OECD TGs were assessed against the physicochemical and toxicological features of graphene and its derivatives (e.g., graphene oxide, reduced graphene oxide, few-layer graphene). The analysis focused on identifying methodological assumptions within TGs (such as solubility, homogeneity, or stability requirements) and evaluating their suitability when applied to 2D materials.
3. **Case study justification: graphene** – Graphene was selected as a model 2D nanomaterial for three reasons:
 - It is the most widely studied 2D material and has extensive commercial applications.
 - It exhibits high variability in structure, functionalization, and reactivity, making it a representative example of the challenges faced when applying conventional OECD TGs.
 - It has been the subject of multiple OECD workshops and initiatives, which allows for direct evaluation of regulatory progress.

The methodology is justified because it integrates both scientific evidence (to capture the complexity of graphene's physicochemical diversity and biological interactions) and regulatory frameworks (to evaluate the degree of fit between existing OECD TGs and the unique testing needs of 2D materials). This combined approach provides a balanced, evidence-based foundation for identifying gaps, highlighting ongoing initiatives, and suggesting directions for future TG development.

OECD Test guidelines and guidance to meet regulatory demands

The Organisation for Economic Cooperation and Development (OECD) plays a central role in facilitating the harmonization of chemical safety assessment practices across its member countries and internationally. To ensure that safety evaluations are scientifically robust and legally defensible, regulatory authorities increasingly rely on OECD Test Guidelines (TGs) and associated Guidance Documents (GDs). These tools provide standardized methodologies and interpretive frameworks for evaluating the physicochemical properties, environmental behaviour, ecotoxicological effects, and human health hazards of chemical substances. Moreover, their use supports transparent



and science-based decision-making processes in both human health and environmental risk assessments. As the regulatory landscape continues to evolve, the OECD framework remains a cornerstone in the development of reliable, harmonized, and efficient safety assessment practices.

OECD Test Guidelines are accepted under the Mutual Acceptance of Data (MAD) system (OECD, 1981). This system allows data generated in one OECD member country to be recognized by others, thereby reducing redundant testing, facilitating international trade, and supporting regulatory compliance in frameworks such as the European Union's REACH regulation (Regulation (EC) No 1907/2006 2006), the United States' Toxic Substances Control Act (TSCA) (Lautenberg, 2016), and similar national schemes. By minimizing the need for repeated studies, particularly those involving vertebrate animals, these guidelines also promote ethical testing practices in line with the 3Rs principle (replacement, reduction, and refinement).

Complementing the Test Guidelines, OECD Guidance Documents provide detailed recommendations on study design, data interpretation, and the application of emerging methodologies. These documents are essential for the appropriate use of new approach methodologies (NAMs), including *in vitro*, *in silico*, and integrated testing strategies. They also support scientifically coherent responses to novel regulatory challenges, such as the safety assessment of nanomaterials.

Graphene itself represents an umbrella term which covers many different materials such as graphene oxide, reduced graphene, few-layer graphene, etc. (Perala *et al.*, 2024) each with distinct physicochemical characteristics depending on synthesis methods, post-processing, and intended use, which affects their interactions with biological and environmental systems.

This variability presents significant regulatory and scientific challenges for testing graphene and other 2D materials (Longhin *et al.*, 2024). From a practical perspective, it is neither economically nor ethically feasible to conduct full toxicological testing on every possible variant, given the virtually infinite combinations of layer number, surface chemistry, functionalization, and production methods. Each modification can alter physicochemical properties in ways that directly influence biological responses, meaning that new variants cannot simply be assumed to behave like their parent material.

From a technical perspective, most commonly accepted OECD Test Guidelines (TGs) and Guidance Documents (GDs) were originally developed for conventional chemicals, where mass is the principal determinant of toxicity (Rasmussen *et al.*, 2019). This assumption does not hold for graphene-based materials. In 2D nanomaterials, parameters such as surface area, surface functional groups, oxidation state, and colloidal stability often govern toxicity more strongly than mass. For example, the same mass concentration of graphene oxide and pristine graphene may result in drastically different cellular uptake, oxidative stress, and immune responses (Ou *et al.*,

2016). Consequently, relying on mass-based dose metrics —as is standard in current OECD TGs— can obscure relevant hazard mechanisms and lead to misleading risk assessments.

To avoid multiple testing of materials which share similar chemical composition but different physico-chemical properties, the OECD produced a general GD with different steps to follow regarding different nanoforms which belong to one particular group of nanomaterials (OECD, 2016b), this GD, however, is too general for 2D materials. In the particular case of graphene, to frame the number of different types into a sizeable group for testing, the Graphene Council has developed a classification framework aimed at promoting consistency, comparability, and transparency in graphene-related research, manufacturing, and regulation. The Graphene Council's system distinguishes graphene materials based on key physical and chemical attributes, including: 1) number of layers: differentiates between monolayer graphene, few-layer graphene (2-10 layers), and multi-layered graphite-like structures; 2) lateral size: indicates flake dimensions, which impact properties like mechanical reinforcement and biological interactions; 3) carbon-to-oxygen ratio: especially important for distinguishing pristine graphene from graphene oxide (GO) and reduced graphene oxide (rGO); 4) purity and defect density: critical for electronic and sensor applications where defects influence conductivity, and, 5) production method: includes methods such as chemical vapor deposition (CVD), liquid-phase exfoliation, or chemical reduction of graphite oxide (The Graphene Council 2019). Hence the aim of the Graphene Council classification framework is to establish a standardized system for defining and distinguishing different forms of graphene, based on measurable parameters such as layer number, lateral size, carbon-to-oxygen ratio, purity, and production method. Its purpose is to promote consistency, comparability, and transparency in research, manufacturing, and regulation, thereby supporting reliable safety assessment and commercialization.

As explained above, this remark challenges the use of such TG and GD for 2D materials, since those were primarily developed for conventional chemicals and bulk materials, but are not always suitable for assessing nanomaterials like graphene. For instance, issues such as dispersion stability, surface area determination, and dose metrics become critical and often lack standardized approaches for 2D materials.

Physicochemical properties and diversity of graphene and other 2D materials

Two-dimensional (2D) materials encompass a broad class of substances with layered atomic structures, each exhibiting distinct physicochemical features that influence their behaviour in biological and environmental systems. These properties —such as surface chemistry, number of layers, lateral dimensions, phase state, and surface charge— not only define the functional potential of the materials but also govern their interactions with cells, organisms, and



ecosystems. Understanding and characterizing these attributes is essential for safe material design and regulatory assessment.

Graphene related materials (GRMs)

Graphene was first isolated in 2004 through mechanical exfoliation of graphite—a technique often referred to as the “Scotch tape method” (Novoselov *et al.*, 2004). This groundbreaking discovery revealed the existence of a truly two-dimensional atomic crystal composed of a single layer of sp^2 -hybridized carbon atoms arranged in a hexagonal lattice. Materials within the graphene group exhibit wide variability in their physicochemical characteristics depending on synthesis method and post-processing. Monolayer graphene, often considered the ‘ideal’ form from a fundamental scientific perspective, exhibits the highest electrical conductivity, mechanical strength, and transparency. However, in practical applications, few-layer graphene, graphene oxide, or reduced graphene oxide are more commonly used, as they are easier to produce at scale and can be tailored for specific functionalities.

The number of atomic layers in a sample affects its surface area and electronic behaviour, while lateral dimensions determine not only physical characteristics but also biological uptake and biodistribution. Smaller graphene flakes tend to disperse better and more readily cross cellular barriers. Furthermore, defect density and edge structure play a pivotal role in dictating reactivity and stability in physiological or environmental media. These variations influence not just technical performance but also toxicity, making precise characterization essential for hazard assessment.

Transition metal dichalcogenides (TMDs)

While molybdenum disulfide (MoS_2) had been used industrially as a lubricant since the 1960s, its semiconducting properties in the 2D limit were only recognized in the early 2010s. In 2010, Mak *et al.* demonstrated that monolayer MoS_2 exhibits a direct bandgap (~ 1.8 eV), unlike its bulk counterpart, which is an indirect semiconductor (Mak *et al.*, 2010). This finding established MoS_2 as the first 2D material with a direct bandgap, making it highly attractive for applications in transistors, photodetectors, and flexible electronics. TMDs such as molybdenum disulfide (MoS_2), tungsten disulfide (WS_2), and tungsten diselenide (WSe_2) consist of a transition metal layer sandwiched between two chalcogen layers. Their properties can vary significantly depending on their crystal phase—most notably between the semiconducting 2H phase and the metallic 1T phase. The phase state affects not only electronic conductivity but also biological reactivity, with metallic forms often generating higher levels of reactive oxygen species (ROS).

In addition, the thickness of TMD layers influences their bandgap, with monolayer MoS_2 exhibiting a direct bandgap that transitions to an indirect one as more layers are added. This affects optical and electronic interactions

with biological systems. Moreover, the edges of TMD nanosheets often exhibit higher catalytic activity and chemical reactivity than their basal planes, contributing to their degradation behavior and interaction with cells. Aged or oxidized MoS₂, for example, has shown elevated cytotoxicity *in vitro*, largely due to oxidative stress and potential ion release.

Hexagonal boron nitride (h-BN)

Hexagonal boron nitride (h-BN) has been known since the 1950s and shares a similar layered structure with graphene, though composed of alternating boron and nitrogen atoms. The exfoliation of h-BN into monolayers was explored following the success of graphene. In 2010, Dean *et al.* demonstrated that h-BN is an ideal substrate for high-mobility graphene devices due to its atomically smooth surface, lack of dangling bonds, and high dielectric breakdown strength (Dean *et al.*, 2010). h-BN is structurally analogous to graphene but is composed of alternating boron and nitrogen atoms. It is electrically insulating, thermally conductive, and chemically inert. Unlike graphene, h-BN has a wide bandgap of approximately 5.9 eV and is used in electronic applications as a dielectric layer or thermal insulator. Its high thermal stability (stable above 900 °C), low surface reactivity, and nanoscale morphology (1–10 nm thick) suggest relatively low biological reactivity. However, comprehensive toxicological data —especially concerning long-term inhalation exposure— are still lacking, necessitating further investigation before regulatory conclusions can be drawn.

MXenes

MXenes represent a newer class of 2D materials derived from MAX phases —layered ternary carbides and nitrides. They were first reported in 2011 by Naguib, Gogotsi, and Barsoum, who synthesized Ti₃C₂T_x by selectively etching aluminum from Ti₃AlC₂ (Naguib *et al.*, 2011). MXenes possess a unique combination of high electrical conductivity and surface tunability due to functional terminations such as –OH, –F, and –O. These features have opened up research into applications in energy storage, EMI shielding, water purification, and emerging toxicological evaluations. MXenes are a class of 2D materials consisting of early transition metal carbides, nitrides, or carbonitrides (e.g., Ti₃C₂T_x), synthesized by selective etching of MAX-phase precursors. Their surfaces are terminated with functional groups such as hydroxyl (–OH), oxygen (–O), and fluoride (–F), which provide hydrophilic properties and influence their electrochemical behavior. Physicochemical characteristics of MXenes include a layer thickness of 1–2 nm, high electrical conductivity, strong negative surface charge, and variable surface terminations. These factors significantly impact their colloidal stability, ion release behavior, and biological interactions. *In vitro* and *in vivo* studies have demonstrated that di-

fferent surface terminations can lead to varying degrees of cytotoxicity and immune response, highlighting the need for thorough characterization prior to safety evaluation.

Black phosphorus (phosphorene)

Black phosphorus (BP), the most stable allotrope of elemental phosphorus, was first synthesized in 1914. However, its exfoliation into few-layer and monolayer sheets —termed phosphorene— was not reported until 2014. That year, Li *et al.* demonstrated that phosphorene could be fabricated into field-effect transistors, exploiting its unique thickness-dependent direct bandgap and high carrier mobility (Li *et al.*, 2014b). Despite its promising electronic properties, phosphorene's instability in ambient conditions remains a significant challenge for both applications and safety testing. BP is a layered semiconductor with a puckered lattice structure and a direct bandgap that depends on the number of layers —ranging from ~0.3 eV in the bulk to ~2.0 eV in monolayers. BP exhibits high carrier mobility and anisotropic optical properties, making it attractive for optoelectronic and biomedical applications. However, its extreme sensitivity to oxygen and moisture leads to rapid degradation, forming oxidized phosphorus species and phosphoric acid. This instability alters surface charge and reactivity, affecting its interaction with biological systems. Degradation products may generate reactive oxygen species or release phosphate ions, which could have pro-oxidant or cytotoxic effects. Therefore, BP requires special handling and stabilization strategies to ensure safety in practical applications. This refined overview emphasizes how specific physicochemical attributes —such as surface functionality, oxidation state, and dimensionality— govern the biological behavior and safety profile of 2D materials. Such diversity underlines the importance of harmonized material characterization, contextual grouping strategies, and fit-for-purpose toxicological testing protocols.

Relevance of existing OECD guidelines to graphene

OECD Test Guidelines (TGs) form a globally recognized foundation for the hazard assessment of chemicals, including nanomaterials. However, the applicability of these TGs to two-dimensional (2D) materials —such as graphene and its derivatives, transition metal dichalcogenides (TMDs), MXenes, and black phosphorus— remains a critical challenge due to the unique physicochemical properties and behaviours of these materials in biological and environmental systems.

Challenges with conventional OECD test guidelines

Most OECD TGs were developed with bulk chemicals or soluble substances in mind. These conventional approaches assume homogeneous dispersion, che-

mical stability, and clear dose-response relationships —assumptions that often do not hold for 2D nanomaterials. 2D materials may agglomerate, settle rapidly, exhibit high surface reactivity, or interact with assay reagents, leading to non-specific effects or false readouts in standard test systems (Fadeel *et al.*, 2018) (table 2).

A fundamental prerequisite for the effective hazard assessment of 2D materials is the comprehensive physicochemical characterization of the test material prior to any biological or environmental testing (table 3). Unlike conventional chemicals, 2D materials exhibit a high degree of variability in structure and behaviour, depending on synthesis method, post-treatment, and functionalization. As a result, understanding their properties is critical not only for selecting appropriate test guidelines but also for ensuring reproducibility and comparability across studies.

One of the most important parameters is the number of atomic layers, as this influences properties such as electronic behavior, surface area, and mechanical flexibility. The lateral size of the material —ranging from tens of nanometers to several microns— can determine cellular uptake and biodistribution, especially in *in vitro* and *in vivo* settings. Additionally, specific surface area plays a key role in determining reactivity, particularly with biological macromolecules, while surface functional groups (such as hydroxyl, carboxyl, or fluorine) affect hydrophilicity, zeta potential, and protein corona formation.

Other critical characteristics include the *zeta potential*, which indicates surface charge and determines how stable graphene dispersions remain in biological or environmental media. Instability or rapid aggregation can lead to inconsistent exposures in toxicity tests. Similarly, the degree of oxidation or doping strongly influences reactivity; for example, oxygen-containing groups on graphene oxide can increase the generation of reactive oxygen species, while doping may alter electron density and biological interactions. In addition, residual metal catalysts or synthesis byproducts —often overlooked in material characterization— can themselves contribute to observed toxic effects, making it difficult to attribute outcomes solely to the graphene material.

Compared to spherical nanoparticles or carbon nanotubes, two-dimensional (2D) materials pose distinct challenges for toxicological testing. Their high aspect ratio and plate-like morphology affect deposition patterns in respiratory tracts and biological tissues, complicating the interpretation of inhalation studies such as OECD TG 403 and 412. Furthermore, variations in functionalization and synthesis can lead to significant differences in hazard profiles, even among materials classified under the same type (Wick *et al.*, 2014). Some 2D materials, including MXenes and black phosphorus, are also prone to rapid transformation under test conditions —such as oxidation or ion release— making consistent exposure difficult to achieve (Teo *et al.*, 2014) (Saraswat *et al.*, 2021). Additionally, these ma-

terials may interfere with *in vitro* assays by adsorbing proteins, quenching assay dyes, or generating reactive oxygen species, thereby complicating the interpretation of standard toxicological endpoints (Lammel *et al.*, 2013). The OECD and other international bodies explicitly recognize that a thorough physicochemical characterization of nanomaterials is essential for reliable safety assessment. For 2D materials such as graphene, this means that properties like layer number, surface chemistry, lateral size, and dispersion stability must be carefully measured and reported, since they directly influence how the material interacts with biological systems and the environment. Without this information, it becomes impossible to interpret toxicological test results or ensure that data are reproducible and comparable across studies characterization for nanomaterials in general, and for 2D materials in particular. Guidance documents such as the Guidance on Sample Preparation (OECD, 2012) and the Developments on the safety of manufactured nanomaterials (OECD, 2016a) emphasize that physicochemical data should inform the interpretation of test results and the selection of relevant test guidelines. Without this information, it is impossible to determine whether observed biological effects are due to the material itself, its impurities, or artifacts introduced by inadequate dispersion or sample handling.

Table 2. OECD test guidelines with limited applicability to 2D materials.

OECD TG	End point	Challenges 2D materials
TG 105	Water solubility.	Assumes chemical solubility, not applicable to insoluble, colloiddally dispersed 2D materials like graphene or MoS ₂ .
TG 106	Adsorption-desorption using a batch equilibrium method.	Designed for dissolved chemicals; not suited for plate-like particles that interact with surfaces through non-equilibrium mechanisms.
TG 403 / TG 412	Acute inhalation toxicity / 28-day inhalation toxicity.	Deposition and clearance behavior of 2D nanosheets not adequately captured; high aspect ratio affects lung retention.
TG 487	<i>In vitro</i> mammalian cell micronucleus test.	2D materials can interfere with fluorescence staining and sediment rapidly, leading to uneven exposure.
TG 417	Toxicokinetics.	Designed for small molecules; does not account for biodistribution or bioaccumulation of large, insoluble nanosheets.
TG 439	<i>In vitro</i> skin irritation: reconstructed human epidermis test method.	2D particles may not penetrate skin models in the same way as soluble chemicals; adsorption onto membranes complicates interpretation.
TG 471	Bacterial reverse mutation test (ames test).	2D particles may not enter bacterial cells; potential for assay interference through adsorption of mutagens or nutrients.

Source: Author's elaboration.

Table 3. Physico chemical characterization of 2D materials and relevant techniques.

Physicochemical property	Relevant techniques
Layer number	Atomic force microscopy (AFM), transmission electron microscopy (TEM), Raman spectroscopy.
Lateral size	Dynamic light scattering (DLS), TEM, scanning electron microscopy (SEM).
Surface area	Brunauer–Emmett–Teller (BET) analysis.
Surface functional groups	X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy (FTIR).
Zeta potential (surface charge)	Zeta potential measurement via electrophoretic light scattering.
Purity / metal content	Inductively coupled plasma mass spectrometry (ICP-MS), thermogravimetric analysis (TGA).
Crystallinity / phase	X-ray diffraction (XRD), Raman spectroscopy.
Colloidal stability / dispersion	UV-vis spectroscopy, DLS, centrifugation-based sedimentation analysis.
Oxidation state / defect density	Raman spectroscopy, XPS, electron paramagnetic resonance (EPR).

Source: Author's elaboration.

Case study graphene: challenges in regulatory testing under OECD guidelines

Graphene is the most used example of a 2D nanomaterial, and represents a valuable case study for examining the challenges of applying OECD Test Guidelines (TGs) to emerging advanced materials (Connolly *et al.*, 2023). Although graphene-based materials are increasingly used in electronics, coatings, biomedical devices, and composite applications, their risk assessment, as discussed in previous sections, still suffers from methodological uncertainties. This complexity arises largely from graphene's unique physicochemical properties, its diversity in structural forms, and its variable behavior in biological and environmental systems.

To illustrate the regulatory challenges posed by graphene within existing OECD frameworks, this section structured around the four classical steps of risk assessment: hazard identification, exposure assessment, dose-response assessment, and risk characterization. The aim is not to perform a complete risk assessment, but rather to highlight where conventional OECD Test Guidelines (TGs) fall short when applied to 2D nanomaterials. By using graphene as a case study, each subsection demonstrates the specific methodological limitations, uncertainties, and adaptations required to ensure reliable safety evaluation. This approach provides a structured way to identify gaps and emphasize the need for tailored testing strategies and new approach methodologies (NAMs) for graphene and related materials (figure 1).

Hazard Identification

Hazard identification for graphene-related materials (GRMs) remains complex due to their structural diversity and context-dependent biological interactions. Conventional OECD Test Guidelines (TGs) provide a useful starting point, but they were not designed to capture the specific mechanisms of action relevant to 2D nanomaterials.

1. Key hazard endpoints.

Studies have reported a range of potential hazards for graphene and its derivatives, including cytotoxicity, oxidative stress, genotoxicity, inflammation, and immune activation. Graphene oxide (GO), for example, tends to generate higher levels of reactive oxygen species (ROS) than pristine graphene due to its oxygen-containing functional groups, leading to greater oxidative stress and membrane damage. Reduced graphene oxide (rGO) and few-layer graphene may exhibit different profiles, reflecting variations in layer number, lateral size, and residual impurities.

2. Variability across graphene types.

A major challenge for hazard identification is that the same label (e.g., “graphene oxide”) may encompass materials with very different surface chemistries, lateral sizes, and defect densities depending on the synthesis method. These variations can alter not only toxicity outcomes but also the uptake pathway (e.g., endocytosis, direct membrane penetration). This variability complicates grouping approaches and makes it difficult to define a “generic hazard profile” for graphene.

3. Mechanistic considerations.

Hazard identification for graphene must go beyond traditional endpoints and consider mechanistic pathways, such as oxidative stress, mitochondrial dysfunction, and pro-inflammatory signaling. Some GRMs have been shown to interfere with DNA and protein interactions, while others adsorb assay dyes or nutrients, leading to false-positive or false-negative results. These mechanistic insights are essential for linking observed effects with real biological relevance, which is a prerequisite for developing OECD-accepted test methods.

4. Challenges with OECD TG applicability.

Current OECD TGs (e.g., TG 403 for acute inhalation, TG 420 for acute oral toxicity, TG 487 for genotoxicity) were designed for soluble chemicals with predictable dose–response patterns. They do not account for GRM-specific issues such as aggregation, poor dispersion in test media, or interactions with assay components. As a result, reported hazards may reflect testing artifacts rather than intrinsic material toxicity, undermining reproducibility and regulatory confidence.

5. Knowledge gaps and future directions.

- Limited *in vivo* data exist on chronic effects, especially for respiratory and immune outcomes.

- Few validated models capture the long-term persistence of graphene in tissues.
- Mechanistic endpoints (e.g., oxidative stress, autophagy, immune modulation) are not explicitly addressed in current OECD TGs.
- Standardized hazard profiles cannot yet be established due to the lack of harmonized characterization and consistent testing protocols.

Future OECD guidance should explicitly integrate mechanistic toxicology, standardized material characterization, and controls for assay interference into hazard identification. This will allow more reliable distinction between true hazard potential and test system artifacts, providing a stronger scientific foundation for risk assessment.

Exposure assessment

Exposure assessment for graphene and other 2D nanomaterials is still in its infancy. While the classical OECD framework considers inhalation, ingestion, and dermal routes, the specific behaviors of graphene-related materials (GRMs) complicate reliable exposure estimation.

1. Human exposure (occupational and consumer).

In occupational settings, inhalation of airborne graphene flakes during production, handling, or disposal represents the most plausible exposure route. However, limited data exist on aerosol formation, particle size distributions, and long-term retention in the respiratory tract. Current OECD inhalation models assume spherical or soluble particles, which do not account for the plate-like morphology and slow clearance of 2D nanosheets.

Consumer exposure may occur via dermal contact (e.g., coatings, wearable electronics, textiles) or ingestion (e.g., accidental release from packaging, biomedical uses). Yet, few standardized studies quantify actual transfer rates through skin or gastrointestinal absorption.

2. Environmental exposure.

Graphene-based materials are increasingly used in composites, paints, and electronics, leading to potential release during product use and disposal. Release pathways include abrasion from coatings, leaching from landfills, or release during incineration and wastewater treatment. Studies indicate that graphene can persist in soils and sediments, but systematic data on environmental concentrations remain scarce. Aquatic exposure is particularly relevant, as graphene oxide disperses more readily in water and may interact with microorganisms, plants, and aquatic organisms.

3. Challenges with current OECD TGs.

Existing OECD exposure models (e.g., those for inhalation or environmental fate) were not designed for insoluble 2D nanomaterials. They assume uniform distribution and solubility, conditions that graphene suspen-

sions rarely meet. Moreover, standard toxicokinetic tests (TG 417) do not capture biodistribution or long-term accumulation of nanosheets in tissues such as lungs, liver, or spleen. This creates major uncertainty in exposure estimates, which are essential for risk characterization.

4. Knowledge gaps and future needs.

- There is a lack of quantitative data on occupational exposure levels to airborne graphene.
- Environmental monitoring data (water, soil, sediments) are almost absent, despite rising production volumes.
- Plant and soil uptake pathways remain poorly characterized, though preliminary studies suggest graphene can affect germination and root development.
- Transformation processes (oxidation, biodegradation, aggregation) that influence bioavailability are insufficiently studied.

5. Moving forward.

Future OECD guidance should prioritize the development of nano-specific exposure scenarios, validated monitoring methods for environmental release, and toxicokinetic approaches that reflect the persistence and distribution of insoluble nanosheets. Exposure assessment for graphene cannot rely on models built for soluble chemicals but must incorporate the unique behaviors of 2D materials to reduce uncertainty and support realistic risk evaluation.

Dose-response assessment

Establishing a meaningful dose–response relationship for graphene-related materials (GRMs) remains one of the most significant challenges for regulatory testing. In conventional OECD TGs, toxicity is typically expressed as a function of administered mass concentration (e.g., mg/L in aquatic tests or mg/kg in oral toxicity studies). However, this approach does not adequately capture the mechanisms of action of 2D nanomaterials.

1. Relevance of alternative dose metrics.

For graphene, parameters such as specific surface area, particle number concentration, layer number, and oxidation state often play a more decisive role in biological interactions than mass alone. For instance, equal mass doses of pristine graphene and graphene oxide may produce vastly different cellular responses, due to differences in surface chemistry, hydrophilicity, and protein corona formation. This makes direct mass-based comparisons unreliable.

2. Influence of dispersion and sedimentation.

Unlike soluble chemicals, graphene suspensions are prone to aggregation, sedimentation, and variable colloidal stability in test media. As a result, the delivered dose (the fraction of material actually interacting with cells or organisms) may differ substantially from the administered dose.

Without harmonized dispersion protocols and validated dosimetry methods, dose-response curves can be inconsistent and difficult to interpret across studies.

3. Non-linear responses and threshold effects.

Evidence suggests that graphene and its derivatives may not follow simple linear dose-response patterns. For example, low concentrations may induce adaptive cellular responses, while higher concentrations trigger oxidative stress or genotoxicity. Such non-monotonic responses challenge the conventional toxicological assumption that “the dose makes the poison” in a straightforward way.

4. Comparative challenges across OECD TGs.

Several OECD TGs (e.g., TG 403 for acute inhalation, TG 487 for *in vitro* genotoxicity) presuppose homogeneous exposure and mass-driven responses. For GRMs, these assumptions are not valid, which can result in under- or overestimation of toxicity. In inhalation studies, for example, the high aspect ratio and plate-like morphology of graphene sheets influence deposition and clearance in ways that are not accounted for in standard OECD models.

5. Future approaches.

Improving dose-response assessment for graphene requires the integration of new dosimetry models (e.g., considering surface area or delivered dose) and the validation of standardized dispersion protocols. Advanced *in vitro* models, combined with computational dosimetry and physiologically based pharmacokinetic (PBPK) modeling for insoluble nanosheets, may provide a more realistic representation of dose-response relationships. Ultimately, incorporating these innovations into OECD TGs will be necessary to achieve reproducibility and regulatory acceptance.

Risk characterization

Integrating hazard and exposure data for graphene remains highly uncertain under current OECD frameworks. While the classical risk assessment paradigm provides a useful structure, several scientific and methodological issues undermine the reliability of risk characterization for 2D nanomaterials.

1. Lack of standardized dose metrics.

Current TGs typically rely on mass-based exposure metrics. However, for graphene materials, toxicity is often driven by parameters such as surface area, oxidation state, or functional groups. Without agreement on the most relevant metric—mass, particle number, or surface area—risk characterization produces inconsistent and potentially misleading outcomes.

2. Variability of graphene-related materials (GRMs)

The diversity of graphene forms (monolayer, few-layer, graphene oxide, reduced graphene oxide) complicates the extrapolation of results across studies. Even materials classified under the same label (e.g., “graphene oxide”)

may differ significantly in synthesis method, lateral size, and purity. This makes grouping and read-across approaches, which are central to regulatory efficiency, difficult to apply without additional OECD guidance.

3. Limited exposure data

Accurate risk characterization requires realistic information on how graphene enters humans, animals, and plants. Yet, data on occupational exposure (e.g., inhalation during production), consumer exposure (e.g., dermal contact in coatings or textiles), and environmental exposure (e.g., release to water and soil) remain scarce. Without such data, risk estimates are based on worst-case assumptions rather than evidence-driven scenarios.

4. Environmental transformation and persistence

Risk characterization must also account for transformations such as oxidation, aggregation, or degradation. These processes can either mitigate or enhance toxicity, but OECD TGs do not provide validated methods for assessing environmental fate or long-term persistence. This gap undermines confidence in predicting ecological impacts.

5. Uncertainty and weight-of-evidence approaches

Given these gaps, risk characterization for graphene currently relies on uncertainty analysis and weight-of-evidence approaches, rather than definitive thresholds of safe exposure. Regulators are increasingly encouraged to combine conventional TGs with new approach methodologies (NAMs)—including *in vitro* and *in silico* models—to reduce uncertainty and capture mechanistic insights such as oxidative stress or immune activation pathways.

6. Implications for regulation

The consequence is that risk characterization for graphene remains provisional, with a lack of consensus on safe exposure limits. This has direct regulatory implications: for example, under REACH, dossiers often rely on read-across or waiving strategies, but these are difficult to justify without agreed grouping rules. A more robust framework combining OECD TG adaptation, NAM integration, and exposure monitoring is therefore essential to reduce uncertainty and enable evidence-based regulation.

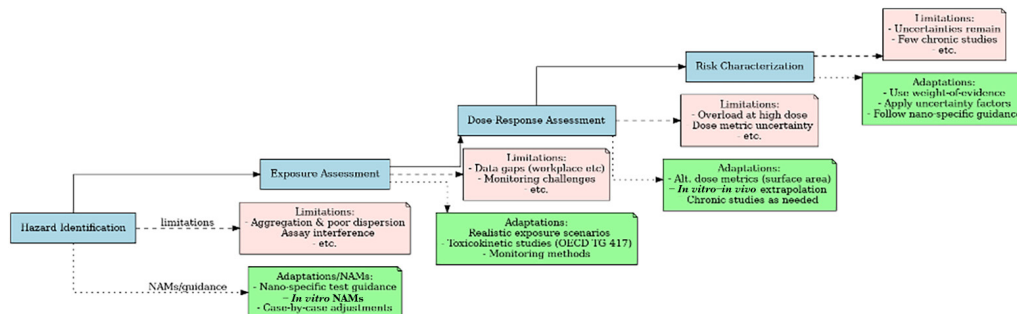
Summary of key shortcomings in OECD guidelines for graphene risk assessment

Based on the current knowledge key adaptations have been identified to allow a full risk assessment of graphene (table 4).

Current efforts and outlook

To address existing gaps in the applicability of current test methods to nanomaterials, the OECD Working Party on Manufactured Nanomaterials (WPMN), along with parallel initiatives such as the Malta Initiative, is actively engaged in conducting case studies, developing nano-specific guidance documents, and

Figure 1. Schematic representation of the risk assessment workflow for GRMs, following the four classical steps.



Hazard identification, exposure assessment, dose-response assessment, and risk characterization are shown in sequence (blue boxes). At each step, key limitations or challenges (pink boxes) are highlighted, alongside corresponding adaptations/new approach methodologies (NAMs) recommended to address those issues (green boxes). The flowchart also notes relevant OECD test guidelines (TGs) for each step.

Table 4. Key issues that require further improvement regarding guideline development for graphene.

Area	Shortcoming
Physicochemical characterization	No standardized guidance on how to define graphene variants (e.g., monolayer vs. few-layer, GO vs. rGO).
Test method validity	Several TGs (e.g., TG 487, TG 417) are not validated for insoluble, high-aspect-ratio materials.
Assay interference	No requirements to assess or mitigate interactions with test reagents or detection systems.
Exposure estimation	Inadequate models for predicting release, aerosol formation, and environmental fate.
Dose metrics	No consensus on whether to use mass, surface area, or number for toxicological dosing.

Source: Author's elaboration.

identifying limitations within existing OECD Test Guidelines. Recent efforts within the OECD's Adverse Outcome Pathways (AOP) framework further aim to incorporate mechanistic insights from nanotoxicology into regulatory decision-making processes.

In this context, stakeholders have emphasized the critical need for standardized reference materials to support the benchmarking of test outcomes, as well as validated dispersion protocols that ensure reproducible and consistent exposure in experimental systems. Moreover, the adoption of new approach methodologies (NAMs) —including high-content imaging and advanced *in vitro* models that more accurately replicate physiological barriers— is increasingly viewed as essential.

Taken together, these developments point toward the value of a tiered, case-by-case approach that strategically combines conventional OECD Test Guidelines with adapted methods and NAMs. Such an approach currently represents the most pragmatic and scientifically sound strategy for the assessment of 2D materials under evolving regulatory frameworks (Oomen *et al.*, 2015; OECD, 2016b).

Future directions for OECD TG development

Future development of OECD Test Guidelines (TGs) must go beyond incremental adaptation and instead embrace the unique challenges posed by graphene and other 2D materials. Several key areas require attention:

1. Standardized material definitions and reference materials.

A first step is the establishment of internationally accepted definitions and reference materials for graphene-related materials (GRMs). Without consensus on parameters such as layer number, surface chemistry, lateral size, and purity, toxicological data cannot be meaningfully compared. The Graphene Council's classification framework provides a foundation, but OECD-endorsed standards are essential for regulatory use.

2. Validated dispersion and dosimetry protocols.

Reproducible testing requires harmonized methods for dispersing graphene in relevant biological and environmental media. Current OECD TGs assume soluble or homogeneously distributed substances, which is not the case for GRMs. New protocols for measuring effective dose (e.g., delivered *vs.* administered concentration, surface area-based metrics) are urgently needed to improve comparability across laboratories.

3. Adaptation of specific test guidelines.

Inhalation (TG 403/412), oral toxicity (TG 420), and aquatic toxicity (TG 201–203) tests need revision to account for aggregation, sedimentation, and high aspect ratios of nanosheets. *In vitro* assays (e.g., TG 487 for genotoxicity) should be updated to include controls for assay interference, such as adsorption of reagents and dyes by graphene surfaces.

4. Integration of new approach methodologies (NAMs).

Traditional TGs rely heavily on animal testing, which is costly, time-intensive, and ethically challenging. NAMs—including advanced *in vitro* models (organ-on-chip, co-culture systems), high-throughput screening, and *in silico* modeling (QSAR, PBPK models for nanosheets)—must be incorporated into OECD guidance. Importantly, NAMs should be validated against real-world exposure scenarios for graphene.

5. Environmental fate and exposure pathways.

Very limited data exist on the release, transformation, and persistence of graphene in water bodies, soil, and air. Future OECD TGs should include guidance for simulating realistic exposure pathways, including occupational exposure during production and environmental release during use and disposal.

For plants and soil organisms in particular, dedicated guidance is lacking.

6. Grouping and read-across approaches.

Given the enormous variability of GRMs, it is unrealistic to test each variant individually. Future guidance should expand the OECD's grouping/read-across framework to 2D materials, enabling the extrapolation of hazard data across materials with shared features (e.g., oxidation state, layer number, surface charge). This approach would reduce redundant testing while maintaining regulatory robustness.

7. Data infrastructure and transparency.

To support regulatory acceptance, OECD should promote open databases that compile physicochemical characterization, exposure, and toxicity data for graphene and 2D materials. Harmonized reporting templates (covering material description, dispersion method, and test conditions) would enhance reproducibility and allow regulators to make evidence-based decisions.

Taken together, these developments point toward a **tiered, case-by-case testing strategy** that combines conventional OECD TGs with adapted methods and NAMs, supported by strong material characterization and grouping principles. Such a framework would not only ensure the safe commercialization of graphene but also serve as a model for other advanced materials in the emerging field of 2D nanotechnology.

Conclusion

Graphene represents a paradigm challenge for regulatory science, requiring a re-evaluation of current OECD guidelines to ensure fit-for-purpose safety assessment. Despite significant advances in material characterization and toxicity testing, there is still a lack of information on how graphene and other 2D nanomaterials enter organisms of humans, animals, and plants. Understanding uptake routes—such as inhalation, ingestion, dermal absorption, and root uptake in plants—remains critical for realistic exposure scenarios but is only partially addressed in current studies.

At the same time, bibliographic evidence highlights that the production and market value of graphene and related nanomaterials have grown substantially in the past decade, with estimated production already reaching thousands of tons annually. These increasing production volumes heighten the probability of release into water bodies and soils during manufacturing, use, and end-of-life disposal. Yet, quantitative data on environmental concentrations remain scarce, and only a limited number of preliminary studies have investigated their persistence, transformation, and toxicity under realistic environmental conditions.

This lack of systematic knowledge underscores the urgency of adapting OECD Test Guidelines. Without validated approaches that consider the diversity of graphene materials, their exposure pathways, and their potential accu-

mulation in ecosystems, regulatory frameworks cannot guarantee safe commercialization. By combining technical insights on test guideline limitations with emerging evidence on production, market growth, and preliminary toxicity studies, this article emphasizes the strategic importance of addressing graphene within international safety assessment frameworks. Coordinated efforts among regulators, industry, and academia are needed to fill these critical data gaps and ensure that innovation in 2D nanomaterials is aligned with human and environmental protection.

Author's contribution

Both authors contributed to: article conception and design, methodological development. Data mining, analysis, and interpretation, preparation of supplementary materials. Writing the original draft, review and final editing of the text

References

- Anasori, Babak, Maria R. Lukatskaya and Yury Gogotsi. (2017). 2D metal carbides and nitrides (MXenes) for energy storage. *Nature Reviews Materials*, 2(2): 16098. <https://doi.org/10.1038/natrevmats.2016.98>.
- Chhowalla, Manish, Hyun Seok Shin, Goki Eda and *et al.* (2013). The chemistry of two-dimensional layered transition metal dichalcogenide nanosheets. *Nature Chemistry*, 5(4): 263-75. <https://doi.org/10.1038/nchem.1589>.
- Connolly, M., G. Moles, F. Candotto Carniel *et al.* (2023). Applicability of OECD TG 201, 202, 203 for the aquatic toxicity testing and assessment of 2D graphene material nanoforms to meet regulatory needs. *NanoImpact*, 29(January): 100447. <https://doi.org/10.1016/j.impact.2022.100447>.
- Dean, C. R., A. F. Young, I. Meric *et al.* (2010). Boron nitride substrates for high-quality graphene electronics. *Nature Nanotechnology*, 5(10): 722-26. <https://doi.org/10.1038/nnano.2010.172>.
- Eggeman, Alexander S., Steffen Illig, Alessandro Troisi, Henning Sirringhaus and Paul A. Midgley. (2013). Measurement of molecular motion in organic semiconductors by thermal diffuse electron scattering. *Nature Materials*, 12(11): 1045-49. <https://doi.org/10.1038/nmat3710>.
- Fadeel, Bengt, Cyrill Bussy, Sonia Merino *et al.* (2018). Safety assessment of graphene-based materials: focus on human health and the environment. *ACS Nano*, 12(11): 10582-620. <https://doi.org/10.1021/acsnano.8b04758>.
- Geim, Andre K. and Irina V. Grigorieva. (2013). Van der Waals heterostructures. *Nature*, 499(7459): 419-25. <https://doi.org/10.1038/nature12385>.
- Lammel, Tobias, Perrine Boisseaux, M. Luisa Fernández-Cruz and José M. Navas. (2013). Internalization and cytotoxicity of graphene oxide and carboxyl graphene nanoplatelets in the human HepG2 cell line. *Particle and Fibre Toxicology*, 10(1): 27. <https://doi.org/10.1186/1743-8977-10-27>.
- Lautenberg, Frank R. (2016). U.S. Congress. Toxic Substances Control Act, 15 U.S.

- Code § 2601-2697 (1976). *Chemical Safety for the 21st Century Act*.
- Li, Hui, Junhao Wu, Zongyou Yin and Hua Zhang. (2014a). Preparation and applications of mechanically exfoliated single-layer and multilayer MoS₂ and WSe₂ nanosheets. *Accounts of Chemical Research*, 47(4): 1067-75. <https://doi.org/10.1021/ar4002312>.
- Li, Likai, Yijun Yu, Guo Jun Ye *et al.* (2014b). Black phosphorus field-effect transistors. *Nature Nanotechnology*, 9(5): 372-77. <https://doi.org/10.1038/nnano.2014.35>.
- Liu, Zhuang, Joshua T. Robinson, Xiao Sun and Hongjie Dai. (2008). PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. *Journal of the American Chemical Society*, 130(33): 10876-77. <https://doi.org/10.1021/ja803688x>.
- Longhin, Eleonora Marta, Ivan Rios-Mondragon, Espen Mariussen *et al.* (2024). Hazard assessment of nanomaterials: how to meet the requirements for (next generation) risk assessment. *Particle and Fibre Toxicology*, 21(1): 54. <https://doi.org/10.1186/s12989-024-00615-4>.
- Mak, Kin Fai, Changgu Lee, James Hone, Jie Shan and Tony F. Heinz. (2010). Atomically thin MoS₂: a new direct-gap semiconductor. *Physical Review Letters*, 105(13): 136805. <https://doi.org/10.1103/PhysRevLett.105.136805>.
- Naguib, Michael, Murat Kurtoglu, Volker Presser *et al.* (2011). Two-dimensional nanocrystals produced by exfoliation of Ti₃AlC₂. *Advanced Materials*, 23(37): 4248-53. <https://doi.org/10.1002/adma.201102306>.
- Novoselov, K. S., A. K. Geim, S. V. Morozov *et al.* (2004). Electric field effect in atomically thin carbon films. *Science*, 306(5696): 666-69. <https://doi.org/10.1126/science.1102896>.
- Novoselov, K. S., A. Mishchenko, A. Carvalho and A. H. Castro Neto. (2016). 2D materials and Van der Waals heterostructures. *Science*, 353(6298): aac9439. <https://doi.org/10.1126/science.aac9439>.
- OECD. (1981). *OECD Council decision of 12 May 1981 concerning the mutual acceptance of data in the assessment of chemicals (C(81)30/Final)*. Organisation for Economic Co-Operation and Development (OECD). <https://legalinstruments.oecd.org/en/instruments/OECD-LEGAL-0194>.
- OECD. (2012). Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. *OECD Series on the safety of manufactured nanomaterials, ENV/JM/MONO(2012)40*. Organisation for Economic Co-Operation and Development (OECD). 36.
- OECD. (2016a). Developments on the safety of manufactured nanomaterials: 2013. *Series on the safety of manufactured nanomaterials ENV/JM/MONO(2016)59*. OECD Publishing. 59.
- OECD. (2016b). Grouping and read-across for the hazard assessment of manufactured nanomaterials. *Series on the safety of manufactured nanomaterials*. OECD Publishing 80. <https://www.oecd.org/chemicalsafety/nanosafety/publications-series-safety-manufactured-nanomaterials.htm>.
- Oomen, Agnes G., Eric A. J. Bleeker *et al.* (2015). Grouping and read-across approaches for risk assessment of nanomaterials. *Environmental Research and Public Health*, 12(10). <https://doi.org/10.3390/ijerph121013415>.

- Ou, Lingling, Bin Song, Huimin Liang *et al.* (2016). Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms. *Particle and Fibre Toxicology*, 13(1): 57. <https://doi.org/10.1186/s12989-016-0168-y>.
- Perala, Ramaswamy Sandeep, Narendhar Chandrasekar, Ramachandran Balaji, Pinky Steffi Alexander, Nik Zulkarnine Nik Humaidi and Michael Taeyoung Hwang. (2024). A comprehensive review on graphene-based materials: from synthesis to contemporary sensor applications. *Materials Science and Engineering: R: Reports*, 159(June): 100805. <https://doi.org/10.1016/j.mser.2024.100805>.
- Rasmussen, Kirsten, Hubert Rauscher, Peter Kearns, Mar González and Juan Riego Sintes. (2019). Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data. *Regulatory Toxicology and Pharmacology*, 104(June): 74-83. <https://doi.org/10.1016/j.yrtph.2019.02.008>.
- Regulation (EC) No 1907/2006. (2006). European Parliament and Council of the European Union. Regulation (EC) No 1907/2006 of 18 December 2006 Concerning the registration, evaluation, authorisation and restriction of chemicals (REACH). *Official Journal of the European Union*, 396: 1-849.
- Saraswat, Vivek, Adil Mushtaq and Sanjay Saxena. (2021). Toxicity and Immunological response of Ti_3C_2 MXene nanosheets *in vitro* and *in vivo*. *Journal of Hazardous Materials*, 420: 126621. <https://doi.org/10.1016/j.jhazmat.2021.126621>.
- Seabra, Aline B., Amanda J. Paula, Renata de Lima *et al.* (2014). Nanotoxicity of graphene and graphene oxide. *Chemical Research in Toxicology*, 27(2): 159-68. <https://doi.org/10.1021/tx400385x>.
- Sun, Zhen, Hao Xie, Shao Tang *et al.* (2015). Black phosphorus nanostructures: recent advances in fabrication and biomedical applications. *Chemical Society Reviews*, 44(24): 8848-59. <https://doi.org/10.1039/C5CS00514H>.
- Tao, Liang, Eugenio Cinquanta, Daniele Chiappe *et al.* (2015). Silicene field-effect transistors operating at room temperature. *Nature Nanotechnology*, 10(3): 227-31. <https://doi.org/10.1038/nnano.2014.325>.
- Teo, Wei Zhe, Elaine L. K. Chng, Zdenek Sofer and Martin Pumera. (2014). Cytotoxicity of exfoliated transition-metal dichalcogenides (MoS_2 , WS_2 , and WSe_2) is lower than that of graphene and its analogues. *Chemistry — A European Journal*, 20(31): 9627-32. <https://doi.org/10.1002/chem.201402680>.
- The Graphene Council. (2019). *Graphene classification framework*. <https://www.thegraphenecouncil.org>.
- Voiry, Damien, Jian Yang and Manish Chhowalla. (2016). Recent strategies for improving the catalytic activity of 2D TMD nanosheets toward the hydrogen evolution reaction. *Advanced Materials*, 28(29): 6197-206. <https://doi.org/10.1002/adma.201600914>.
- Wick, Peter, Annelies E. Louw-Gaume, Melanie Kucki *et al.* (2014). Classification framework for graphene-based materials. *Angewandte Chemie International Edition*, 53(30): 7714-18. <https://doi.org/10.1002/anie.201403335>.
- Yang, Kai, Huan Gong, Xiaoyan Shi, Jia Wan, Yuxiao Zhang and Zhuang Liu. (2013). *In vivo* biodistribution and toxicology of functionalized nano-graphene oxide in

mice after oral and intraperitoneal administration. *Biomaterials*, 34(11): 2787-95. <https://doi.org/10.1016/j.biomaterials.2012.12.039>.

Zhi, Chunyi, Yoshio Bando, Chengchun Tang *et al.* (2010). Boronnitride nanotubes. *Materials Science and Engineering: R: Reports*, 70(3-6): 92-111. <https://doi.org/10.1016/j.mser.2010.06.015>.