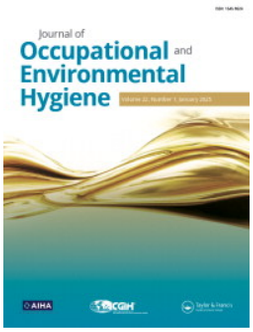

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REPORT

Application of the Tier 3 NIOSH occupational exposure banding process for the graphene family of nanomaterials: A case study

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ABSTRACT

Graphene is a class of two-dimensional (2D) nanomaterials composed of single or multiple layers of carbon atoms. To date, there are limited clinical data and no epidemiological research available to assess graphene toxicity in humans. Despite the growing amount of animal toxicity data, there are currently no occupational exposure limits (OELs) for any type of graphene nanomaterial published by international authoritative organizations to ensure their safe handling within workplaces. In the absence of consensus OELs for graphene, the National Institute for Occupational Safety and Health (NIOSH) occupational exposure banding process was used to assign an occupational exposure band (OEB). The NIOSH banding process is organized into a three-tiered system and is a resource for occupational safety and health (OSH) professionals to guide risk management and exposure control decisions when OELs are not available. To the authors' knowledge, there are no Globally Harmonized System of Classification and Labeling of Chemicals (GHS) H-codes/statements available for graphene to conduct a Tier 1 analysis. Even though data were available from authoritative sources for three of nine health endpoints, the data were insufficient to support banding in a Tier 2 assessment. Therefore, a Tier 3 assessment using the NIOSH banding process was applied to the graphene family of nanomaterials (GFN) as a case study based on the specific physicochemical and toxicological properties with uncertainty factor adjustments. The band assignment was replicated by three individuals with advanced toxicology and industrial hygiene knowledge to ensure a consistent outcome. The results found that three of the six endpoints banded were "E," representing an air concentration $\leq$0.01 mg/m^3, while the other three ranged from "A" to "C." This indicates that the graphene materials evaluated may have potential effects at low exposure concentrations ($\leq$0.01 mg/m^3). These findings suggest an OEB may be a suitable option for OSH professionals attempting to mitigate risk for GFN in the absence of an OEL and may provide a reasonable initial estimate for recommended workplace exposure and control measures.

KEYWORDS

Hazard evaluation; health effect endpoints; nanomaterials; occupational exposure banding

Introduction

Graphene is a class of materials commonly referred to as the graphene family of nanomaterials (GFN) based on their surface properties, number of layers, and size. There is limited agreement on the specific nomenclature for this family of nanomaterials, but they can generally be categorized as monolayer or pristine (single atom layer thick), very few layers (vFLG, 1–3 layers of carbon), few layer (FLG, 2–5 layers), multilayer (MLG, 2–10 layers), graphene nanoplatelets (GNP, stacks of graphene sheets > 10 layers), or graphene quantum dots (GQDs) which consist of one or a few layers of graphene sheets <100 nm in their lateral dimension (Sanchez et al. 2012; Wick et al. 2014; Chen et al. 2017; ISO 2017; Paszkiewicz and Szymczyk 2019). In addition to carbon layers, GFN can be categorized based on their oxygen content which includes graphene oxide (GO, which is a compound of carbon, oxygen, and hydrogen) and reduced graphene oxide (rGO, which has fewer oxygen groups; Smith et al. 2019). Furthermore, graphene can be

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functionalized for specific applications by adding elements to the surface or edges, such as graphene nanoflakes (GNFs) functionalized with carboxylic acid groups (Lamb et al. 2019).

Over the last decade, graphene has attracted tremendous attention due to its remarkable electronic, chemical, and mechanical properties. These properties facilitate their widespread application in many areas including semiconductors, composites, sensors, energy storage, functional ink, polymer additives, tires, coatings, and many more (Park et al. 2017). While the number of workers exposed to GFN in the U.S. is unknown, the quantity of this two-dimensional nanomaterial produced or used by ten surveyed companies was estimated at around 50,000 kg/year (Babik et al. 2018). As the utilization of graphene continues to grow with further expansion into industrial applications, the potential for workplace exposures throughout the materials' life cycle is expected to successively increase.

Workplace exposure to graphene likely occurs through several routes: inhalation, dermal, ocular, and oral (Pelin et al. 2018). However, inhalation is considered the major route of human exposure to GFN in occupational settings (Han et al. 2015; Pelin et al. 2018). To date, only one clinical research study and no epidemiologic research or case studies are available to assess potential health effects in humans. A double-blind randomized controlled study conducted on human participants reported that acute inhalation of GO (the only type of GFN material tested) was well tolerated with no impact on human health (Andrews et al. 2024). Therefore, most current information on the potential toxicity of graphene comes from experimental animal studies. *In vivo* studies of rodents exposed to GFN using various routes of dosing methods provide evidence of graphene uptake in the blood causing potential toxicity in various organs and cells such as the liver (Syama et al. 2017; Amrollahi-Sharifabadi et al. 2018), kidney (Patlolla et al. 2016; Amrollahi-Sharifabadi et al. 2018), heart (Krajnak et al. 2019), genome (Bengtson et al. 2017; El-Yamany et al. 2017), neurons (Mendonça et al. 2015, 2016a, 2016b), testes (Nirmal et al. 2017), eye (Wu et al. 2016), and the brain (Amrollahi-Sharifabadi et al. 2018). However, there is an exception with the lung where the health effects may occur following inhalation without requiring uptake in the blood (Schinwald et al. 2012; Ma-Hock et al. 2013).

Despite the growing amount of data from animal studies, there are no occupational exposure limits (OELs) for any type of GFN published by international authoritative organizations to ensure safe handling in workplaces. However, an OEL was developed by independent researchers based on data from a sub-chronic inhalation toxicity study of GO that used a lung dosimetry model to derive an OEL of 18 $\mu g/m^3$ (Lee et al. 2019). Additionally, a benchmark concentration (BMC) for graphene nanoplatelet exposure of 212 $\mu g/m^3$ was developed by Spinazzè et al. (2019) using an experimental probabilistic approach based on limited short-term inhalation exposure data. In the absence of consensus OELs, occupational safety and health (OSH) professionals require additional information on how to manage graphene exposure risks within the workplace.

The National Institute for Occupational Safety and Health (NIOSH) occupational exposure banding process was developed to fill in knowledge gaps and serve as a starting point to inform risk management decisions when an authoritative OEL is not available (NIOSH 2019). Occupational exposure banding assigns chemicals into health protective bands based on toxicological potency information on selected health effect endpoints (NIOSH 2019). The NIOSH occupational exposure banding process is organized into a three-tiered system, where each tier has different requirements related to data availability and expertise of the users (NIOSH 2019). Tier 1 is a screening-level process based on the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals hazard codes/statements (NIOSH 2019). The same technical criteria for each toxicological endpoint are applicable for both Tier 2 and Tier 3 (NIOSH 2019). While Tier 2 involves assigning the occupational exposure band (OEB) based on quantitative and qualitative information from authoritative sources and reviews, Tier 3 relies on a comprehensive evaluation of health effects studies to assign an OEB (NIOSH 2019).

Previously, a case study using the NIOSH Tier 1 and Tier 2 banding process was completed to derive an OEB for Bisphenol A (Hines et al. 2019). The authors are unaware of any published literature that has completed a Tier 3 assessment. Dissemination of the Tier 3 banding process serves as an important step to demonstrate the application of the process and, most importantly, to provide valuable information to OSH professionals managing graphene exposure risks within the workplace. Therefore, the objective of this case study was to use available data from appropriate toxicological studies to apply a Tier 3 assessment using the NIOSH banding process for GFN.

Methods

NIOSH banding process—Tier assessment

Following the NIOSH banding process, the authors attempted to apply Tier 1 and 2 assessments. To the authors' knowledge, there are no GHS H-codes/statements for GFN to complete a Tier 1 analysis. Data were available from authoritative sources, such as the European Chemicals Agency (ECHA), for three of the 9 health endpoints (respiratory sensitization, eye irritation, and skin irritation) with a Total Determinant Score (TDS) of 20. However, a TDS of less than 30 indicates that the data for GFN are insufficient to support banding in a Tier 2 assessment (NIOSH 2019). Therefore, a Tier 3 assessment of the NIOSH banding process was applied for GFN.

Literature review of toxicological studies

A literature review was completed using the Stephen B. Thacker Centers for Disease Control and Prevention (CDC) Library to obtain dose-response information from animal toxicology studies. The literature search was completed in May 2023 across a variety of databases which included Medline, ProQuest, Web of Science, Toxline, and Scopus. The data collected from these studies included both qualitative and quantitative data on nine standard toxicological health endpoints: (1) carcinogenicity; (2) reproductive/developmental toxicity; (3) specific target organ toxicity-repeated exposure (STOT-RE); (4) genotoxicity; (5) respiratory sensitization; (6) skin sensitization; (7) acute toxicity; (8) skin corrosion and irritation; and (9) eye damage/irritation (Table 1). The keywords used for the literature search can be found in the supplemental materials. All complete *in vivo* animal toxicological studies were included in this Tier 3 assessment. Studies that only had an abstract or were written in a language other than English were excluded. In total, 7,470 potential records were identified through the literature search (Figure 1). After reading the titles and abstracts, 2,249 articles were removed because they did not meet the inclusion criteria of this study. Out of the 5,221 articles screened, 5,163 were excluded because they were systematic reviews, *in vitro* studies, or duplicates. Out of the remaining 56 available articles, 32 studies were not utilized for band assignment because they were either (a) studies that utilized an intravenous route of administration, (b) STOT-RE endpoint studies that were under 28 days, (c) respiratory sensitization endpoint studies that did not report T helper 2 (Th 2) cells, cytokines such as interleukin (IL) IL-3, IL-4, IL-5, IL-6, IL-8, IL-13 or IL-18, and immunoglobulin E (IgE) (Robinson et al. 1992; Robinson 2000; Boverhof et al. 2008; Sanders and Mishra 2016; Sutton et al. 2019; Gibb and Sayes 2022), or (d) acute toxicity studies that were not expressed as LD_{50} or LC_{50}. Finally, the remaining 24 eligible articles (with two articles having two different endpoints) were used to derive an OEB in this Tier 3 assessment.

Toxicological study evaluation

The 24 eligible articles on graphene toxicity from experimental animal studies were compiled and analyzed to compare hazard potency for nine health endpoints. Rodent and rabbit toxicological data were used in this case study because human dose-response data were not available for GFN. These published articles were evaluated to determine whether both quantitative and qualitative toxicity information was available to assign an OEB for graphene following the methodology outlined in the Tier 3 NIOSH banding process (NIOSH 2019). In evaluating the results from the toxicological studies, consideration was given to many factors, including the physical and chemical properties of the graphene material under study such as the type of graphene, particle size, and physical form of the material as well as the specific attributes of the studies including animal species, dosing levels, route and duration of exposure, nature of pathologic changes, and alteration in metabolic responses.

Band selection

OEBs for graphene were selected for each of the nine health endpoints in rodent and rabbit studies by following the NIOSH Tier 3 assessment recommendations. For each endpoint, graphene potency estimates were determined, and endpoints were assigned to exposure bands A, B, C, D, or E (Table 1; NIOSH 2019). Each endpoint exposure band represents an order of magnitude decrease in an airborne concentration range with the "A" OEB being the highest concentration range and "E" OEB being the lowest concentration range (Table 2).

After compiling and assessing the graphene toxicity data and assigning an OEB for each endpoint, the appropriate overall OEB for GFN was determined by considering all endpoints together (NIOSH 2019). In cases where given endpoints were classified into more than one OEB because of having multiple potency

Table 1. Data selection criteria for a Tier 3 assessment following the NIOSH occupational exposure banding process.

Health Endpoint	Criteria for Selecting Endpoint and Assigning a Band
Acute Toxicity	Acute lethality data expressed as LD_{50} or LC_{50}
	Routine experimental animals, e.g., rats, mice, rabbits, guinea pigs, etc. Exclude chickens, frogs, etc.
	Route of administration: oral, dermal, or inhalation. Exclude subcutaneous, intraperitoneal, intravascular routes.
	Single dose. Exclude multiple dose studies.
	Exclude inhalation studies where exposure duration not reported. If exposure duration other than four hours, adjust LC_{50} using ten Berge equation.
	Exclude LD_{50} or LC_{50} values preceded by a greater than (>) symbol.
	Exclude LD_{50} or LC_{50} values presented as a range of concentrations when values in the range fall within occupational exposure bands B-E, except when the range reports values separately for male and female, in which case the low end of range is used for banding.
Specific Target Organ Toxicity-Repeated Exposure (STOT-RE)	NOAEL or BMDL value from a study of at least 28 days.
	If study duration 90 days or longer, reported NOAEL or BMDL is used.
	If study duration 28 days but less than 90 days, NOAEL is divided by 3 to estimate a 90-day equivalent NOAEL.
	If no NOAEL or BMDL values are available, use LOAEL, if available, divided by 10 to estimate a NOAEL equivalent.
	If multiple NOAELs or BMDLs are available for an exposure route, use the lowest route-specific value.
Genotoxicity	Availability of genotoxicity data from *in vivo* assays and mammalian assays supported by *in vitro* and nonmammalian assays.
	Consistent results in a diverse array of assays that evaluate different types of effects on genetic material
Skin Corrosion/Irritation	Assessment based on a chemical in its pure form unless exposure banding targeted at a specific product with diluted or non-concentrated chemical.
Eye Damage/Irritation	NIOSH has not recommended band assignments based on potency information (e.g., dose-response data, Draize scores, etc.) for the eye damage/irritation endpoint.
	Instead, NIOSH recommends assigning bands based on qualitative data
Skin Sensitization	**Qualitative**
	Human patch testing for sensitization
	Quantitative
	LLNA EC3
	GMPT
	Buehler guinea pig test
Carcinogenicity	**Quantitative**
	Potency information: slope factor, inhalation risk unit, tumorigenic dose (TD_{05}), or concentration (TC_{05})
	Qualitative
	Assessment based on authoritative reviews.
Reproductive/Developmental	Internationally accepted test guideline (i.e., GLP or OECD) studies preferred.
	NOAEL, BMDL or BMCL values that assess:
	Developmental toxicity
	Perinatal and postnatal toxicity
	One-generation or two generation toxicity
	Reproductive/developmental toxicity
	Combined repeated dose toxicity study with reproductive/developmental toxicity.
	Short- or long-term repeated dose toxicity (i.e., impairment of reproductive function in the absence of significant generalized toxicity).
	If no NOAEL or BMDL values are available, use LOAEL, if available, divided by 10 to estimate a NOAEL equivalent.
Respiratory Sensitization	Markers such as high levels of T helper 2 (Th 2) cells, cytokines such as interleukin (IL) IL-3, IL-4, IL-5, IL-6, IL-8, IL-13 and IL-18, and immunoglobulin E (IgE).

Adapted from NIOSH OEB Process for Chemical Risk Management (2019). LD_{50} = Lethal dose, 50%. LC_{50} = Lethal concentration, 50%.

estimates, the most protective OEB was chosen (NIOSH 2019).

Quantitative endpoints

For the STOT-RE and reproductive/developmental toxicity endpoints, OEBs were derived using no observed adverse effect level (NOAEL), and lowest observed adverse effect level (LOAEL) data from rodent studies (NIOSH 2019). The NOAEL is defined as the highest dose at which there are no observed adverse effects; whereas the LOAEL is the lowest dose at which there are observed adverse effects (Klaassen and Watkins 2010; Kale et al. 2022). A NOAEL estimate from a rodent study with an exposure duration of 90 days or longer could be used to directly assign an OEB. If a NOAEL estimate was derived from a rodent study of 28 to 89 days of exposure duration, then that estimate was divided by a factor of three to derive a point of departure (POD) estimated to be equivalent to a 90-day exposure (NIOSH 2019). If NOAEL values were not available, LOAEL values were divided by 10 to estimate a NOAEL equivalent (NIOSH 2019).

Qualitative endpoints

For endpoints such as skin and eye irritation, genotoxicity, and skin and respiratory sensitization, a categorical outcome was evaluated on a qualitative or semi-quantitative basis to describe the presence of the effect of the outcome such as no effect, mild, or severe (NIOSH 2019). The skin sensitization endpoint considers the local lymph node assay (LLNA), the guinea pig maximization test (GPMT), and the Buehler assay. The genotoxicity endpoint relies on the overall judgment of genotoxicity studies from a literature review. For eye irritation, NIOSH recommends assigning bands based on qualitative data. Studies that evaluated allergic reactions and inflammation in the lungs were also considered for banding in respiratory sensitization (NIOSH 2019).

Band replication

The band assignment in this case study was independently replicated by three individuals with expertise in toxicology and industrial hygiene to ensure reliable outcomes during the banding process. Each of the individuals held at least a master's degree in industrial hygiene, or related field of study, possess the professional credential of Certified Industrial Hygienist (CIH), and had between 8 and 20 years of professional experience. After describing the procedures of the study, each individual performing the replication was provided with the literature review and a summary of the key information needed to select health-effect endpoints, determine the TDS, and assign an OEB. An overall study OEB (i.e., Final Band) was selected based upon majority agreement between the replicates. In instances where there was no majority agreement, the middle band was selected. If the middle band could not be assigned, the more protective band was selected. The rationale for the selection of the health-effect endpoints and OEB assignment for each replication is presented in detail within the supplemental materials (Supplementary Tables S1–S3). The NIOSH OEB process suggested using Klimisch scores if the banding results for a particular endpoint did not agree on an OEB selection (NIOSH 2019). Klimisch et al. (1997) developed a scoring system to assess the reliability of data to guide the evaluation of the quality of toxicological data to be used in risk assessments (see additional information in Supplementary Tables S1 and S2). Instead of using Klimisch scores in this case study, the replication process was adopted to determine OEB reliability based on majority agreement among experienced assessors.

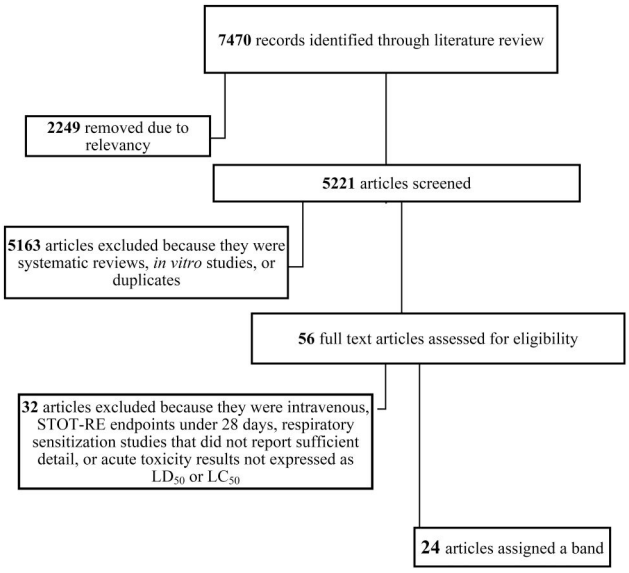

Figure 1. Flow diagram for selection of studies in the literature review.

Results

Toxicity data for GFN were identified for seven of the nine Tier 3 endpoints including STOT-RE, respiratory sensitization, acute toxicity, reproductive and developmental toxicity, eye irritation, skin sensitization, and genotoxicity. No data were available for the skin corrosion/irritation and cancer endpoints.

Summary of available toxicological studies

The 56 available graphene toxicological articles were summarized to provide a general overview of graphene *in vivo* toxicity. Most studies on graphene toxicity were conducted using airway exposure, such as nose-only inhalation, intratracheal instillation, and

Table 2. Airborne concentration ranges associated with NIOSH occupational exposure bands.

Occupational Exposure Band	Airborne Target Range for Particles (mg/m^3)	Airborne Target Range for Gas or Vapors (ppm)
A	>10	>100
B	>1 to 10	>10 to 100
C	>0.1 to 1	>1 to 10
D	>0.01 to 0.1	>0.1 to 1
E	≤0.01	≤0.1

Adapted from the NIOSH Occupational Exposure Banding Process for Chemical Risk Management (2019).

pharyngeal aspiration (Supplementary Tables S1–S3). In addition to the airway exposure, studies were conducted using intraperitoneal injection, intravenous injection, oral gavage, intravitreal injection, application in the conjunctival sac of the right eye, and application to the dorsal skin of each ear (Table 3).

Stot-re

Bronchoalveolar lavage analysis demonstrated low toxicity following a 28-day repeated nose-only GNP inhalation study in Sprague-Dawley rats at the tested doses of 0.12, 0.47, and 1.88 mg/m^3 compared to the controls (Kim et al. 2016). These results were supported by Shin et al. (2015) who reported that the 5-day repeated exposure to graphene nanoflakes via nose-only inhalation in rats only had a minimal toxic effect at the concentrations (0.68 and 3.86 mg/m^3) and time points used in this study. Another inhalation study conducted by Ma-Hock et al. (2013) observed inflammatory microgranuloma in the lungs after 10 mg/m^3 of GNP head-nose exposure in male Wistar rats. Additionally, El-Yamany et al. (2017) found that GO nanosheets have the potential to accumulate in lung tissue and induce pulmonary injury following an intraperitoneal administration of 250 and 500 µg/kg in mice. Similarly, Park et al. (2017) observed cytoskeletal damage in the lung and systemic suppression of antigen-presenting cells after an intratracheal installation of 5 mg/kg of GNP in mice. Wu et al. (2022) reported that the intranasal administration of nitrogen-doping GQD (N-GQDs) and amino-modified GQD (A-GQDs) at a dosage of 1 mg/kg caused lung fibrotic effects in mice. Furthermore, Durán et al. (2017) demonstrated that GO-induced spleen inflammation following intraperitoneal administration of 5 mg/kg in rats. On the other hand, no toxic effects

Table 3. Summary of graphene family nanomaterials toxicological studies selected.

Endpoint	Reference	Type of graphene	Size (nm)	Species	Dosing route	Study duration (Days)
STOT-RE	Durán et al. (2017)	GO	0.7–1.2	Rat	Intraperitoneal	30
	El-Yamany et al. (2017)	GO	7.6	Mice	Intraperitoneal	7, 28, and 56
	Kim et al. (2016)	GNP	0.35–0.38	Rat	Nose-only inhalation	1, 28, and 90
	Kurantowicz et al. (2015)	GO	3–4	Rat	Intraperitoneal	28
	Park et al. (2017)	GNP	3–4	Mice	Intratracheal	90
	Strojny et al. (2015)	GO	3–4	Rat	Intraperitoneal	28 and 84
	Wu et al. (2022)	GQD, NGQD, AGQD	1.6–5-8.	Mice	Intratracheal	28
	Yang et al. (2013)	GO, nGO-PEG, RGO-PEG; nRGO-PEG	0.94–5.66	Mice	Oral	1, 7, and 30
Respiratory Sensitization	Han et al. (2015)	GO	10–120	Rat	Nose-only inhalation	1, 7, and 14
	Park et al. (2015)	GNP	3–4	Mice	Intratracheal	1, 7, 14, and 28
	Roberts et al. (2016)	GNP	8–25	Mice	Pharyngeal aspiration	0, 1 , 7, 30, and 60
	Schinwald et al. (2012)	GO	10	Mice	Pharyngeal	1
	Shurin et al. (2014)	GO	0.61	Mice	Pharyngeal	28 and 29
Genotoxicity	Bengtson et al. (2017)	GO and rGO	200–800	Mice	Intratracheal	1, 3, 28 and 90
	Durán et al. (2017)	GO	0.7–1.2	Rat	Intraperitoneal	30
	El-Yamany et al. (2017)	GO	7.6	Mice	Intraperitoneal	7, 28, and 56
	Mohamed et al. (2021)	GO	62.5	Mice	Oral	5
Skin Sensitization	Kim et al. (2021)	GNP	N/A	Mice	Intraperitoneal	1, 2, and 3
	Sosa et al. (2023)	FLG and GO	171	Mice	Dermal	3
Eye Irritation	Lin et al. (2015)	G-OH	1.3	Rabbits	Intravitreous injection	56
	Wu et al. (2016)	GO	1.2	Rabbits and rats	Conjunctival sac application	0, 1, 2, 3, and 5
	Yan et al. (2012)	GO	1	Rabbits	intravitreally injection	2, 4, and 49
Reproductive/ Developmental	Fu et al. (2015)	GO	1.8	Mice	Oral	21 and 38
	Liang et al. (2015)	GO	4	Mice	Intraperitoneal	30 and 60
	Nirmal et al. (2017)	GO	0.8–2	Mice	Intraperitoneal	15 and 130
	Zhang et al. (2019)	GQD	5.25	Mice	Oral	1 and 10

GO: graphene oxide; rGO: reduced graphene oxide; GNP: graphene nanoplatelets; FLG: few layers graphene; GQD: graphene quantum dots; MLG: multi-layered graphene; A-GQDs: amino-modified graphene quantum dot; GO-PSS: nanographene oxide functionalized with poly sodium 4-styrenesulfonate; PEG-nGO: PEGylated graphene oxide nanoparticle; GNF: graphene nanoflakes; nGO-PEG: PEGylated nano-GO; nGO-PEG: PEGylated nano-graphene oxide; RGO-PEG: PEGylated reduced graphene oxide; nRGO-PEG: PEGylated nano reduced graphene oxide; G-OH: Hydroxylated graphene; GNF: graphene nanoflakes; STOT-RE: Specific Target Organ Toxicity-Repeated Exposure; N/A: not available.

on blood and no effects on growth were observed in the liver and spleen in rats after an intraperitoneal administration of 4 mg/kg of GO (Kurantowicz et al. 2015). Although these reports suggested mixed results, a minimal NOAEL of 1.88 mg/m^3 for lung effects in rats was found (Kim et al. 2016).

Respiratory sensitization

Reports of respiratory sensitization for graphene were the most common outcome. Schinwald et al. (2012, 2014) observed inflammogenic response in both the lung and the pleural space following a pharyngeal aspiration of GNP and unoxidized multilayered graphene platelets (GPs) in mice. Similarly, Han et al. (2015) reported inflammation of the lung at a dose of 3.76 mg/m^3 after a nose-only inhalation of GO in rats. Park et al. (2015) investigated the effect of intratracheally instilled GNP in mice and reported that doses of 2.5 and 5 mg/kg induced a sub-chronic inflammatory response. Another intratracheal instillation of 5 mg/kg of GO in mice caused dose-dependent acute lung injury characterized mainly by cell injury, lung edema, and neutrophil infiltration (Li et al. 2013). Additionally, Lee et al. (2017) observed a significant acute neutrophilic inflammation which resolved over time following a 1 mg/rat intratracheal instillation of all types of GNPs. Li et al. (2018) studied the pulmonary toxicity of GO and rGO after oropharyngeal aspiration exposure and reported that 2 mg/kg of GO showed moderate effects, while 2 mg/kg of rGO induced low levels of lung inflammation in rats. Furthermore, Mao et al. (2016) observed that exposure to 50 µg of FLG caused moderate pulmonary edema in mice following an intratracheal instillation. Altogether, these studies found that graphene exposure through multiple airway administration methods in rodents can lead to lung inflammation. However, pulmonary inflammation alone was not considered for banding respiratory sensitization endpoints.

Genotoxicity

The genotoxicity of graphene nanomaterials has been tested in acute and sub-chronic bioassays in mice and rats. Intraperitoneal treatment of GO at doses of 10, 50, 100, 250, and 500 µg/kg once weekly for 7, 28, and 56 days induced chromosomal aberrations in bone marrow cells and DNA fragmentation in lung cells that were time and dose-dependent (El-Yamany et al. 2017). Another study by Mohamed et al. (2021) confirmed the effect of GO in mice bone marrow cells in a dose-dependent manner along with a histological lesion such as apoptosis, necrosis, inflammations, and cell degeneration in the liver and brain tissue sections following an oral gavage at doses of 10, 20, or 40 mg/kg for 5 days. In addition, Bengtson et al. (2017) demonstrated that at a dose of 18 µg/mouse, both GO and rGO induced a significant increase of DNA damage in bronchoalveolar lavage (BAL) cells at days 3 and 28 for GO and days 28 and 90 for rGO following intratracheal administration. Moreover, the genotoxicity of GO was observed in rats following an intraperitoneal administration of 5.0 mg/kg as compared to rats receiving the dose of GO 0.5 mg/kg and the negative control (Durán et al. 2017). Similarly, Altwaijry et al. (2022) showed single and double-stranded DNA breaks at 1 to 2 h following an intravenous injection of 5.0 mg/kg PEGylated GO (Polyethylene glycol (PEG) conjugated to the GO). All these reports suggested that GO may have a genotoxic potential that increases with the dose.

Skin sensitization

Skin sensitization was evaluated in mice following the application of GNP (working concentration: 25, 50, and 100% v/v, respectively) to the dorsal skin of each ear and intraperitoneal administration of 20 mg/mL GNP using the murine local lymph node assay (LLNA): BrdU-FCM *in vivo* test (Kim et al. 2021). It was found that GNPs did not induce skin sensitization at any concentration. The absence of skin sensitization was confirmed by Sosa et al. (2023) after daily exposure to FLG or GO through an application of the dorsal skin of each ear at a working concentration range of 0.4–40 mg/mL for three consecutive days. These studies suggest that graphene nanomaterials may not be a skin sensitizer.

Eye irritation

The potential as an eye irritant for GO has been demonstrated through *in vivo* rodent experiments. Wu et al. (2016) studied the toxicity of GO exposure in the eyes of Sprague–Dawley rats by evaluating the lesions of conjunctiva, cornea, and iris following ocular instillation. They concluded that GO did not cause acute eye irritation because no evidence of corneal opacity, conjunctival redness, abnormality of the iris, or chemosis was observed at 12.5 mg/mL or 25 mg/mL, 1 h post application (Wu et al. 2016). However, mild corneal opacity and conjunctival redness were observed in 25 and 50% of rats following short-term repeated GO treatment of 50 and 100 mg/mL, respectively (Wu et al. 2016). Furthermore, the authors found that the GO-induced effects were reversible as no effects were observed at 24-, 48-, and 72-hr post-

GO exposure (Wu et al. 2016). Similarly, Lin et al. (2015) reported moderate damage to eyesight following intravitreal injection of 50 µL hydroxylated graphene (G-OH) into the right eye of rabbits. On the other hand, Yan et al. (2012) demonstrated that 0.1, 0.2, and 0.3 mg GO intravitreally injected eyes showed little change in eyeball appearance and eyesight in rabbits. These reports suggested mixed results for graphene as a potential eye irritant.

Reproductive and developmental toxicity

Liang et al. (2015) studied GO reproductive system toxicity in male mice given 25 mg/kg via tail vein injection and intraperitoneal injection of 24 and 60 mg/kg for 5 days and observed no decrease in fertility. Similarly, Zhang et al. (2019) showed that high doses of GQDs administered via oral gavage (300 mg/kg), or intravenous injection (150 mg/kg) produced no discernible short- and long-term toxic effects on male reproductive ability and health of offspring. Contradicting results were found by Akhavan et al. (2015), who observed a significant reduction in sperm viability and motility in male mice following intravenous administration of GO at a concentration below 0.4 mg/kg. The semen of the GO-treated mice (containing the damaged spermatozoa) was observed to change the fertility, gestation ability, and multi-production capability of female mice. Another study by Nirmal et al. (2017) in rats demonstrated a dose-dependent reduction in total sperm count after 15 and 30 days of exposure to GO via intraperitoneal injection. The authors observed no sperm dysfunction or testis damage associated with the low dose of 0.4 mg/kg and mid-dose 2.0 mg/kg, whereas a high dose of 10.0 mg/kg induced considerable testis damage (Nirmal et al. 2017). Additionally, Fu et al. (2015), demonstrated that an oral administration of GO at a concentration of 0.5 mg/mL or 150 mg/kg every day hurts the development of mice in the lactation period and an increase in body weight, body length, and tail length in mice. This study was corroborated by Xu et al. (2015), who observed evidence of abortions after an intravenous administration of rGO in pregnant mice with low (6.25 mg/kg) or medium doses (12.5 mg/kg) at a late stage (~20 days) of gestation and death of nearly all mice following intravenous injection with a high dose (25 mg/kg) of rGO in the late stage of gestation. Despite the contradicting results, these studies suggest that graphene may be a reproductive and developmental toxicant. Based on studies in rodents, a NOAEL of 300 mg/kg-day was identified for male reproductive ability and health of the offspring in mice (Zhang et al. 2019).

Acute toxicity

No adverse health effects of graphene have been reported in humans. Andrews et al. (2024) reported that no heart rate, blood pressure, lung function, or inflammatory markers were affected following 2 h inhalation exposure of 200 µg/m^3 GO in a double-blind randomized controlled study in humans. On the other hand, acute toxicity potential for GFN has been demonstrated through *in vivo* rodent studies. Duch et al. (2011) showed that 50 µg/mouse of pristine graphene and GO induced severe and persistent lung injury with excessive inflammation in C57BL/6 mice following 24 h intratracheal administration. The same dose of 50 µg/mouse GNP produced granulomatous lesions in the bronchiole lumen and near the alveolar region 24 h after pharyngeal aspiration (Schinwald et al. 2012). In addition, Singh et al. (2011) observed extensive pulmonary thromboembolism occluding lung vessels only 15 min following intravenous administration of rGO and GO at a dose of 250 µg/kg in mice. Another oropharyngeal aspiration investigation of rGO and GO at a 40 µg dose observed physiological changes and alterations in reactive oxygen species and gene expression that may lead to cardiovascular dysfunction following a one-day exposure (Krajnak et al. 2019). Together these data suggest the potential for acute toxicity for rGO and GO in rodents but possibly limited adverse health effects in humans. However, the NIOSH banding criteria require lethality data (LC50 or LD50) which were not available in any of the previously discussed animal studies for GFN.

Tier 3 overall OEB assignment by endpoint

The NIOSH banding process was followed for the nine health endpoints using the rodent and rabbit potency estimates to assign an OEB for GFN. Banding results for six health endpoints are listed in Table 4.

Stot-re

For the STOT-RE endpoint, NOAELs/LOAELs derived from rodent studies performed under internationally accepted test guidelines were used. There were eight different studies used for the banding process and eight different types of graphene were evaluated in those studies. Two studies resulted in band "E" and one study in band "C." However, five studies were not banded by two replicators because of insufficient information. The overall band "E" ($\leq$0.01 mg/m^3) was assigned to GFN,

Table 4. Occupational exposure band estimates for graphene family of nanomaterials by endpoints.

				Assigned band by replicate			
Endpoint	Reference	Type of graphene	PoD From the study	1	2	3	FB
STOT-RE2	Durán et al. (2017)	GO	NOAEL = 0.05 mg/kg	E*	N/A	N/A	N/A
	El-Yamany et al. (2017)	GO	NOAEL = 0.01 mg/kg	E*	N/A	E*	E*
	Kim et al. (2016)	GNP	NOAEL = 1.88 mg/m^3	C	C	C	C
	Kurantowicz et al. (2015)	GO	NOAEL = 4 mg/kg	N/A	N/A	D	N/A
	Park et al. (2017)	GNP	LOAEL = 5 mg/kg	D	N/A	N/A	N/A
	Strojny et al. (2015)	GO	NOAEL = 4 mg/kg	N/A	N/A	D	N/A
	Wu et al. (2022)	GQD, NGQD, AGQD	NOAEL = 0.1 mg/kg	E*	C	E*	E*
	Yang et al. (2013)	GO, nGO-PEG, RGO-PEG and nRGO-PEG	NOAEL = 100 mg/kg	N/A	N/A	C	N/A
	Overall STOT-RE Band			**E**			
Respiratory Sensitization	Han et al. (2015)	GO	IL-18	E	C	N/A	E
	Park et al. (2015)	GNP	IL-6	E	E	N/A	E
	Roberts et al. (2016)	GNP	IL-5, IL-10, and IL-13	E	N/A	E	E
	Schinwald et al. (2012)	GO	IL-8	E	E	N/A	E
	Shurin et al. (2014)	GO	IL-4, IL-5, IL-13	E	E	C	E
	Overall Respiratory Sensitization Band			E			
Genotoxicity	Bengtson et al. (2017)	GO and rGO	Positive genotoxicity	E	C	E	E
	Durán et al. (2017)	GO	Positive genotoxicity	E	C	E	E
	El-Yamany et al. (2017)	GO	Positive genotoxicity	E	E	E	E
	Mohamed et al. (2021)	GO	Positive genotoxicity	E	E	E	E
	Overall Genotoxicity Band			E			
Skin Sensitization	Kim et al. (2021)	GNP	Negative LLNA test	A	N/A	A	A
	Sosa et al. (2023)	FLG and GO	Negative LLNA test	A	N/A	A	A
	Overall Skin Sensitization Band			A			
Eye Irritation	Lin et al. (2015)	G-OH	Moderate damage on eyesight	B	B	A	B
	Wu et al. (2016)	GO	Mild to moderate corneal opacity	B	B	B	B
	Yan et al. (2012)	GO	Nonirritant	A	A	A	A
	Overall Eye Irritation Band			B			
Reproductive/Developmental	Fu et al. (2015)	GO	NOAEL = 30 mg/kg	C	N/A	C	C
	Liang et al. (2015)	GO	NOAEL = 60 mg/kg	B	N/A	B	B
	Nirmal et al. (2017)	GO	NOAEL = 10 mg/kg	C	A	C	C
	Zhang et al. (2019)	GQD	NOAEL = 60 mg/kg	B	N/A	B	B
	Overall Reproductive/ Developmental Band			C			

GO: graphene oxide; rGO: reduced graphene oxide; GNP: graphene nanoplatelets; FLG: few layers graphene; GQD: graphene quantum dots; MLG: multi-layered graphene; A-GQDs: amino-modified graphene quantum dot; GO-PSS: nanographene oxide functionalized with poly sodium 4-styrenesulfonate; PEG-nGO: PEGylated graphene oxide nanoparticle; GNF:graphene nanoflakes; nGO-PEG: PEGylated nano-GO; nGO-PEG: PEGylated nano-graphene oxide; RGO-PEG: PEGylated reduced graphene oxide; nRGO-PEG: PEGylated nano reduced graphene oxide G-OH: Hydroxylated graphene; GNF:graphene nano-flakes; LOAEL: lowest adverse effect level; NOAEL: no observable adverse effect level; STOT-RE: Specific Target Organ Toxicity-Repeated Exposure; PoD: point of departure; N/A: not assigned a band; IL: interleukin; *: Band assignments that would be an order-of-magnitude or more below band E.

which is the most stringent band. Two of the eight STOT-RE bands were based on lung effects, two were based on blood and immune toxicity, and four were based on internal organ (liver and kidney) toxicity.

Respiratory sensitization

The presence of high levels of T helper 2 (Th 2) cells, cytokines such as interleukin (IL) IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, and IL-18, and immunoglobulin E (IgE), which are important mediators of allergic responses were considered for the respiratory sensitization endpoint. Evidence of various interleukins were found for all the graphene nanomaterials including three GOs and two GNPs. Therefore, the overall band "E" was assigned.

Genotoxicity

The genotoxicity endpoint refers to changes in genetic material which includes mutations and other DNA or chromosome level changes. Qualitative data (e.g., *in vivo* heritable germ cell mutagenicity tests and *in vivo* somatic cell mutagenicity tests) were considered for banding this endpoint. All the data available for GO and rGO observed genotoxicity in rodent bioassays; therefore, the overall band "E" was assigned.

Skin sensitization

For skin sensitization, both quantitative (e.g., LLNA tests) and qualitative data (e.g., NIOSH SK-SEN notation) were considered for this endpoint. Only qualitative skin sensitization data were available for GNP, FLG, and GO. The overall band "A" (>10 mg/m^3) was assigned for both FLG and GNP because the skin sensitization results using the LLNA test were negative.

Eye irritation

The eye irritation endpoints were based on serious eye damage or irritation studies performed under an internationally accepted test. NIOSH OEB guidance

recommends assigning bands based on qualitative data instead of quantitative dose-response potency information from eye damage/irritation studies (NIOSH 2019). Two studies of GO and G-OH materials resulted in band "B" (>1 to 10 mg/m^3) based on mild to moderate eye irritation and another GO study resulted in band "A" based on nonirritating effects on the eye. The overall OEB selected for GFN for eye irritation was band "B."

Reproductive and developmental toxicity

These endpoints are based on adverse effects on sexual function and fertility in adult male and female test animals, and the developmental toxicity in the offspring observed in rodent bioassay data from three GO materials and one GQD. Based on these criteria, two studies of GO were assigned to band "C" (>0.1 to 1 mg/m^3), another GO study resulted in band "B", and the GQD was assigned to band "B." Therefore, the overall band for reproductive and developmental toxicity was "C."

Overall OEB assignment by material

In total 11 different GFN materials were included in this banding process, which included 18 studies on GOs, five for GNPs, two for GQD, and one each for FLG, rGO, NGQD, AGQD, G-OH, nGO-PEG, RGO-PEG, and nRGO-PEG. The overall OEB for GFN assigned by different independent replicates was "E," representing an exposure air concentration of $\leq$ 0.01 mg/m^3. The GO materials examined had four different bands assigned, including "E" for STOT-RE, respiratory sensitization, and genotoxicity, "C" for reproductive and developmental toxicity endpoints, "B" for eye irritation and reproductive and developmental toxicity endpoints, and "A" for skin sensitization endpoint. The rGO materials were banded "E" for respiratory sensitization and genotoxicity health endpoint. GNP materials received a band "E" for the respiratory sensitization endpoint, "C" for the STOT-RE endpoint, and "A" for skin sensitization. FLG nanomaterials were also banded "A" for skin sensitization. Functionalized graphene nanomaterials such as NGQD and AGQD were assigned a band "E" for STOT-RE endpoints. GQD was assigned a band "E" based on STOT-RE and "B" for reproductive and developmental toxicity endpoints. Finally, a band "B" was assigned for G-OH based on the eye irritation endpoint.

Discussion

The aim of this case study was to apply a Tier 3 assessment using the NIOSH banding process to derive an OEB for GFN. The NIOSH banding process is a resource for OSH professionals to guide risk management and exposure control decisions when OELs are not available. The information provided by OEBs, together with exposure assessment data, can be used to determine the acceptability of air sampling results and evaluate the need for controlling exposures (NIOSH 2019).

Currently, there are insufficient published data for GFN to perform a Tier 1 or Tier 2 assessment. The NIOSH banding process recommends that nine standard toxicological endpoints be considered when conducting a Tier 3 assessment (NIOSH 2019). During this Tier 3 assessment for GFN, data for two of the nine endpoints (skin corrosion/irritation and carcinogenicity) were not available while the data for acute toxicity was insufficient to band. Therefore, the remaining six endpoints were pursued here for banding. The overall OEB for GFN assessed was "E," representing a target exposure air concentration of $\leq$ 0.01 mg/m^3. This result is comparatively similar to the recommended OEL of 18 μg/m^3 by Lee et al. (2019) but lower than the BMC of 212 μg/m^3 developed by Spinazzè et al. (2019).

The results of this banding process were replicated by three individuals with advanced toxicology and industrial hygiene knowledge which was a strength of this study and should be considered for use in future Tier 3 assessments as well. There were discrepancies between OEBs among the replicates. However, there was only one study that did not have at least a two-thirds agreement among the assessors. The discrepancies were mainly due to difficulties locating NOAEL/LOAEL values in the articles, and therefore being unable to assign a band.

In this present study, the OEBs were assigned based on the specific physicochemical and toxicological properties of graphene nanomaterials with uncertainty factor adjustments. The results found that the overall band for GFN is band "E" which indicates that the materials evaluated could pose health effects at relatively low concentrations. OEB estimates for GO (El-Yamany et al. 2017), GO (Durán et al. 2017), and GQD (Wu et al. 2022) with NOAEL values of 0.01, 0.05, and 0.1 mg/kg-day, respectively, were lower than the current NIOSH band "E" representing a NOAEL of $\leq$1 mg/kg-day (Table 4). This may suggest the need for a risk manager to consider limiting exposures to a level lower than the "E" band based on the effect

levels presented in the data (External Draft Review NIOSH 2021). This suggestion was supported by the United Kingdom Health and Safety Executive (HSE) which recommended applying control approach three for dust in air exposure level of $\leq 0.001\,mg/m^3$ (HSE 2009). Other international occupational hazard band frameworks such as the International Organization for Standardization (ISO) and the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) have corroborated these findings by shifting by a factor of 10 difference which corresponds to an air concentration of $\leq 0.001\,mg/m^3$ for nanoparticles (ANSES 2010; ISO 2016).

There are no known reports or case studies of adverse health effects in workers using or producing GFN. The only controlled human exposure study available to date reported that no heart rate, blood pressure, lung function, or inflammatory markers were affected within 14 young healthy volunteers following 2-hr inhalation exposure of $200\,\mu g/m^3$ GO in repeated visits (Andrews et al. 2024). By contrast, the literature review identified short-term and sub-chronic studies in rats and mice, many of which indicated that graphene may have the potential to cause non-cancerous adverse lung effects including pulmonary edema, pulmonary inflammation, and granulomatous lesions in the bronchiole lumen and near the alveolar region following inhalation, intratracheal instillation, or pharyngeal aspiration (Schinwald et al. 2012, 2014; Park et al. 2015; Mao et al. 2016; El-Yamany et al. 2017; Lee et al. 2017).

The NIOSH occupational exposure banding process did not guide sampling methods or the relevant aerosol size fraction to sample to protect workers from potentially harmful exposures to particulates. Rather, OSH professionals applying a Tier 3 assessment should decide on the sampling method and size fraction for the chemical of interest. For GFN, it may be advisable to sample at least at the respirable size fraction because the derived OEB was primarily based on adverse lung effects in animals observed near the alveolar region (Schinwald et al. 2012, 2014; El-Yamany et al. 2017; Li et al. 2018). The derived OEB for GFN is designed to be used as an 8-hr time-weighted average (TWA) exposure range (NIOSH 2019).

There are currently no consensus air sampling methods for GFN. However, there has been a growing agreement on methodologies for other carbonaceous nanomaterials, such as carbon nanotubes, as well as some graphene nanomaterials which have used elemental carbon as a marker for exposure (Lee et al. 2016; Vaquero et al. 2019; Guseva Canu et al. 2020; Lovén et al. 2021; McCormick et al. 2021). The exposure sampling methods for carbonaceous nanomaterials have generally consisted of a sample collected for elemental carbon mass using NIOSH method 5040, or equivalent, coupled with a sample for electron microscopy to verify and further describe the size and morphology of the collected aerosol (Birch 2016; Eastlake et al. 2016; Dahm et al. 2018).

NIOSH has previously evaluated the consistency of the banding process for chemical substances by comparing OEBs with existing OELs. This evaluation demonstrated that the NIOSH Tier 1 and Tier 2 banding process resulted in a band that included the OEL or was more conservative than the OEL 91% and 98% of the time, respectively (NIOSH 2019). By contrast, the NIOSH Tier 3 banding process has never been evaluated. The guidance states that expert judgment should be used providing OSH professionals with a wide latitude for decision making. The NIOSH Tier 3 banding process allows for the use of *in vitro* data, specifically for the genotoxicity endpoint. However, it was not considered in this study because *in vitro* data lacked the inherent complexity of organ systems, and extrapolating the results of *in vitro* assays to predict the toxicokinetics of graphene in workers may lead to a misinterpretation of the data (Saeidnia et al. 2015). Despite the NIOSH banding framework recommending the use of NOAEL values derived from studies featuring oral, dermal, and/or inhalation exposures in experimental animals, other routes of exposure such as intratracheal instillation and intraperitoneal delivery were also considered in this Tier 3 assessment due to a lack of current oral and inhalation studies. Intratracheal instillation is widely used to test the respiratory toxicity of a substance as an alternative to inhalation in animal testing (Driscoll et al. 2000). The toxicokinetics of substances administered via intraperitoneal injection are considered to be like those seen after oral administration because they are absorbed primarily through the portal circulation and therefore must pass through the liver before reaching the other organs (Klaassen and Watkins 2010). The NIOSH banding process could be repeated for GFN once more toxicological data are available to demonstrate the assumptions made during this application.

The NIOSH banding process guidance recommends the use of NOAEL/LOAEL or benchmark dose level (BMDL) values when outcomes are expressed as quantitative information and/or potency data (NIOSH 2019). Although the BMDL approach typically offers

more accurate potency estimations since it utilizes the full dose range of data (Boots et al. 2021; EFSA Scientific Committee 2022; EPA 2012), it was not considered in this paper because many of the studies used for the STOT-RE and reproductive/developmental endpoints were not amenable to BMDL modeling.

Conducting a Tier 3 assessment using the NIOSH banding process was challenging and required individuals with advanced knowledge in toxicology and industrial hygiene. One of the major challenges faced during this assessment was identifying NOAEL/LOAEL values from animal toxicology studies. To find such a dose from nonclinical studies can often be challenging, as well as subjective, due to difficulties in distinguishing between adverse and non-adverse effects (Kale et al. 2022). Another challenge encountered during the NIOSH Tier 3 banding process was selecting the criteria for the respiratory sensitization endpoint. While pulmonary inflammation is recommended by the NIOSH banding process, it may not be a sufficient endpoint for evaluating respiratory sensitization. Therefore, other respiratory sensitization markers such as high levels of T helper 2 (Th 2) cells, immunoglobulin E (IgE) antibodies, or the presence of certain cytokines were considered for banding this health endpoint.

Limitations

Limitations in the data include a lack of graphene solubility data from acute to chronic endpoints. Given the uncertainties in the relationship of solubility to particle toxicity, NIOSH recommends that in the absence of data to the contrary, all nanoscale particles should be treated in the same manner without regard to solubility (NIOSH 2019). Another limitation of this study is the lack of relevant data for GFN across some toxicological endpoints such as acute toxicity, skin corrosion/irritation, and carcinogenicity. Generally, lacking toxicological data across all endpoints may lead to the banding process resulting in a band that is not the most protective. In addition, the use of intratracheal instillation as an alternative to inhalation constitutes a limitation as it bypasses the upper respiratory tract such as nasal and oral passages, pharynx, and larynx, thus making it difficult to compare with the results obtained using inhalation exposure (Driscoll et al. 2000). These limitations highlight the need for additional sub-chronic and chronic repeated dose inhalation studies which would provide critical information for future risk assessments.

Conclusion

The derived overall OEB for GFN from this case study that applied the NIOSH banding process was a band "E," representing an air concentration of $\leq$ 0.01 mg/m^3. The OEB can be used in combination with exposure information to inform workplace risk management decisions. This derived band is voluntary and only serves as a consideration for OSH professionals until a traditional and more thorough risk assessment is performed by an authoritative public health agency to develop an OEL. Furthermore, additional sub-chronic and chronic repeated dose inhalation animal studies and research on the solubility of graphene nanomaterials would benefit future risk assessments on these materials.

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Disclaimer

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

References

Akhavan O, Ghaderi E, Hashemi E, Akbari E. 2015. Dose-dependent effects of nanoscale graphene oxide on reproduction capability of mammals. Carbon. 95:309–317. doi: 10.1016/j.carbon.2015.08.017.

Altwaijry N, Ain QT, Alnuwaysir H, Alamro A, Alghamdi A, Haq SH. 2022. A time-course evaluation of DNA damage and neurotoxicity induced by PEGylated graphene oxide nanoparticle in swiss albino mice. J Biomed Nanotechnol. 18(4):1180–1186. doi: 10.1166/jbn.2022.3306.

Amrollahi-Sharifabadi M, Koohi MK, Zayerzadeh E, Hablolvarid MH, Hassan J, Seifalian AM. 2018. In vivo toxicological evaluation of graphene oxide nanoplatelets for clinical application. Int J Nanomedicine. 13:4757–4769. doi: 10.2147/IJN.S168731.

Andrews JPM, Joshi SS, Tzolos E, Syed MB, Cuthbert H, Crica LE, Lozano N, Okwelogu E, Raftis JB, Bruce L, et al. 2024. First-in-human controlled inhalation of thin graphene oxide nanosheets to study acute cardiorespiratory responses. Nat Nanotechnol. 19(5):705–714. doi: 10.1038/s41565-023-01572-3.

ANSES. 2010. Development of a specific control banding tool for nanomaterials. Maisons-Alfort Cedex (France): Agence nationale de sécurité sanitaire. French Agency for Food, Environmental and Occupational Health & Safety. Request no. 2008-SA-0407. https://www.anses.fr/en/system/files/AP2008sa0407RaEN.pdf.

Babik K, Dahm M, Dunn KH, Dunn KL, Schubauer-Berigan M. 2018. Characterizing workforces exposed to current and emerging non-carbonaceous nanomaterials in the U.S. J Occup Environ Hyg. 15(1):44–56. doi: 10.1080/15459624.2017.1376252.

Bengtson S, Knudsen KB, Kyjovska ZO, Berthing T, Skaug V, Levin M, Koponen IK, Shivayogimath A, Booth TJ, Alonso B, et al. 2017. 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(6):e0178355. doi: 10.1371/journal.pone.0178355.

Birch ME. 2016. Monitoring diesel exhaust in the workplace. In: Andrews R, O'Connor PF, editors. NIOSH Manual of Analytical Methods (NMAM). 5th ed., Chapter DL. Cincinnati (OH): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH).

Boots TE, Kogel AM, Drew NM, Kuempel ED. 2021. Utilizing literature-based rodent toxicology data to derive potency estimates for quantitative risk assessment. Nanotoxicology. 15(6):740–760. doi: 10.1080/17435390.2021.1918278.

Boverhof DR, Billington R, Gollapudi BB, Hotchkiss JA, Krieger SM, Poole A, Wiescinski CM, Woolhiser MR. 2008. Respiratory sensitization and allergy: current research approaches and needs. Toxicol Appl Pharmacol. 226(1):1–13. doi: 10.1016/j.taap.2007.10.008.

Chen F, Gao W, Qiu X, Zhang H, Liu L, Liao P, Fu W, Luo Y. 2017. Graphene quantum dots in biomedical applications: recent advances and future challenges. Front Lab Med. 1(4):192–199. doi: 10.1016/j.flm.2017.12.006.

Dahm MM, Schubauer-Berigan MK, Evans DE, Birch ME, Bertke S, Beard JD, Erdely A, Fernback JE, Mercer RR, Grinshpun SA, et al. 2018. Exposure assessments for a cross-sectional epidemiologic study of US carbon nanotube and nanofiber workers. Int J Hyg Environ Health. 221(3):429–440. doi: 10.1016/j.ijheh.2018.01.006.

Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, Schlesinger RB. 2000. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. Toxicol Sci. 55(1):24–35. doi: 10.1093/toxsci/55.1.24.

Duch MC, Budinger GR, Liang YT, Soberanes S, Urich D, Chiarella SE, Campochiaro LA, Gonzalez A, Chandel NS, Hersam MC, et al. 2011. Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. Nano Lett. 11(12):5201–5207. doi: 10.1021/nl202515a.

Durán M, Durán N, Fávaro WJ. 2017. In vivo nanotoxicological profile of graphene oxide. J Phys Conf Ser. 838:012026. doi: 10.1088/1742-6596/838/1/012026.

Eastlake A, Beaucham C, Martinez K, Dahm M, Sparks C, Hodson L, Geraci C. 2016. Refinement of the nanoparticle emission assessment technique into the Nanomaterial Exposure Assessment Technique (NEAT 2.0). J Occup Environ Hyg. 13(9):708–717. doi: 10.1080/15459624.2016.1167278.

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernández-Jerez AF, Bennekou SH, Koutsoumanis K, Lambré C, Machera K, et al. 2022. Guidance on the use of the benchmark dose approach in risk assessment. EFSA J. 20(10):e07584. doi: 10.2903/j.efsa.2022.7584.

El-Yamany NA, Mohamed FF, Salaheldin TA, Tohamy AA, Abd El-Mohsen WN, Amin AS. 2017. Graphene oxide nanosheets induced genotoxicity and pulmonary injury in mice. Exp Toxicol Pathol. 69(6):383–392. doi: 10.1016/j.etp.2017.03.002.

EPA. 2012 Jun. Benchmark dose technical guidance. Washington (DC): Risk Assessment Forum U.S. Environmental Protection Agency. EPA/100/R-12/001.

Fu C, Liu T, Li L, Liu H, Liang Q, Meng X. 2015. Effects of graphene oxide on the development of offspring mice in lactation period. Biomaterials. 40:23–31. doi: 10.1016/j.biomaterials.2014.11.014.

Gibb M, Sayes C. 2022. An in vitro alveolar model allows for the rapid assessment of chemical respiratory sensitization with modifiable biomarker endpoints. Chem Biol Interact. 368:110232. doi: 10.1016/j.cbi.2022.110232.

Guseva Canu I, Batsungnoen K, Maynard A, Hopf NB. 2020. State of knowledge on the occupational exposure to carbon nanotubes. Int J Hyg Environ Health. 225:113472. doi: 10.1016/j.ijheh.2020.113472.

Han SG, Kim JK, Shin JH, Hwang JH, Lee JS, Kim TG, Lee JH, Lee GH, Kim KS, Lee HS, et al. 2015. Pulmonary responses of Sprague-Dawley rats in single inhalation

exposure to graphene oxide nanomaterials. Biomed Res Int. 2015:376756–376759. doi: 10.1155/2015/376756.

Hines CJ, Lentz TJ, McKernan L, Rane P, Whittaker C. 2019. Application of the draft NIOSH occupational exposure banding process to bisphenol A: a case study. J Occup Environ Hyg. 16(2):120–128. doi: 10.1080/15459624.2018.1542495.

[HSE] Health and Safety Executive. 2009. The technical basis for COSHH essentials: easy steps to control chemicals. Health and Safety Executive. http://www.hse.gov.uk/pubns/guidance/coshh-technical-basis.pdf.

[ISO] International Organization for Standardization. 2016. Nanotechnologies—overview of available frameworks for the development of occupational exposure limits and bands for nano-objects and their aggregates and agglomerates (NOAAs). Geneva (Switzerland): ISO. ISO Technical Report ISO/TR 18637. https://www.iso.org/standard/63096.html.

[ISO] International Organization for Standardization. 2017. Nanotechnologies—vocabulary—part 13: graphene and related two dimensional (2D) materials. First edition 2017-09. ISO/TS 80004-13:2017. https://standards.iteh.ai/catalog/standards/sist/4c93ccd3-8f8a-4567-b42c9c87a-f206af6/iso-ts-80004-13-2017.

Kale VP, Bebenek I, Ghantous H, Kapeghian J, Singh BP, Thomas LJ. 2022. Practical considerations in determining adversity and the no-observed-adverse-effect-level (NOAEL) in nonclinical safety studies: challenges, perspectives and case studies. Int J Toxicol. 41(2):143–162. doi: 10.1177/10915818211073047.

Kim JK, Shin JH, Lee JS, Hwang JH, Lee JH, Baek JE, Kim TG, Kim BW, Kim JS, Lee GH, et al. 2016. 28-Day inhalation toxicity of graphene nanoplatelets in Sprague-Dawley rats. Nanotoxicology. 10(7):891–901. doi: 10.3109/17435390.2015.1133865.

Kim SH, Hong SH, Lee JH, Lee DH, Jung K, Yang JY, Shin HS, Lee J, Jeong J, Oh JH. 2021. Skin sensitization evaluation of carbon-based graphene nanoplatelets. Toxics. 9(3):62. doi: 10.3390/toxics9030062.

Klaassen CD, Watkins JB, III. 2010. Casarett and Doull's essentials of toxicology. 2nd ed. New York (NY): McGraw-Hill/Medical Pub. Div.

Klimisch HJ, Andreae M, Tillmann U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol. 25(1):1–5. doi: 10.1006/rtph.1996.1076.

Krajnak K, Waugh S, Stefaniak A, Schwegler-Berry D, Roach K, Barger M, Roberts J. 2019. Exposure to graphene nanoparticles induces changes in measures of vascular/renal function in a load and form-dependent manner in mice. J Toxicol Environ Health A. 82(12):711–726. doi: 10.1080/15287394.2019.1645772.

Kurantowicz N, Strojny B, Sawosz E, Jaworski S, Kutwin M, Grodzik M, Wierzbicki M, Lipińska L, Mitura K, Chwalibog A. 2015. Biodistribution of a high dose of diamond, graphite, and graphene oxide nanoparticles after multiple intraperitoneal injections in rats. Nanoscale Res Lett. 10(1):398. doi: 10.1186/s11671-015-1107-9.

Lamb J, Fischer E, Rosillo-Lopez M, Salzmann CG, Holland JP. 2019. Multi-functionalised graphene nanoflakes as tumour-targeting theranostic drug-delivery vehicles. Chem Sci. 10(38):8880–8888. doi: 10.1039/c9sc03736e.

Lee JH, Han JH, Kim JH, Kim B, Bello D, Kim JK, Lee GH, Sohn EK, Lee K, Ahn K, et al. 2016. Exposure monitoring of graphene nanoplatelets manufacturing workplaces. Inhal Toxicol. 28(6):281–291. doi: 10.3109/08958378.2016.1163442.

Lee JK, Jeong AY, Bae J, Seok JH, Yang JY, Roh HS, Jeong J, Han Y, Jeong J, Cho WS. 2017. The role of surface functionalization on the pulmonary inflammogenicity and translocation into mediastinal lymph nodes of graphene nanoplatelets in rats. Arch Toxicol. 91(2):667–676. doi: 10.1007/s00204-016-1706-y.

Lee YS, Sung JH, Song KS, Kim JK, Choi BS, Yu IJ, Park JD. 2019. Derivation of occupational exposure limits for multi-walled carbon nanotubes and graphene using subchronic inhalation toxicity data and a multi-path particle dosimetry model. Toxicol Res (Camb). 8(4):580–586. doi: 10.1039/c9tx00026g.

Li R, Guiney LM, Chang CH, Mansukhani ND, Ji Z, Wang X, Liao YP, Jiang W, Sun B, Hersam MC, et al. 2018. Surface oxidation of graphene oxide determines membrane damage, lipid peroxidation, and cytotoxicity in macrophages in a pulmonary toxicity model. ACS Nano. 12(2):1390–1402. doi: 10.1021/acsnano.7b07737.

Li Y, Yuan H, von Dem Bussche A, Creighton M, Hurt RH, Kane AB, Gao H. 2013. Graphene microsheets enter cells through spontaneous membrane penetration at edge asperities and corner sites. Proc Natl Acad Sci USA. 110(30):12295–12300. doi: 10.1073/pnas.1222276110.

Liang S, Xu S, Zhang D, He J, Chu M. 2015. Reproductive toxicity of nanoscale graphene oxide in male mice. Nanotoxicology. 9(1):92–105. Erratum in: Nanotoxicology. 2016;10(8):1204. doi: 10.3109/17435390.2014.893380.

Lin M, Zou R, Shi H, Yu S, Li X, Guo R, Yan L, Li G, Liu Y, Dai L. 2015. Ocular biocompatibility evaluation of hydroxyl-functionalized graphene. Mater Sci Eng C Mater Biol Appl. 50:300–308. doi: 10.1016/j.msec.2015.01.086.

Lovén K, Franzén SM, Isaxon C, Messing ME, Martinsson J, Gudmundsson A, Pagels J, Hedmer M, NanoLund. 2021. Emissions and exposures of graphene nanomaterials, titanium dioxide nanofibers, and nanoparticles during downstream industrial handling. J Expo Sci Environ Epidemiol. 31(4):736–752. doi: 10.1038/s41370-020-0241-3.

Ma-Hock L, Strauss V, Treumann S, Küttler K, Wohlleben W, Hofmann T, Gröters S, Wiench K, van Ravenzwaay B, Landsiedel R. 2013. Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black. Part Fibre Toxicol. 10(1):23. doi: 10.1186/1743-8977-10-23.

Mao L, Hu M, Pan B, Xie Y, Petersen EJ. 2016. Biodistribution and toxicity of radio-labeled few layers graphene in mice after intratracheal instillation. Part Fibre Toxicol. 13(1):7. doi: 10.1186/s12989-016-0120-1.

McCormick S, Niang M, Dahm MM. 2021. Occupational exposures to engineered nanomaterials: a review of workplace exposure assessment methods. Curr Environ Health Rep. 8(3):223–234. doi: 10.1007/s40572-021-00316-6.

Mendonça MC, Soares ES, de Jesus MB, Ceragioli HJ, Batista ÂG, Nyúl-Tóth Á, Molnár J, Wilhelm I, Maróstica MR, Jr, Krizbai I, et al. 2016a. PEGylation of reduced

graphene oxide induces toxicity in cells of the blood-brain barrier: an in vitro and in vivo study. Mol Pharm. 13(11):3913–3924. doi: 10.1021/acs.molpharmaceut.6b00696.

Mendonça MC, Soares ES, de Jesus MB, Ceragioli HJ, Ferreira MS, Catharino RR, da Cruz-Höfling MA. 2015. Reduced graphene oxide induces transient blood-brain barrier opening: an in vivo study. J Nanobiotechnol. 13(1):78. doi: 10.1186/s12951-015-0143-z.

Mendonça MC, Soares ES, de Jesus MB, Ceragioli HJ, Irazusta SP, Batista ÂG, Vinolo MA, Maróstica Júnior MR, da Cruz-Höfling MA. 2016b. Reduced graphene oxide: nanotoxicological profile in rats. J Nanobiotechnol. 14(1):53. doi: 10.1186/s12951-016-0206-9.

Mohamed HRH, Welson M, Yaseen AE, El-Ghor A. 2021. Induction of chromosomal and DNA damage and histological alterations by graphene oxide nanoparticles in Swiss mice. Drug Chem Toxicol. 44(6):631–641. doi: 10.1080/01480545.2019.1643876.

NIOSH. 2019. Technical report: the NIOSH occupational exposure banding process for chemical risk management. By Lentz TJ, Seaton M, Rane P, Gilbert SJ, McKernan LT, Whittaker C. Cincinnati (OH): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 2019-132, doi: 10.26616/NIOSHPUB2019132.

NIOSH. 2021. External review draft. Approaches to developing occupational exposure limits or bands for engineered nanomaterials: user guide and technical report. NIOSH Docket Number 342, CDC-2021-0067.

Nirmal NK, Awasthi KK, John PJ. 2017. Effects of nano-graphene oxide on testis, epididymis and fertility of Wistar rats. Basic Clin Pharmacol Toxicol. 121(3):202–210. doi: 10.1111/bcpt.12782.

Park EJ, Lee GH, Han BS, Lee BS, Lee S, Cho MH, Kim JH, Kim DW. 2015. 2015. Toxic response of graphene nanoplatelets in vivo and in vitro. Arch Toxicol. 89(9):1557–1568. doi: 10.1007/s00204-014-1303-x.

Park EJ, Lee SJ, Lee K, Choi YC, Lee BS, Lee GH, Kim DW. 2017. Pulmonary persistence of graphene nanoplatelets may disturb physiological and immunological homeostasis. J Appl Toxicol. 37(3):296–309. doi: 10.1002/jat.3361.

Paszkiewicz S, Szymczyk A. 2019. Chapter 6 – Graphene-based nanomaterials and their polymer nanocomposites. In: Karak N, editor. Nanomaterials and polymer nanocomposites – Raw materials to applications. Cambridge (MA): Elsevier Inc. p. 177–216. doi: 10.1016/B978-0-12-814615-6.00006-0.

Patlolla AK, Randolph J, Kumari SA, Tchounwou PB. 2016. Toxicity evaluation of graphene oxide in kidneys of Sprague-Dawley rats. Int J Environ Res Public Health. 13(4):380. doi: 10.3390/ijerph13040380.

Patlolla AK, Rondalph J, Tchounwou PB. 2017. Biochemical and histopathological evaluation of graphene oxide in Sprague-Dawley rats. Austin J Environ Toxicol. 3(1):1021.

Pelin M, Sosa S, Prato M, Tubaro A. 2018. Occupational exposure to graphene-based nanomaterials: risk assessment. Nanoscale. 10(34):15894–15903. doi: 10.1039/c8nr04950e.

Roberts JR, Mercer RR, Stefaniak AB, Seehra MS, Geddam UK, Chaudhuri IS, Kyrlidis A, Kodali VK, Sager T, Kenyon A, et al. 2016. Evaluation of pulmonary and systemic toxicity following lung exposure to graphite nanoplates: a member of the graphene-based nanomaterial family. Part Fibre Toxicol. 13(1):34. doi: 10.1186/s12989-016-0145-5.

Robinson DS. 2000. Th-2 cytokines in allergic disease. Br Med Bull. 56(4):956–968. doi: 10.1258/0007142001903625.

Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med. 326(5):298–304. doi: 10.1056/NEJM199201303260504.

Saeidnia S, Manayi A, Abdollahi M. 2015. From in vitro experiments to in vivo and clinical studies; pros and cons. Curr Drug Discov Technol. 12(4):218–224. doi: 10.2174/1570163813666160114093140.

Sanchez VC, Jachak A, Hurt RH, Kane AB. 2012. Biological interactions of graphene-family nanomaterials: an interdisciplinary review. Chem Res Toxicol. 25(1):15–34. doi: 10.1021/tx200339h.

Sanders NL, Mishra A. 2016. Role of interleukin-18 in the pathophysiology of allergic diseases. Cytokine Growth Factor Rev. 32:31–39. doi: 10.1016/j.cytogfr.2016.07.001.

Schinwald A, Murphy F, Askounis A, Koutsos V, Sefiane K, Donaldson K, Campbell CJ. 2014. Minimal oxidation and inflammogenicity of pristine graphene with residence in the lung. Nanotoxicology. 8(8):824–832. doi: 10.3109/17435390.2013.831502.

Schinwald A, Murphy FA, Jones A, MacNee W, Donaldson K. 2012. Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. ACS Nano. 6(1):736–746. doi: 10.1021/nn204229f.

Shin JH, Han SG, Kim JK, Kim BW, Hwang JH, Lee JS, Lee JH, Baek JE, Kim TG, Kim KS, et al. 2015. 5-Day repeated inhalation and 28-day post-exposure study of graphene. Nanotoxicology. 9(8):1023–1031. doi: 10.3109/17435390.2014.998306.

Shurin MR, Yanamala N, Kisin ER, Tkach AV, Shurin GV, Murray AR, Leonard HD, Reynolds JS, Gutkin DW, Star A, et al. 2014. Graphene oxide attenuates Th2-type immune responses but augments airway remodeling and hyperresponsiveness in a murine model of asthma. ACS Nano. 8(6):5585–5599. doi: 10.1021/nn406454u.

Singh SK, Singh MK, Nayak MK, Kumari S, Shrivastava S, Grácio JJ, Dash D. 2011. Thrombus inducing property of atomically thin graphene oxide sheets. ACS Nano. 5(6):4987–4996. doi: 10.1021/nn201092p.

Smith AT, LaChance AM, Zeng S, Liu B, Sun L. 2019. Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. Nano Mater Sci. 1(1):31–47. doi: 10.1016/j.nanoms.2019.02.004.

Sosa S, Tubaro A, Carlin M, Ponti C, Vázquez E, Prato M, Pelin M. 2023. Assessment of skin sensitization properties of few-layer graphene and graphene oxide through the Local Lymph Node Assay (OECD TG 442B). NanoImpact. 29:100448. doi: 10.1016/j.impact.2022.100448.

Spinazzè A, Cattaneo A, Borghi F, Del Buono L, Campagnolo D, Rovelli S, Cavallo DM. 2019.

Probabilistic approach for the risk assessment of nanomaterials: a case study for graphene nanoplatelets. Int J Hyg Environ Health. 222(1):76–83. doi: 10.1016/j.ijheh.2018.08.011.

Strojny B, Kurantowicz N, Sawosz E, Grodzik M, Jaworski S, Kutwin M, Wierzbicki M, Hotowy A, Lipińska L, Chwalibog A. 2015. Long term influence of carbon nanoparticles on health and liver status in rats. PLoS One. 10(12):e0144821. doi: 10.1371/journal.pone.0144821.

Sutton BJ, Davies AM, Bax HJ, Karagiannis SN. 2019. IgE antibodies: from structure to function and clinical translation. Antibodies (Basel). 8(1):19. doi: 10.3390/antib8010019.

Syama S, Paul W, Sabareeswaran A, Mohanan PV. 2017. Raman spectroscopy for the detection of organ distribution and clearance of PEGylated reduced graphene oxide and biological consequences. Biomaterials. 131:121–130. Erratum in: Biomaterials. 2021;276:121054. doi: 10.1016/j.biomaterials.2017.03.043.

Vaquero C, Wendelbo R, Egizabal A, Gutierrez-Cañas C, López de Ipiña J. 2019. Exposure to graphene in a pilot production plant. J Phys Conf Ser. 1323(1):012005. doi: 10.1088/1742-6596/1323/1/012005.

Wick P, Louw-Gaume A, Kucki M, Krug H, Kostarelos K, Fadeel B, Dawson K, Salvati A, Vazquez E, Ballerini L, et al. 2014. Classification framework for graphene-based materials. Angew Chem Int Ed Engl. 53(30):7714–7718. doi: 10.1002/anie.201403335.

Wu T, Wang X, Chen M, Zhang X, Zhang J, Cheng J, Kong L, Tang M. 2022. Respiratory exposure to graphene quantum dots causes fibrotic effects on lung, liver, and kidney of mice. Food Chem Toxicol. 163:112971. doi: 10.1016/j.fct.2022.112971.

Wu W, Yan L, Wu Q, Li Y, Li Q, Chen S, Yang Y, Gu Z, Xu H, Yin ZQ. 2016. Evaluation of the toxicity of graphene oxide exposure to the eye. Nanotoxicology. 10(9):1329–1340. doi: 10.1080/17435390.2016.1210692.

Xu S, Zhang Z, Chu M. 2015. Long-term toxicity of reduced graphene oxide nanosheets: effects on female mouse reproductive ability and offspring development. Biomaterials. 54:188–200. doi: 10.1016/j.biomaterials.2015.03.015.

Yan L, Wang Y, Xu X, Zeng C, Hou J, Lin M, Xu J, Sun F, Huang X, Dai L, et al. 2012. Can graphene oxide cause damage to eyesight? Chem Res Toxicol. 25(6):1265–1270. doi: 10.1021/tx300129f.

Yang K, Gong H, Shi X, Wan J, Zhang Y, Liu Z. 2013. In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. Biomaterials. 34(11):2787–2795. doi: 10.1016/j.biomaterials.2013.01.001.

Zhang D, Zhang Z, Wu Y, Fu K, Chen Y, Li W, Chu M. 2019. Systematic evaluation of graphene quantum dot toxicity to male mouse sexual behaviors, reproductive and offspring health. Biomaterials. 194:215–232. doi: 10.1016/j.biomaterials.2018.12.001.